Development and Characterization of a Multiplexed RT-PCR Species Specific Assay for Bovine and one for Porcine Foot-and-Mouth Disease Virus Rule-Out

SUPPLEMENTAL MATERIALS

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Executive summary

Lawrence Livermore National Laboratory (LLNL), in collaboration with the Department of Homeland Security (DHS) and the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Services (APHIS) has developed advanced rapid diagnostics that may be used within the National Animal Health Laboratory Network (NAHLN), the National Veterinary Services Laboratory (Ames, Iowa) and the Plum Island Animal Disease Center (PIADC). This effort has the potential to improve our nation's ability to discriminate between foreign animal diseases and those that are endemic using a single assay, thereby increasing our ability to protect animal populations of high economic importance in the United States. Under 2005 DHS funding we have developed multiplexed (MUX) nucleic-acid-based PCR assays that combine foot-and-mouth disease virus (FMDV) detection with rule-out tests for two other foreign animal diseases Vesicular Exanthema of Swine (VESV) and Swine Vesicular Disease (SVD) and four other domestic viral diseases Bovine Viral Diarrhea Virus (BVDV), Bovine Herpes Virus 1 (BHV-1 or Infectious Bovine Rhinotracheitus IBR), Bluetongue virus (BTV) and Parapox virus complex (which includes Bovine Papular Stomatitis Virus BPSV, Orf of sheep, and Pseudocowpox). Under 2006 funding we have developed a Multiplexed PCR [MUX] porcine assay for detection of FMDV with rule out tests for VESV and SVD foreign animal diseases in addition to one other domestic vesicular animal disease vesicular stomatitis virus (VSV) and one domestic animal disease of swine porcine reproductive and respiratory syndrome (PRRS). We have also developed a MUX bovine assay for detection of FMDV with rule out tests for the two bovine foreign animal diseases malignant catarrhal fever (MCF), rinderpest virus (RPV) and the domestic diseases vesicular stomatitis virus (VSV), bovine viral diarrhea virus (BVDV), infectious bovine rhinotracheitus virus (BHV-1), bluetongue virus (BTV), and the Parapox viruses which are of two bovine types bovine papular stomatitis virus (BPSV) and psuedocowpox (PCP).

This document provides details of signature generation, evaluation, and testing, as well as the specific methods and materials used. A condensed summary of the development, testing and performance of the multiplexed assay panel was presented in a 126 page separate document, entitled "Development and Characterization of A Multiplexed RT-PCR Species Specific Assay for Bovine and one for Porcine Foot-and-Mouth Disease Virus Rule-Out". This supplemental document provides additional details of large amount of data collected for signature generation, evaluation, and testing, as well as the specific methods and materials used for all steps in the assay development and utilization processes. In contrast to last years' effort, the development of the bovine and porcine panels is pending additional work to complete analytical characterization of FMDV, VESV, VSV, SVD, RPV and MCF. The signature screening process and final panel composition impacts this effort. The unique challenge presented this year was having strict predecessor limitations in completing characterization, where efforts at LLNL must preceed efforts at PIADC, such challenges were alleviated in the 2006 reporting by having characterization data from the interlaboratory comparison and at Plum Island under AgDDAP project. We will present an addendum at a later date with additional data on the characterization of the porcine and bovine multiplex assays when that data is available.

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INTRODUCTION

The Chemical and Biological (CB) Assay Development Group at Lawrence Livermore National Lab (LLNL) followed a proven assay development process involving computational signature identification followed by wet chemistry bench-screening to identify specific and sensitive signatures that underlie robust, reliable detection assays. We used our computational DNA signature generation system to identify the genome regions of vesicular disease-causing viruses that are unique to each agent. We mined the resulting sequence for triplet oligonucleotides that met stringent parameters [e.g. melt temperature, amplicon length, GC content of oligos, etc.] for the development of candidate Real-time RT- PCR signatures expected to produce robust assays. These "in-silico" down-selected signatures were then put through our rigorous wet chemistry bench-screening process that seeks to identify signatures unique to Bluetongue Virus.

Multiplexed assays provide many advantages over conventional detection methods. In the event of an outbreak, the use of these multiplexed assay panels can provide rapid, sensitive, specific and cost effective means of handling high volumes of samples. Custom tailored assay panels can greatly improve response time and provide rapid results, which can help, reduce the impact of infectious disease outbreaks. The use of bead-based liquid arrays has proven to be a well adapted and versatile technology that can be custom tailored to rapidly screen for both DNA and RNA in a single tube, while also allowing for multi-loci detection.

The LLNL bench screening process involves over 7500 individual reactions [2500 samples run in triplicate] per signature run against background, target, and near-neighbor panels. The most promising signatures that are shown to be both sensitive and specific are accepted to form a Real-time RT- PCR assay. The signatures comprising multiple Real-time RT- PCR assays targeting multiple organisms are then sent forward for multiplexed assay development. Through a series of step-wise tests, each signature is tested then merged into a multiplexed panel. At this phase of development, signatures are re-screened (as a complete panel) against environmental backgrounds as well as target nucleic acids. Performance of each signature is characterized in multiplex assay formats and the results of the performance of each of the domestic animal disease signatures are reported herein.

This document is organized by agent, where each agent section reports sequential assay development step in the development process. This document contains not only new development efforts for FY06, but also previous years development as it is applicable to the development of species-specific assay panels (Parapox, BHV, BVD, BTV, FMDV, VESV, and SVD). The order of the agents in this document is respective to their panel membership. The first 8 agents are bovine-panel agents, and the last 5 are porcine panel agents, with overlap of FMDV and VSV which belong to both assay panels. Each sections begins with an objective statement, followed by background information on the agent, then the bioinformatics and computations summary which provides detailed information on signature generation and attributes, followed by the gel and real-time PCR screening report, and lastly a report covering the multiplexed PCR assay development effort. Content and organization of this material has been standardized such that each agent report contains the same general information.

1. BLUE TONGUE VIRUS (BOVINE PANEL)

OBJECTIVE: We were tasked by the Department of Homeland Security to develop specific and reliable Real-time RT- PCR signatures for Bluetongue virus (BTV), among other major agriculturally-impacting viruses that are clinically indistinguishable from foot and mouth disease virus [FMDV]. The purpose of these assays (collectively with other virus-specific signatures developed in parallel) is to develop a species specific (i.e. bovine, porcine) multiplexed detection assay for Foot-and-Mouth disease rule-out for use in veterinary diagnostic laboratories. This work uses some previously designed BTV signatures that will be re-evaluated in this work as well as some newly designed signatures. The assays were designed to meet two major criteria. First, they would be able to discriminate among multiple viral agents whose disease symptoms mimic those of Foot and Mouth Disease Virus [FMDV]. Secondly, these signatures would be capable of detecting all twenty-four serotypes of BTV, referred to herein as "PAN". This document describes the development of Real-time RT-PCR and multiplexed [MUX] PCR signatures to potentially detect all serotypes of Bluetongue Virus.

1.1. BACKGROUND AND ETIOLOGY OF BTV

Bluetongue is an infectious, noncontagious arthropod borne viral disease primarily of domestic and wild ruminants. Infection with bluetongue virus is common worldwide but is usually subclinical or mild in most infected ruminants. Bluetongue is almost exclusively a disease of sheep, particularly the fine-wool and mutton breeds, although white-tailed deer (*Odocoileus virginianus*), and pronghorn (*Antilocapra americana*) and desert bighorn sheep (*Ovis canadensis*) may develop severe clinical disease in North America.

Bluetongue virus is the type-species of the genus Orbivirus in the family Reoviridae. There are 24 serotypes worldwide, although not all serotypes exist in any one geographic area, e.g., only 5 serotypes (2, 10, 11, 13, and 17) have been reported in the USA. Distribution throughout the world parallels the spatial and temporal distribution of vector species of *Culicoides* biting midges, which are the only significant natural transmitters of the virus. Of more than 1,400 *Culicoides* species worldwide, fewer than 20 are actual or possible vectors of bluetongue virus. Continued cycling of the virus among competent *Culicoides* vectors and susceptible ruminants is critical to viral ecology. In the USA, the principal biologic vector is *C variipennis sonorensis* , which limits distribution of the virus to southern and western regions. In Australia the principal vector is *C brevitarsis* , while in Africa, Europe, and the Middle East it is *C imicola*. In each geographic region, secondary vector species may attain local importance. Vectors become infected with bluetongue virus by imbibing blood from infected vertebrates; transovarial transmission has not been reported. High affinity of the virus to blood cells, especially the sequestering of viral particles in invaginations of RBC membranes, contributes to prolonged viremia in the presence of neutralizing antibody. The extended viremia in cattle (up to 9 wk), and the host preference of most vector species of *Culicoides* for cattle, provides a mechanism for year-round transmission in domestic ruminants. Mechanical transmission by other bloodsucking insects is of minor significance. Bluetongue virus is not contagious, and concentrations in secretions and excretions are minimal, making oral or aerosol transmission unlikely. However,

semen from viremic bulls can serve as a source of infection for cows through natural service or artificial insemination. Embryo transfer is regarded as safe, provided that donors are not viremic and an appropriate washing procedure for embryos is used. Accidental infection has been reported in dogs in the USA following administration of a modified live virus vaccine that was contaminated with the virus. Serologic evidence of infection with bluetongue virus has been found in large carnivores in Africa, perhaps as a result of ingesting virus-infected viscera[1].

1.2. BTV SIGNATURE COMPREHENSIVE ASSAY SUMMARY

Bioinformatics Summary

2005 BTV Assay development

Virus name: Bluetongue Virus
Signature generation reference: Single genome for domestic strains (BTV 2,10,11,13,17) sequenced at LLNL
Level of discrimination: Species; domestic strains (pan US), hits all 5 domestic serotypes
Number of computational signatures developed: 8
Number of Signatures forwarded to gel PCR screening: 8
Number of Signatures forwarded to real-time PCR screening: 5

2006 BTV Assay development

Organism name: Bluetongue Virus
Project name: PAN Bluetongue Virus
Level of discrimination: Species
Number of Initial Signatures: 27
Number of Signatures forwarded to PCR gel screening: 27
Number of Signatures forwarded to Real-time RT-PCR screening: 14

Real-time PCR Screening Summary

2005 BTV Assay development

1. Final signatures down-selected in real-time RT-PCR screening (5).

LLNL Signature Designation	Sequence
1759930.F	TCAAGACGAATGAATGAGGAGAA
1759930.R	AACCAGATTGCTTGGGTTCG
1759930.P	ACCCACGCCCTGCCATATCCAGTAAT
1759931.F	AGGGATTTGCGATATGAAGGTT

[1] Source: The Merck Veterinary Manual
http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/54700.htm&word=blue%2ctongue%2cvirus

1759931.R	CGTTGTATCAAGGTTCCAATAGCC
1759931.P	ATCAAAGCCAAATCGCTTCCATCCGTT
1759932.F	GCACCCTATATGTTTCCAGACCA
1759932.R	CAGCTAACTCTTCAGCCACACG
1759932.P	ACAGAAGATGATGATTGGCCCACGAGTTAG
1759933.F	AGAATTCAGGATGGGCAGGA
1759933.R	GCACAATTCCCATCCCCTTA
1759933.P	CCATCACACCATTATACTGTACCCGCGTAGC
1759935.F	TGATCAAGCGTTCATCCGAG
1759935.R	AACTCCCGCATCAGCATCTC
1759935.P	CTCCTCCTCCAACCTTTCCATCTCCTCTGT

2. Summary of wet-bench screening in signature down-selection.

	Soils	Eukaryotes	Prokaryotes	Aerosols[1]	Near Neighbors	Target
Gel Screening	5	5	5	0	2	5
Real-time PCR Screening	15	16	13	2	0	5

[1]There are 752 pooled samples in each Aerosol Block.

Screening summary: Some cross-reactions were observed in gel screening with near neighbors, however, no cross reactions seen in real-time PCR screening.

2006 BTV Assay development

TABLE 3. Final signatures down-selected in real-time RT-PCR screening (5).

#	LLNL Signature	Sequence	#	LLNL Signature	Sequence
1	1810199_F	CACATGTCGCTTAATTTGTCTTAACC	4	1810205_F	TCAATTTTGGTAGAATTTGTTCATTCA
	1810199_R	GCGGAGAAGGCTGCATT		1810205_R	GCGGAGAAGGCTGCATTC
	1810199_P	ACGAAACGCTTCCGCGTACGATG		1810205_P	ACGAAACGCTTCCGCGTACGATG
2	1810200_F	TTAAGCCTCCTAGGTCACTTTTCAA	5	1810207_F	CAAACACAAAAGGCGGAGAAG
	1810200_R	AAAGCTGCATTCGCATCGT		1810207_R	GGCGTTTAATCTGTCTTAGTCTTACGT
	1810200_P	CACATCATCACGAAACGCTTCTGCG		1810207_P	TCGCATCGTACGCAGAAGCGTTTC
3	1810201_F	TAATGATGCGGTGAGGATGAGT			
	1810201_R	CGCCACTCTACCTACTGATCTTAGG			
	1810201_P	AGTCCCGCTAGATGGTTTCGAATTACCAT			

TABLE 4. Summary of wet-bench screening in assay down-selection.

	Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors	Target
Gel Screening	5	5	5	none	3	5
Real-time RT-	45	54	16	3 Aerosol	3	5

PCR Screening				Blocks		

Note: There are 752 pooled samples in each Aerosol Block.

TABLE 5. Relative limit of detections for each of the five successful Pan BTV signatures. The five signatures were screened against each of the 24 BTV strains using 2 pg, 0.2 pg, 20 fg and 2 fg total RNA, for relative limit of detection (qualitative comparison). The assays were performed in triplicate and values below are the average Ct value, where the limit of detection is defined as the point at which no Ct values are detected. The "score" indicates how many strains of BTV that particular signature was able to detect.

	Limit of Detection Summary (units in pg)				
	1810205 (B1)	**1810207 (B3)**	**1810199 (A7)**	**1810200 (A8)**	**1810201 (A9)**
BTV1	0.2	<0.002	0.02	<0.002	0.2
BTV2	0.02	<0.002	0.02	0.02	0.2
BTV3	0.02	<0.002	0.02	0.2	N
BTV4	0.2	<0.002	0.2	0.2	N
BTV5	0.02	<0.002	0.02	2	N
BTV6	0.02	0.02	0.02	2	N
BTV7	<0.002	<0.002	<0.002	<0.002	<0.002
BTV8	<0.002	<0.002	<0.002	<0.002	0.02
BTV9	<0.002	<0.002	0.02	0.2	N
BTV10	<0.002	<0.002	2	<0.002	N
BTV11	0.02	0.02	<0.002	<0.002	0.02
BTV12	0.2	0.2	<0.002	2	N
BTV13	<0.002	0.02	<0.002	<0.002	0.02
BTV14	<0.002	0.02	0.2	0.02	N
BTV15	<0.002	0.02	0.2	N	0.02
BTV16	<0.002	0.02	N	N	N
BTV17	<0.002	0.02	<0.002	<0.002	0.2
BTV18	0.02	0.02	0.02	2	N
BTV19	<0.002	0.2	0.2	0.02	0.02
BTV20	2	0.2	0.2	0.2	0.02
BTV21	N	N	2	2	2
BTV22	<0.002	0.02	<0.002	<0.002	<0.002
BTV23	<0.002	2	0.02	2	0.2
BTV24	2	0.02	0.02	N	N
Score	23/24	23/24	23/24	21/24	13/24

Multiplexed PCR Screening Summary

2005 BTV Assay development

TABLE 6. Backgrounds screening in multiplexed format for BTV at LLNL.

	Soils	Prokaryotes	Eukaryotes	Aerosols[1]	Near Neighbors[2]	Targets
Mux PCR Screening	54	135	16	0	43* (0)	5

[1]-There are 752 pooled samples in each Aerosol Block. [2]Near-neighbors indicated (43) apply to the near-neighbor panel that is not uniquely specific for BTV, but for the other panel constituents that were screened concurrently.

TABLE 7. Signature summary for BTV multiplexed signatures in the bovine panel.

LLNL Signature Designation	MUX Group ID	Gene ID	Limit of detection[1]	Targets Screened	Near Neighbors Screened
BTV_1759932	BTV_1759932	BTVs1gp1 (VP1)/2943156	$5x10^{-3}$ - $5x10^{1}$ $TCID_{50}$/rxn	4	4

[1] The relative "Limit of detection" is described as a range of lowest and highest sensitivity determined by multiple target strains screened and is described further in the Multiplex results summary report section.

2006 BTV Assay development

TABLE 8. Backgrounds screening in multiplexed format for BTV at LLNL.

	Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors[2]	Targets
Mux PCR Screening	54	135	16	0	43* (0)	5

[1]-There are 752 pooled samples in each Aerosol Block. [2]Near-neighbors indicated (43) apply to the near-neighbor panel that is not uniquely specific for PRRS, but for the other panel constituents that were screened concurrently.

TABLE 9. Signature summary for BTV multiplexed assays in the bovine panel.

LLNL Signature Designation	MUX Group ID	Gene ID	Limit of detection[1]	Targets Screened	Near Neighbors Screened
BTV_1759932	BTV_1759932	BTVs1gp1 (VP1)/2943156	$5x10^{-3}$ - $5x10^{1}$ $TCID_{50}$/rxn	4	4
BTV10_1810199	BTV10_1810199	S10 Nonstructural Protein	$1x10^{0}$ - $1x10^{3}$ $TCID_{50}$/rxn	4	4
BTV10_1810205	BTV10_1810205	S10 Nonstructural Protein	$1x10^{0}$ - $1x10^{3}$ $TCID_{50}$/rxn	4	4
BTV10_1810207	BTV10_1810207	S10 Nonstructural Protein	$1x10^{0}$ - $1x10^{3}$ $TCID_{50}$/rxn	4	4

[1] The relative "Limit of detection" is described as a range of lowest and highest sensitivity determined by multiple target strains screened and is described further in the Multiplex results summary report section.

1.3. BTV SIGNATURE BIOINFORMATICS AND GENERATION INFORMATION (**2005)**

Bioinformatics process 2005:

The LLNL Bioinformatics team have developed a computational process that uses available DNA sequence information for a given target organism to generate candidate DNA signature sequences that are conserved and unique. Signatures are primarily used to underlie reliable and specific pathogen detection assays. Evidence of the success of the process is that several of the Real-time RT-PCR signatures that are used in the national BioWatch monitoring system were

generated by this process, and to date, has resulted in no false positives from a total of over 5 million assays performed.

The general approach is the following: All available complete genomes of different strains of the target species are compared using a multiple genome alignment programs. A *consensus gestalt* is formed from the alignments that contain the sequence conserved amongst all target inputs. (This step is bypassed if only one target sequence is available).

To determine that organism-conserved sequence which does not occur in any other sequenced microbial organism, the consensus gestalt is compared against our immense, continuously updated database of microbial organism. A customized algorithm accomplishes this electronic subtraction, and the result is a *uniqueness gestalt* that is mined for potential signature candidates. A final computational screening is done to verify that cross-reactions are not detected.

Bluetongue virus is an FMDV look alike that occurs in cattle. It encodes 10 genes on separate segments and to date there are 26 serotypes. The goal of this project was to develop assays to detect the domestic serotypes, 2, 10, 11, 13, and 17. We ran the computational process on all available sequence data for the domestic serotypes, and were able to generate conserved unique signatures on only three of the segments, 1, 8 and 9. These are designed to detect all domestic serotypes, however, will not necessarily discriminate against the exotic serotypes.

Target Organism Information.
Organism name: Bluetongue Virus.
Type: dsRNA virus.
Genome size: 10 segments (19186 bp total).

TABLE 10. List of main parameters and settings used in generating candidate signatures using Primer 3 signature generation tool.

Primer3 Parameters	
Parameters	**Standard Settings**
PRIMER_OPT_SIZE	**20**
PRIMER_MIN_SIZE	**18**
PRIMER_MAX_SIZE	**27**
PRIMER_PRODUCT_OPT_SIZE	**100**
PRIMER_PRODUCT_SIZE_RANGE	**71-600**
PRIMER_OPT_TM	**62**
PRIMER_MIN_TM	**61**
PRIMER_MAX_TM	**63**
PRIMER_MIN_GC	**20**
PRIMER_MAX_GC	**80**
PRIMER_PICK_INTERNAL_OLIGO	**1**
PRIMER_INTERNAL_OLIGO_OPT_SIZE	**31**
PRIMER_INTERNAL_OLIGO_MIN_SIZE	**18**
PRIMER_INTERNAL_OLIGO_MAX_SIZE	**36**

PRIMER_INTERNAL_OLIGO_OPT_TM	**72**
PRIMER_INTERNAL_OLIGO_MIN_TM	**71**
PRIMER_INTERNAL_OLIGO_MAX_TM	**73**
Number of Primers/Probe Set generated:	**8**

Primer/Probe Set Generation Information

Method: Alignment and All Microbe Database subtraction.

Note: The eight Bluetongue signatures were generated from three separate kpath runs and they are listed below with their corresponding genome sequences information used for the alignment.

TABLE 11. K-path run id: 85629. Total Number of Genome Sequences for alignment: 5.

	Description	GI Number	Sequence Length (bp)	K-path ID
1	Bluetongue virus (serotype 17 / isolate USA) segment 1 from LLNL on Feb 14 2005 1:58PM	n/a	3862	85629
2	Bluetongue virus (serotype 10 / American isolate) segment 1 from LLNL on Feb 14 2005 1:51PM	n/a	3703	85629
3	Bluetongue virus (serotype 2 / isolate USA) segment 1 from LLNL on Feb 14 2005 2:01PM	n/a	3857	85629
4	Bluetongue virus (serotype 11 / isolate USA) segment 1 from LLNL on Feb 14 2005 1:53PM	n/a	3755	85629
5	Bluetongue virus (serotype 13 / isolate USA) segment 1 from LLNL on Feb 14 2005 1:56PM	n/a	3830	85629

TABLE 12. K-path run id: 85636. Total Number of Genome Sequences for alignment: 5.

	Description	GI Number	Sequence Length (bp)	K-path ID
1	Bluetongue virus (serotype 17 / isolate USA) segment 8 from LLNL on Feb 14 2005 2:00PM	n/a	1074	85636
2	Bluetongue virus (serotype 10 / American isolate) segment 8 from LLNL on Feb 14 2005 1:51PM	n/a	1085	85636
3	Bluetongue virus (serotype 11 / isolate USA) segment 8 from LLNL on Feb 14 2005 1:54PM	n/a	977	85636
4	Bluetongue virus (serotype 13 / isolate USA) segment 8 from LLNL on Feb 14 2005 1:57PM	n/a	1090	85636

TABLE 13. K-path run id: 85637. Total Number of Genome Sequences for alignment: 4.

	Genome Description	GI Number	Sequence Length (bp)	K-path ID
1	Bluetongue virus (serotype 17 / isolate USA) segment 9 from LLNL on Feb 14 2005 2:00PM	n/a	997	85637
2	Bluetongue virus (serotype 10 / American isolate) segment 9 from LLNL on Feb 14 2005 1:52PM	n/a	897	85637
3	Bluetongue virus (serotype 2 / isolate USA) segment 9 from LLNL on Feb 14 2005 2:03PM	n/a	1029	85637
4	Bluetongue virus (serotype 11 / isolate USA) segment 9 from LLNL on Feb 14 2005 1:54PM	n/a	808	85637

Signature Information

Source: LLNL
Project name: Bluetongue Virus-pan US.
Level of discrimination: Species.
Number of initial signatures: 8
Number of signatures forwarded to bench-screening: 8
Number of final signatures forwarded to multiplex: 5

Signature list.
Note: For a listing of computationally predicted product sequences, please see attached document "BTV Taqsim Run Data".

Taqsim description
We used a computational TaqMan simulator program, "Taqsim", to identify all potential targets for each signature from all sequences available in genbank. Taqsim is a BLAST-based comparison of each signature as a triplet against all sequences in genbank to identify the targets that are predicted to produce a TaqMan reaction at 57 degrees primer annealing and 67 degrees for probe annealing (these temperatures are according to Primer 3 oligo Tm calculations). The first column of the excel spreadsheets list the signatures by the name, and the following columns report the predicted genbank targets, coordinates of signature region, amplicon size, and accession #'s for each target.

All sequences are listed in the 5'→ 3' direction.

TABLE 14a-e. Signature bioinformatics (a) 1759930 (b) 1759931 (c) 1759935 (d) 1759932 (e) 1759933 (a)

Target Organism	Blue tongue virus
Forward Primer	1759930
FWD Primer Length (bp)	23
FWD Primer TM (^{o}C)	61
FWD Primer GC Content (%)	39
Forward Sequence	TCAAGACGAATGAATGAGGAGAA
Reverse Primer	1759930
Rev Primer Length (bp)	20
Rev Primer TM (^{o}C)	62
Rev Primer GC Content (%)	50
Reverse Sequence	AACCAGATTGCTTGGGTTCG
Probe Name	1759930
Probe Length (bp)	27
Probe TM (^{o}C)	61
Probe GC Content (%)	33
Probe Sequence	ACCCACGCCCTGCCATATCCAGTAAT
Probe strand	Minus

Predicted Product Size	264

(b)

Target Organism	Blue tongue virus
Forward Primer	1759931
FWD Primer Length (bp)	22
FWD Primer TM (°C)	61
FWD Primer GC Content (%)	40
Forward Sequence	AGGGATTTGCGATATGAAGGTT
Reverse Primer	1759931
Rev Primer Length (bp)	24
Rev Primer TM (°C)	62
Rev Primer GC Content (%)	45
Reverse Sequence	CGTTGTATCAAGGTTCCAATAGCC
Probe Name	1759931
Probe Length (bp)	28
Probe TM (°C)	61
Probe GC Content (%)	32
Probe Sequence	ATCAAAGCCAAATCGCTTCCATCCGTT
Probe strand	minus
Predicted Product Size	362

(c)

Target Organism	Bluetongue
Forward Primer	1759935
FWD Primer Length (bp)	20
FWD Primer TM (°C)	62
FWD Primer GC Content (%)	50
Forward Sequence	TGATCAAGCGTTCATCCGAG
Reverse Primer	1759935
Rev Primer Length (bp)	20
Rev Primer TM (°C)	62
Rev Primer GC Content (%)	55
Reverse Sequence	AACTCCCGCATCAGCATCTC
Probe Name	1759935
Probe Length (bp)	31
Probe TM (°C)	62
Probe GC Content (%)	32
Probe Sequence	CTCCTCCTCCAACCTTTCCATCTCCTCTGT
Probe strand	minus
Predicted Product Size	332

(d)

Target Organism	Bluetongue
Forward Primer	1759932
FWD Primer Length (bp)	23
FWD Primer TM (°C)	62
FWD Primer GC Content (%)	47
Forward Sequence	GCACCCTATATGTTTCCAGACCA
Reverse Primer	1759932
Rev Primer Length (bp)	22
Rev Primer TM (°C)	62
Rev Primer GC Content (%)	54
Reverse Sequence	CAGCTAACTCTTCAGCCACACG
Probe Name	1759932
Probe Length (bp)	31
Probe TM (°C)	62
Probe GC Content (%)	35
Probe Sequence	ACAGAAGATGATGATTGGCCCACGAGTTAG
Probe strand	plus
Predicted Product Size	271

(e)

Target Organism	Bluetongue
Forward Primer	1759933
FWD Primer Length (bp)	20
FWD Primer TM (°C)	61
FWD Primer GC Content (%)	50
Forward Sequence	AGAATTCAGGATGGGCAGGA
Reverse Primer	1759933
Rev Primer Length (bp)	20
Rev Primer TM (°C)	61
Rev Primer GC Content (%)	50
Reverse Sequence	GCACAATTCCCATCCCCTTA
Probe Name	1759933
Probe Length (bp)	32
Probe TM (°C)	61
Probe GC Content (%)	31
Probe Sequence	CCATCACACCATTATACTGTACCCGCGTAGC
Probe strand	minus
Predicted Product Size	187

TABLE 15a-b.(a) Reference Genomes used for Gene Information. (b) Target region gene information from annotated genomes available.

(a)

	Genome Description	GI Number	Sequence Length (bp)
1	Segment 1 reference genome	50253391/NC006023.1	3944
2	Segment 8 reference genome	50253377/NC_006007.1	1125
3	Segment 9 reference genome	50253379/NC_006008.1	1049

(b)

					Gene Location		Target Region Location	
kpath Run Id	Primer	Segment	Gene	Description	Start	End	Start	End
85629	1759930	1	BTVs1gp1 (VP1)/2943156	Hypothetical protein	12	3920	966	1229
85629	1759931	1	BTVs1gp1 (VP1)/2943156	Hypothetical protein	12	3920	1881	2242
85629	1759932	1	BTVs1gp1 (VP1)/2943156	Hypothetical protein	12	3920	3276	3546
85636	1759933	8	BTVs8gp1 (NS2)/2943149	Viral inclusion body matrix protein	20	1084	218	404
85637	1759935	9	BTVs9gp1 (VP6)/2943150	binding protein	16	1005	50	381

1.4. BTV SIGNATURE BIOINFORMATICS AND GENERATION INFORMATION (**2006)**

Bioinformatics process:

The LLNL Bioinformatics team have developed a computational process that uses available DNA sequence information for a given target organism to generate candidate DNA signature sequences that are conserved and unique. Signatures are primarily used to underlie reliable and specific pathogen detection assays. Evidence of the success of the process is that several of the Real-time RT- PCR signatures that are used in the national BioWatch monitoring system were generated by this process, and to date, has resulted in no false positives from a total of over 5 million assays performed.

The general approach is the following: All available complete genomes of different strains of the target species are compared using a multiple genome alignment programs. A *consensus gestalt* is formed from the alignments that contain the sequence conserved amongst all target inputs. (This step is bypassed if only one target sequence is available).

To determine that organism-conserved sequence which does not occur in any other sequenced microbial organism, the consensus gestalt is compared against our immense, continuously updated database of microbial organism. A customized algorithm accomplishes this electronic

subtraction, and the result is a *uniqueness gestalt* that is mined for potential signature candidates. A final computational screening is done to verify that cross-reactions are not detected.

Target Organism Information:
Organism name: *Bluetongue Virus*
Type: dsRNA virus
Genome size: 19186 bp.

Primer/Probe Set Generation Information

Minimal clustering method was used to generate the PAN Bluetongue signatures. This method finds a set with the minimum number of signatures that would detect all strains.

TABLE 16. List of main parameters and settings used in generating candidate signatures using Primer 3 signature generation tool.

Primer3 Main Parameters	
Parameters	**Standard Settings**
PRIMER_OPT_SIZE	20
PRIMER_MIN_SIZE	18
PRIMER_MAX_SIZE	27
PRIMER_PRODUCT_OPT_SIZE	100
PRIMER_PRODUCT_SIZE_RANGE	71-600
PRIMER_OPT_TM	62
PRIMER_MIN_TM	61
PRIMER_MAX_TM	63
PRIMER_MIN_GC	20
PRIMER_MAX_GC	80
PRIMER_PICK_INTERNAL_OLIGO	1
PRIMER_INTERNAL_OLIGO_OPT_SIZE	31
PRIMER_INTERNAL_OLIGO_MIN_SIZE	18
PRIMER_INTERNAL_OLIGO_MAX_SIZE	36
PRIMER_INTERNAL_OLIGO_OPT_TM	72
PRIMER_INTERNAL_OLIGO_MIN_TM	71
PRIMER_INTERNAL_OLIGO_MAX_TM	73
Number of Primers/Probe Set generated:	**27**

Signature Information

Source: LLNL
Project name: PAN *Bluetongue Virus*
Level of discrimination: Species.

Number of Initial Signatures: 27
Number of Signatures forwarded to gel bench-screening: 27
Number of Signatures forwarded to real-time RT-PCR TaqMan screening: 14
Number of Final real-time RT-PCR Signatures: 5

Taqsim description

We used a computational Real-time RT- PCR simulator program, "Taqsim", to identify all potential targets for each signature from all sequences available in Genbank. Taqsim is a BLAST-based comparison of each signature as a triplet against all sequences in Genbank to identify the targets that are predicted to produce a Real-time RT- PCR reaction at 57 degrees primer annealing and 67 degrees for probe annealing (these temperatures are according to Primer 3 oligo Tm calculations). The first column of the excel spreadsheets list the signatures by the name, and the following columns report the predicted Genbank targets, coordinates of signature region, amplicon size, and accession #'s for each target.

Note: For a listing of computationally predicted product sequences and potential cross targets for each signature, please see Appendix II, Taqsim Run Data.

Signature bioinformatics

TABLE 17. Detailed signature bioinformatics for final assays.

Signature	Size (bp)		Sequence	Length (bp)	T_m (°C)	GC (%)
1810199	78	F	CACATGTCGCTTAATTTGTCTTAACC	26	54	39
		R	GCGGAGAAGGCTGCATT	17	51	59
		P(+)[1]	ACGAAACGCTTCCGCGTACGATG	23	60	57
1810200	118	F	TTAAGCCTCCTAGGTCACTTTTCAA	25	55	40
		R	AAAGCTGCATTCGCATCGT	19	53	47
		P(+)[1]	CACATCATCACGAAACGCTTCTGCG	25	60	52
1810201	96	F	TAATGATGCGGTGAGGATGAGT	22	54	46
		R	CGCCACTCTACCTACTGATCTTAGG	25	57	52
		P(+)[1]	AGTCCCGCTAGATGGTTTCGAATTACCATTA	31	61	42
1810205	103	F	TCAATTTTGGTAGAATTTGTTCATTCA	27	52	26
		R	GCGGAGAAGGCTGCATTC	18	53	61
		P(+)[1]	ACGAAACGCTTCCGCGTACGATG	23	60	57
1810207	85	F	CAAACACAAAAGGCGGAGAAG	21	53	48
		R	GGCGTTTAATCTGTCTTAGTCTTACGT	27	56	41
		P(+)[1]	TCGCATCGTACGCAGAAGCGTTTC	24	61	54

[1] Indicates that the probe is located of the forward strand, or positive strand of the RNA.

Target Region Gene Information

TABLE 18a-b. (a) Reference genomes used for gene information. (b) Gene information for each signature (a)

Signature	Genome Description	GI Number	Sequence Length (bp)
1810199	Bluetongue virus serotype 10 non-structural protein NS3 and non-structural protein NS3A genes, complete cds.	3643705	822
1810200	Bluetongue virus serotype 10 non-structural protein NS3 and non-structural protein NS3A	3643705	822

	genes, complete cds.		
1810201	Bluetongue virus serotype 10 non-structural protein NS3 and non-structural protein NS3A genes, complete cds.	3643705	822
1810205	Bluetongue virus 2 nonstructural protein NS3/NS3A (S10) gene, complete cds	4959686	785
1810207	Bluetongue virus 2 NS3 (S10) gene, complete cds	21637336	690

(b)

			Gene Location		**Target Region Location**	
Kpath Signature ID	**Gene/ID**	**Description**	**Start**	**End**	**Start**	**End**
1810199	S10	non-structural protein NS3	20	709	236	313
	S10	non-structural protein NS3A	59	709	236	313
1810200	S10	non-structural protein NS3	20	709	242	359
	S10	non-structural protein NS3A	59	709	242	359
1810201	S10	non-structural protein NS3	20	709	634	729
	S10	non-structural protein NS3A	59	709	634	729
1810205	S10	non-structural protein NS3/NS3A	1	690	217	319
1810207	S10	non-structural protein NS3	1	690	205	289

1.5. BTV GEL AND REAL-TIME PCR SCREENING REPORT **2005** DEVELOPMENT

2005 Wet-lab screening process. To ensure extremely high selectivity and sensitivity, we have established a screening protocol that rigorously screens candidate DNA signatures to select the optimal sets for eventual field use. Candidate signature primer pairs that survive computational screening proceed to wet chemistry screening utilizing standard PCR with agarose gel electrophoresis. This step ensures that the primers will react across strains representative of the diversity of the pathogen (avoid false negative), but will not react with the nucleic acids of other organisms that could be present in a sample (avoid false positives). The collection of purified nucleic acid templates used in this screening process is listed below:

1) Strain panels representative of the diversity of the target organism in nature. We have a panel of 5 Bluetongue virus strains
2) Near neighbors-organisms that are closely related at the genetic level and therefore have the greatest likelihood of cross-reaction. We screened the candidate signatures against two near-neighbor isolates of EHD.
3) Eukaryotic DNA that may carry over from sample collection procedures.

4) Prokaryotic DNA that would be expected to be present in environmental samples and may also be present in Bovine and Porcine oral cavities.
5) Fifteen soil samples from around the country representing diverse climates and contain a complex mixture of organisms from diverse environmental collections
6) Aerosol samples: 752 samples collected from aerosol collectors and pooled for background testing purposes.

Based on data from primer pair screening, a set of **8** specific and reliable signatures were then further tested for suitability for real-time TaqMan fluorogenic PCR detection protocols. The selected signatures showed a robust signal in all target reactions.

TABLE 19. List of near-neighbors screened. Virus was un-titered and extracted as total nucleic acid.

Organism	Serotype/Isolate	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	Use stock titer	Titer Method
Epizootic Hemorrhagic Disease Virus 1	Type 1, Georgia	CAHFS	Unknown	Unknown	12/2006	At CAHFS	Unknown	Unknown
Epizootic Hemorrhagic Disease virus 2	Type 2, Alberta 041 ODV 0301	NVSL	Unknown	Vero M/12, BHK/8, BHK 137	12/2006	At CAHFS	Unknown	Unknown

Note: CAHFS: California Animal Health and Food Safety Laboratory; Located on the campus of the University of California, Davis

TABLE 20. List of targets screened. Virus was un-titered and extracted as total nucleic acid.

Organism	Serotype/Isolate	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	Use stock titer	Titer Method
Bluetongue Virus	2	CAHFS	Un-titered	Unknown	12/2006	Trizol	Un-titered	Unknown
Bluetongue Virus	10	CAHFS	Un-titered	Unknown	12/2006	Trizol	Un-titered	Unknown
Bluetongue Virus	11	CAHFS	Un-titered	Unknown	12/2006	Trizol	Un-titered	Unknown
Bluetongue Virus	13	CAHFS	Un-titered	Unknown	12/2006	Trizol	Un-titered	Unknown
Bluetongue Virus	17	CAHFS	Un-titered	Unknown	12/2006	Trizol	Un-titered	Unknown

2005 Bluetongue Virus (BTV) - Gel Screening Report

TABLE 21. Nucleic acid extracts used to challenge the initial set of candidate signatures. These select backgrounds are used to determine which signatures will be down-selected against in the screening process. No cross-reactions were observed.

Nucleic Acid Extract Type	Description/ID
Soil Extract	D000402

Soil Extract	D000109
Soil Extract	D000510
Soil Extract	D000101
Soil Extract	D000107
Prokaryotic DNA Extract	*B. cereus*
Prokaryotic DNA Extract	*B. burgdorferi*
Prokaryotic DNA Extract	*C. burnetti*
Prokaryotic DNA Extract	*E. coli*
Prokaryotic DNA Extract	*H. influenzae*
Eukaryotic DNA Extract	Porcine
Eukaryotic DNA Extract	Flea
Eukaryotic DNA Extract	Equine
Eukaryotic DNA Extract	Bovine
Eukaryotic DNA Extract	Sheep

TABLE 22. List of signatures screened in gel format against available targets and near neighbors.

Signature ID	Forward Primer	Reverse Primer
1758843	GCACCCTATATGTTTCCAGACCA	ACGACATCCTTTCTTAATATCACATCA
1759929	CAACATATGGCATTGGGATGA	AGGGTAGCATGCTGCGAAA
1759930	TCAAGACGAATGAATGAGGAGAA	AACCAGATTGCTTGGGTTCG
1759931	AGGGATTTGCGATATGAAGGTT	CGTTGTATCAAGGTTCCAATAGCC
1759932	GCACCCTATATGTTTCCAGACCA	CAGCTAACTCTTCAGCCACACG
1759933	AGAATTCAGGATGGGCAGGA	GCACAATTCCCATCCCCTTA
1759934	CGCGAATTACGGCAAAAGAT	TTTTCCAACCCGCTAGTTCC
1759935	TGATCAAGCGTTCATCCGAG	AACTCCCGCATCAGCATCTC

TABLE 23. Results and product size for target and near neighbor screening of signatures when challenged against 200pg of extracted nucleic acid. Signatures that cross-reacted with the near-neighbor EHD were dropped from further screening, thus leaving a remaining signatures set of 5.

	1758843	**1759929**	**1759930**	**1759931**	**1759932**	**1759933**	**1759934**	**1759935**
Predicted Product Size	137	108	264	362	271	187	161	332
Bluetongue Virus-2	140	90	**260**	**360**	**270**	**190: multibands**	160: multibands	**330: multibands**
Bluetongue Virus-10	140	100: 100, 500	**260**	**360**	**270**	**190: 180, 500**	160: 200	**330: 100, 140**
Bluetongue Virus-11	140	100: 200, 500	**260**	**360**	**270**	**190: 500**	200	**330**
Bluetongue Virus-13	140	100	**260**	**360**	**270**	**190: 180**	160: 220	**330: multibands**
Bluetongue Virus-17	140	100: 200, 500	**260**	**360**	**270**	**190: 500**	160: 280	**330**

Epizootic Hemorrhagic Disease virus-1	150: multibands	100: multibands	0	0	200: multibands	0	160: multibands	0
Epizootic Hemorrhagic Disease virus-2	140: multibands	100: multibands	0	0	250: multibands	0	160: multibands	0

2005 Bluetongue Virus (BTV) - Real-time PCR Screening Report

TABLE 24. List of signatures screened against Backgrounds in Real-time PCR Format.

1759930	F	TCAAGACGAATGAATGAGGAGAA
	R	AACCAGATTGCTTGGGTTCG
	P	ACCCACGCCCTGCCATATCCAGTAAT
1759931	F	AGGGATTTGCGATATGAAGGTT
	R	CGTTGTATCAAGGTTCCAATAGCC
	P	ATCAAAGCCAAATCGCTTCCATCCGTT
1759932	F	GCACCCTATATGTTTCCAGACCA
	R	CAGCTAACTCTTCAGCCACACG
	P	ACAGAAGATGATGATTGGCCCACGAGTTAG
1759933	F	AGAATTCAGGATGGGCAGGA
	R	GCACAATTCCCATCCCCTTA
	P	CCATCACACCATTATACTGTACCCGCGTAGC
1759935	F	TGATCAAGCGTTCATCCGAG
	R	AACTCCCGCATCAGCATCTC
	P	CTCCTCCTCCAACCTTTCCATCTCCTCTGT

TABLE 25a-d. Real-time RT- PCR background screening, all performed in triplicate against an extensive list of soil, Prokaryotic and Eukaryotic extracted DNAs in addition to 3 aerosol blocks, each containing 752 samples. All signatures passed real-time RT- PCR background screening. None of the 5 signatures produced an amplicon when screened against the listed backgrounds. All signatures moved forward to be screened against the available BTV targets and near neighbors.

(a) Total of 15 soils screened

D000558	D000086	D000564	D000101	D00028	D000401	D000051	D000405
D000559	D000098	D000019	D000102	D000036	D000403	D000054	

(b) Total of 16 Eukaryotes screened

Monkey	Sheep	Tick	Chicken	Rat	Drosophila	Rabbit	Mosquito
Human	Dog	Mouse	Cat	Pig	Bovine	Equine	Flea

(c) Total of 13 Prokaryotes screened

E. coli	*B. cereus*	*S. typhimurium*	*B. subtilis*	*E. herbicola*
B. globigii	*S. pneumonia*	*L. monocytogenes*	*H. influenza*	
P. aeruginosa	*C. burnetti*	*B. burgdorferi*	*S. aureus*	

(d) Aerosols screened

Aerosol Block	Signature Screened	Number of Samples in Block
BB 080403 4	1759930, 1759931, 1759932, 1759933, 1759935	752
BB 081903 3	1759930, 1759931, 1759932, 1759933, 1759935	752
Total:		**1504 samples**

TABLE 26. Results of signatures crossed with LLNL targets in Real-time RT- PCR. Screenings were performed in triplicate by challenging each PCR reaction with 200pg of extracted nucleic acid. The actual Ct values for each signature against the listed target is reported.

Isolate tested[1]	1759930	1759931	1759932	1759933	1759935
Bluetongue Virus-2	22.3, 22.3, 22	24.4, 24.2, 25.2	21.8, 21.7, 21.5	30.3, 30.7, 29.6	23.4, 23.8, 25.2
Bluetongue Virus-10	21.6, 21.6, 21.8	24.5, 25.3, 24	20.3, 20.2, 20.1	20.7, 20.5, 20.8	23.7, 23.5, 23.8
Bluetongue Virus-11	21.6, 21.3, 21.7	23.8, 24.8, 24.5	20.8, 20.9, 20.9	20.9, 20.5, 20.4	23.3, 23.7, 24
Bluetongue Virus-13	21.5, 21.6, 21.5	23, 23.3, 22.7	20.3, 20.4, 20.2	20.4, 20.4, 20.2	24.2, 23.1, 23.9
Bluetongue Virus-17	21.6, 22.1, 21.7	23.6, 25.3, 23.8	20.9, 20.7, 21.7	20.2, 20.2, 20.2	23.5, 23.2, 25.4

[1] For information on isolates tested please refer to TABLE 9 above.
[2]The number of Ct values denotes the number of replicate screenings against the particular template

2005 Bluetongue Virus (BTV) - LOD Report

TABLE 27. To assess the relative limits of detection for each Real-time PCR signature developed, a dilution series of target was made across 4 logs of the detection range. The diluted targets were then tested with each signature using the standard Real-time PCR protocol in triplicate and average Ct values are reported for each dilution. It is expected that the 10-fold dilutions will result in a 3.2 CT shift per log, in some cases where replicates contained an outlier point, this data may not show an exact 3.2 CT shift, but is reported irregardless. [Note: The LODs reported here are relative LODs comparing one signature with another shown here as a dilution factor of 10-log serial dilutions from untitered virus.]. The CT value at the LOD is highlited in green

Dilution series	1759930	1759931	1759932	1759933	1759935
BTV2 1:10	26	27.2	24.8	28.5	30.3
BTV2 1:100	29.6	**32.3**	27.8	**32**	**27.6**
BTV2 1:1K	**32.2**	N/A	**32.6**	N/A	N/A
BTV2 1:10K	N/A	N/A	N/A	N/A	N/A
BTV10 1:10	25.1	26.1	23.6	24.8	28.2
BTV10 1:100	28.4	**30.5**	26.8	28.3	**32.7**
BTV10 1:1K	33.4	N/A	**30.6**	**30.7**	N/A

BTV10 1:10K	32.8	N/A	N/A	N/A	N/A
BTV11 1:10	26.4	26.5	24.3	24.4	27.1
BTV11 1:100	30.1	**31.1**	27.3	27.6	36
BTV11 1:1K	**34.7**	N/A	**31.5**	**31.1**	35.7
BTV11 1:10K	N/A	N/A	N/A	N/A	35.7
BTV13 1:10	25.7	**27.5**	23.4	24.5	27.7
BTV13 1:100	29.3	N/A	27.4	28	31.8
BTV13 1:1K	33.8	N/A	**32.8**	**33.4**	33.8
BTV13 1:10K	32.9	N/A	N/A	N/A	32.9
BTV17 1:10	27.2	28	24.7	25	27.9
BTV17 1:100	31.2	33.2	27.7	27.7	25.9
BTV17 1:1K	33.5	**35.9**	**32.8**	**31.5**	**33.9**
BTV17 1:10K	31.6	N/A	N/A	N/A	N/A

TABLE 28. Summary Table of Signature LODs on various BTV strains as indicated by raw data in TABLE 18 above.

Isolate	**Signature (LOD reported as highest dilution to give Ct Value)**				
	1759930	**1759931**	**1759932**	**1759933**	**1759935**
BTV-2	1:1K	1:100	1:1K	1:100	1:10
BTV-10	1:1K	1:100	1:1K	1:1K	1;100
BTV-11	1:1K	1:100	1:1K	1:1K	1:10
BTV-13	1:1K	1:10	1:1K	1:1K	1:100
BTV-17	1:1K	1:100	1:1K	1:1K	1:100

1.6. BTV GEL AND REAL-TIME PCR SCREENING REPORT **2006** DEVELOPMENT

2006 Wet-lab screening process. To ensure extremely high selectivity and sensitivity, we have established a screening protocol that rigorously screens candidate DNA signatures to select the optimal sets for eventual field use. Candidate signature primer pairs that survive computational screening proceed to wet chemistry screening utilizing standard PCR with agarose gel electrophoresis. This step ensures that the primers will react across strains representative of the diversity of the pathogen (avoid false negative), but will not react with the nucleic acids of other organisms that could be present in a sample (avoid false positives). The collection of purified DNA templates used in this screening process is listed below:

1) Strain panels representative of the diversity of the target organism in nature.
2) Near neighbors-organisms that are closely related at the genetic level and therefore have the greatest likelihood of cross-reaction.
3) Eukaryotic DNA that may carry over from sample collection procedures.
4) Prokaryotic DNA that would be expected to be present in environmental samples and may also be present in Bovine and Porcine oral cavities.
5) Forty five soil samples from around the country representing diverse climates and contain a complex mixture of organisms from diverse environmental collections

6) Aerosol samples: 752 samples collected from aerosol collectors and pooled for background testing purposes.

2006 Pan Bluetongue Virus (BTV) TaqMan Screening Report

Bluetongue is a double stranded RNA virus in the genus *Orbivirus* and the family *Reoviridae.* The Bluetongue disease is known to mostly affect sheep. The LLNL Bioinformatics team generated 27 unique signatures for bench screening. The signatures are designed to identify segments 5 or 10 of all 24 know serotypes of Bluetongue. Serotypes 2, 10, 11, 13 and 17 are domestic strains, the remaining serotypes are foreign and were screened by Bill Wilson's group at the USDA in Laramie, WY. It is predicted that no single signature will be able to detect all serotypes. The goal of this testing is to develop a unique set of signatures that will be treated as one test to identify any of the 24 serotypes of Bluetongue.

TABLE 29. List of targets screened in multiplexed PCR format. All BTV strains from Bill Wilson (ARS arbovirus research lab Laramie WY) were purified double stranded BTV RNA from virus with unknown titers.

Virus	Serotype/Isolate or Strain	Unique ID	Source[1]	Previous Passage History	Previous Passages History	Extraction date/ID	Extraction Method	Use stock titer	Titer Method
BTV	BTV-10	lot #001 ODV 0001 Dec 5, 2000	NVSL, Lot 9901 from ABADL, Denver, CO	(NVSL)BHK1(LLNL)BHK1	ECE/1, BHK-21/5	12/14/06	Trizol	$6.32x10^5$ $TCID_{50}$/0.1 mL	Reed-Meunch
BTV	BTV-11	lot#002 ODV0101 Nov 30, 2001	NVSL, Lot 9701 from ABADL, Denver, CO	(NVSL)BHK?(LLNL)BHK1	unknown	12/14/06	Trizol	$6.32x10^5$ $TCID_{50}$/0.1 mL	Reed-Meunch
BTV	BTV-13	no lot or strain given	NVSL, Lot 9701 from ABADL, Denver, CO	unknown	unknown	12/14/06	Trizol	$3.56x10^3$ $TCID_{50}$/0.1 mL	Reed-Meunch
BTV	BTV-17	lot#004 ODV 0201 Nov 28, 2002	NVSL Lot 9802 from ABADL, Denver, CO	(NVSL)BHK?(LLNL)BHK1	BHK-21/4, ECE/1, BHK-21/10, Vero-M/1	12/14/06	Trizol	$1.12x10^5$ $TCID_{50}$/0.1 mL	R Reed-Meunch
BTV	BTV-2	no lot given	NVSL, Lot 9701 from ABADL, Denver, CO	(NVSL)BHK2(LLNL)BHK2	BHK/2, ECE/1, BHK/2,Vero M/1, BHK/3	12/14/06	Trizol	$6.32x10^5$ $TCID_{50}$/0.1 mL	Reed-Meunch
BTV	BT-1 (Prototype)	600558	Bill Wilson:USDA, ARS	Unknown, BHK-2	+BHK-1	Unknown	Unknown	N/A	N/A
BTV	BT 2	600557	Bill Wilson:USDA	Unknown, BHK-2	+BHK-1	Unknown	Unknown	N/A	N/A
BTV	BT3	600565	Bill	Unknown,	+BHK-1	Unknown	Unknown	N/A	N/A

	(Prototype)		Wilson:USDA	BHK-2					
BTV	BT4 (Prototype)	600566	Bill Wilson:USDA	Unknown, BHK-2	+BHK-1	Unknown	Unknown	N/A	N/A
BTV	BT5 (Prototype)	600567	Bill Wilson:USDA	LK-6; FET LK1; Vero-1	+BHK-3 (12/7/83); (12/8/86); (12/14/86)	Unknown	Unknown	N/A	N/A
BTV	BT6 (Prototype)	600568	Bill Wilson:USDA	ECE 2; LK-8; Vero-1	+BHK-3 (12/7/83); (4/28/86); (12/16/86)	Unknown	Unknown	N/A	N/A
BTV	BTY7 (Prototype)	600561	Bill Wilson:USDA	Unknown; BHK-1	+BHK-1	Unknown	Unknown	N/A	N/A
BTV	BT8 (Prototype)	600570	Bill Wilson:USDA	Sheep 2, ECE-1,BHK-4; LK-8; Vero-1	+BHK-2 (12/7/83); (4/2/87)	Unknown	Unknown	N/A	N/A
BTV	BT9 (Prototype)	600571	Bill Wilson:USDA	Unknown, BHK-2	+BHK-1	Unknown	Unknown	N/A	N/A
BTV	BT 10 (SV119)	BT-8	Bill Wilson:USDA	Unknown, BHK-2	+BHK-1	Unknown	Unknown	N/A	N/A
BTV	BT 11 (SV122)	TX Station (BT318)	Bill Wilson:USDA	Unknown, BHK-2	+BHK-1	Unknown	Unknown	N/A	N/A
BTV	BT12 (Prototype)	600604	Bill Wilson:USDA	Sheep-3; ECE-3; LK-8; Vero-1	+BHK-2 (12/7/83); (12/17/86)	Unknown	Unknown	N/A	N/A
BTV	BT 13 (SV121)	67-41B (BT 290)	Bill Wilson:USDA	Unknown, BHK-2	+BHK-1	Unknown	Unknown	N/A	N/A
BTV	BT14 (Prototype)	600576	Bill Wilson:USDA	Unknown, BHK-2	+BHK-1	Unknown	Unknown	N/A	N/A
BTV	BT15 (Prototype)	600577	Bill Wilson:USDA	Unknown	+BHK-1	Unknown	Unknown	N/A	N/A
BTV	BT16 (Prototype)	600578	Bill Wilson:USDA	Unknown	+BHK-1	Unknown	Unknown	N/A	N/A
BTV	BT 17 (SV120)	63-66B (BT 183)	Bill Wilson:USDA	Unknown	+BHK-1	Unknown	Unknown	N/A	N/A
BTV	BT18 (Prototype)	600578	Bill Wilson:USDA	Unknown	+BHK-1	Unknown	Unknown	N/A	N/A
BTV	BT19 (Prototype)	600579	Bill Wilson:USDA	Unknown	+BHK-1	Unknown	Unknown	N/A	N/A
BTV	BT20 (Prototype)	NVSL 021 ODV 9901	Bill Wilson:USDA	Unknown, BHK-2	+BHK-1	Unknown	Unknown	N/A	N/A
BTV	BT21	NVSL 019	Bill	Unknown,	+BHK-1	Unknown	Unknown	N/A	N/A

	(Prototype)	ODV 9301	Wilson:USDA	BHK-2					
BTV	BT22 (Prototype)	NSVL 022 ODV 9301	Bill Wilson:USDA	Unknown, BHK-2	+BHK-1	Unknown	Unknown	N/A	N/A
BTV	BT23 (Prototype)	NVSL 023 ODV 8901	Bill Wilson:USDA	Unknown, BHK-2	+BHK-1	Unknown	Unknown	N/A	N/A
BTV	BT24 (Prototype)	NVSL 024 ODV 9101	Bill Wilson:USDA	Unknown, BHK-2	+BHK-1	Unknown	Unknown	N/A	N/A

[1]Sources listed include: The National Veterinary Service Laboratory (NVSL); California Animal Health and Food Safety Laboratory, (CAHFS) Davis, CA; Plum Island Animal Disease Center (PIADC); and other external collaborators as indicated.

TABLE 30. List of near neighbors screened in multiplexed PCR format. All EHDV strains were purified double stranded BTV RNA from virus with unknown titers.

Virus	Serotype/Isolate or Strain	Unique ID	Source[1]	Previous Passage History	ABADRL Passages since 1983	Extraction date/ID	Extraction Method	Use stock titer	Titer Method
EHDV-1	Type 1, Santa Barbara 2001 isolate	N/A	CAHFS	unknown	N/A	unknown	Trizol	N/A	N/A
EHDV-1	Type 1, Georgia	N/A	CAHFS	unknown	N/A	unknown	Trizol	N/A	N/A
EHDV-1	Type 1, NJ,	040 ODV 0401	NVSL	NHK pass 133	N/A	unknown	Trizol	N/A	N/A
EHDV-2	Alberta	041 ODV 0301	NVSL	Vero M/12, BHK/8, BHK pass 137	N/A	unknown	Trizol	N/A	N/A
EHDV-1 (SV123)	New Jersey (3TD) Deer, NJ 1955	BHK-6; Vero-6	Bill Wilson:USDA	+BHK-3 (12/7/83); (12/11/86); (12/18/86)	EHDV-1 (SV123)	Unknown	Purescript RNA kit (Gentra, MN)	N/A	N/A
EHDV-2 (SV124)	Alberta (59) Deer-1;	BHK-4; Vero-5	Bill Wilson:USDA	+BHK-3 (12/7/83); (12/8/86); (12/19/86)	EHDV-2 (SV124)	Unknown	Purescript RNA kit (Gentra, MN)	N/A	N/A
EHDV-3	NVSL 047 ODV 0001 Nigeria	MB 7, BHK-6	Bill Wilson:USDA	+BHK-1	EHDV-3	Unknown	Purescript RNA kit (Gentra, MN)	N/A	N/A
EHDV-4	NVSL 046 ODV 9201 Nigeria	MB8, BHK-3	Bill Wilson:USDA	+BHK-1	EHDV-4	Unknown	Purescript RNA kit (Gentra MN)	N/A	N/A
EHDV-5	NVSL CSIRO 157	BHK-21/266	Bill Wilson:USDA	+BHK-1	EHDV-5	Unknown	Purescript RNA kit (Gentra MN)	N/A	N/A
EHDV-6	NVSL C6753	N/A	Bill Wilson:USDA	BHK-21/266 ,	+BHK-1	Unknown	Purescript RNA kit (Gentra MN)	N/A	N/A
EHDV-7	NVSL CS775	N/A	Bill Wilson:US	BHK-21/266	+BHK-1	Unknown	Purescript RNA kit	N/A	N/A

			DA				(Gentra MN)		
EHDV-8	NVSL DPP059	N/A	Bill Wilson:USDA	BHK-21/266	+BHK-1	Unknown	Purescript RNA kit (Gentra MN)	N/A	N/A

[1]Sources listed include: The National Veterinary Service Laboratory (NVSL); California Animal Health and Food Safety Laboratory, (CAHFS) Davis, CA; Plum Island Animal Disease Center (PIADC); and other external collaborators as indicated.

TABLE 31. Screening summary for BTV at LLNL

	Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors	Targets
Gel Screening	5	5	5	none	3	5
Real-time RT-_PCR Screening	45	54	16	3_Aerosol Blocks[1]	3	29

[1-]There are 752 pooled samples in each Aerosol Block.

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Background gel screening was carried out in duplicate as 25ul reactions in 96 well PCR plates on MJ thermal cyclers. Each reaction mix consisted of 1X PCR Buffer [200mM Tris-HCL (pH 8.4), 500mM KCl], 1.5mM $MgCl_2$, 0.8mM each dNTP, 80ng BSA, 0.4uM each forward and reverse primers, 0.75U Platinum *Taq* DNA polymerase, PCR water and a variable amount of extracted DNA template. A total of 5ng of soil DNA or 1ng of Eukaryotic DNA or 200pg of Prokaryotic DNA was added to each 25ul reaction mix. Background template data and extraction protocols are available upon request.

Because the target is RNA, we used the Clontech Powerscript RT-PCR kit. Each reaction was performed in triplicate as per the manufacturer's suggested protocol, replacing probe with PCR water, in a 25ul volume with a total of 200pg TRIZOL extracted RNA.

Reaction products were visualized by gel electrophoresis on 4% agarose gels. PCR product sizes are listed as visual estimates based on a 20bp ladder that was run on each gel for reference. I f a signature screened against a background produced a PCR product size that fell below 100 base pairs greater in size than the predicted product size for a signature screened against its target, the signature was dropped from further screening. The theory behind this selection process is that a much larger than target PCR product would not cause inhibitory PCR competition. However, a PCR product of correct size or smaller would inhibit PCR through competition.

TABLE 32a-b. List of 27 signatures that were screened in gel format. Of these signatures 16 (a) were designed to identify segment 10 of BTV and 11 signatures (b) were designed to detect segment 5.

(a) 16 signatures designed to identify BTV segment 10

PAN_BTV10_1810193_F	GCACCCTCCCCCGTTATAC

PAN_BTV10_1810193_R	AATGACGCGGTGAGGATGA
PAN_BTV10_1810193_P	CCTACTGTCCTCAGGTTAATGGCATTTCGAA
PAN_BTV10_1810194_F	GATTGAAGAAGAAGCGAGCAATC
PAN_BTV10_1810194_R	TTTATCTTAAAGGCCACACTCATATCG
PAN_BTV10_1810194_P	NOT SCREENED
PAN_BTV10_1810195_F	GCCACACTCATATCGCTTGAAA
PAN_BTV10_1810195_R	CGTACGCGGAAGCGTTTC
PAN_BTV10_1810195_P	NOT SCREENED
PAN_BTV10_1810196_F	AAAGTGTCGCTGCCATGCTAT
PAN_BTV10_1810196_R	GACATCGCTTTATCCAATATTTCAAG
PAN_BTV10_1810196_P	CCACCGAGATATGCTCCGAGTGCC
PAN_BTV10_1810197_F	GAGCAACGACTGCCGCTAT
PAN_BTV10_1810197_R	GCGGAGAAGGCTGCATTC
PAN_BTV10_1810197_P	NOT SCREENED
PAN_BTV10_1810198_F	TAATGATGCGGTGAGGATGAG
PAN_BTV10_1810198_R	GCGCCACTCTACCTACTGATCTT
PAN_BTV10_1810198_P	CCCGCTAGATGGTTTCGAACTACCATTAACC
PAN_BTV10_1810199_F	CACATGTCGCTTAATTTGTCTTAACC
PAN_BTV10_1810199_R	GCGGAGAAGGCTGCATT
PAN_BTV10_1810199_P	ACGAAACGCTTCCGCGTACGATG
PAN_BTV10_1810200_F	TTAAGCCTCCTAGGTCACTTTTCAA
PAN_BTV10_1810200_R	AAAGCTGCATTCGCATCGT
PAN_BTV10_1810200_P	CACATCATCACGAAACGCTTCTGCG
PAN_BTV10_1810201_F	TAATGATGCGGTGAGGATGAGT
PAN_BTV10_1810201_R	CGCCACTCTACCTACTGATCTTAGG
PAN_BTV10_1810201_P	AGTCCCGCTAGATGGTTTCGAATTACCATTA
PAN_BTV10_1810202_F	GATCACTTTTCAGTTTGGGTAGAATCT
PAN_BTV10_1810202_R	CGTACGCAGAAGCGTTTCG
PAN_BTV10_1810202_P	TGGCGTTTAATTTGTCTCAACCTCACATCA
PAN_BTV10_1810203_F	GTGCAACGCAAACACAAAAAG
PAN_BTV10_1810203_R	GCAACCACAGCAGCCACTA
PAN_BTV10_1810203_P	NOT SCREENED
PAN_BTV10_1810204_F	ATAATGATGCGGTGAGGATGAG
PAN_BTV10_1810204_R	GCATACCCTCCCCCGTTAG
PAN_BTV10_1810204_P	TGATCTCACGATGCAGACTCCTACTGCTG
PAN_BTV10_1810205_F	TCAATTTTGGTAGAATTTGTTCATTCA
PAN_BTV10_1810205_R	GCGGAGAAGGCTGCATTC
PAN_BTV10_1810205_P	ACGAAACGCTTCCGCGTACGATG
PAN_BTV10_1810206_F	CTACTGGTAGCTGCCGTGGTT
PAN_BTV10_1810206_R	CATTTATCTTGAAGGCCACACTCA
PAN_BTV10_1810206_P	NOT SCREENED
PAN_BTV10_1810207_F	CAAACACAAAAGGCGGAGAAG
PAN_BTV10_1810207_R	GGCGTTTAATCTGTCTTAGTCTTACGT
PAN_BTV10_1810207_P	TCGCATCGTACGCAGAAGCGTTTC
PAN_BTV10_1810208_F	GAGGGCATAGGCGCACTT

(b) 11 signatures designed to identify BTV segment 5

PAN_BTV5_1810182_F	GATTGCTTCACGGCCTCAT
PAN_BTV5_1810182_R	TTGGCAAAGGAGGCAATGT
PAN_BTV5_1810182_P	TGCACCCCGCACCGCTTC
PAN_BTV5_1810183_F	CTTCGTCAGCTCCCATCTCA
PAN_BTV5_1810183_R	GGCCTTGATTACAACTGCGATT
PAN_BTV5_1810183_P	CGCGTCCGAGCATGAAAATACCCTC
PAN_BTV5_1810184_F	AATGGATGATTTTGCGAAGCAT
PAN_BTV5_1810184_R	CTCTTCAGCATAACCAACTCTCAGAT
PAN_BTV5_1810184_P	CAGGTACGCCTCGATGGCGATACAAC
PAN_BTV5_1810185_F	ACCTGTTGGAACCCTTCCAAA
PAN_BTV5_1810185_R	TAATAGGTATGCTTCGATGGCTATACA
PAN_BTV5_1810185_P	AGCTGCTGCACATACCTGACCTCTTCAGC
PAN_BTV5_1810186_F	ACCTGTTGGAACCCTTCCAAA
PAN_BTV5_1810186_R	GCGAAGCATTTTAATAGGTATGCTT
PAN_BTV5_1810186_P	NOT SCREENED
PAN_BTV5_1810187_F	GTACCAATCAGAGAAGGCCAAATC
PAN_BTV5_1810187_R	ATTTGCCTCTCTACTTCCCGTTTT
PAN_BTV5_1810187_P	NOT SCREENED
PAN_BTV5_1810188_F	TCTTTCTGAATTAGCCAGTGCAGAT
PAN_BTV5_1810188_R	AGCGGTGTGGAGTGCAACT
PAN_BTV5_1810188_P	TCACGGCCTCATCCATCATTCCACT
PAN_BTV5_1810189_F	CGGAATGCTTTTATTGGTTCCT
PAN_BTV5_1810189_R	CAATGGGATGTGTGCGAAA
PAN_BTV5_1810189_P	NOT SCREENED
PAN_BTV5_1810190_F	CACCGATCCTGATGATCCAA
PAN_BTV5_1810190_R	GGCGATGAGAACTGGCAAA
PAN_BTV5_1810190_P	CCCTCACGAATGATGATCATAGGTAATAGCCA
PAN_BTV5_1810191_F	ATCCATAACACACGAAACAGAACAG
PAN_BTV5_1810191_R	GCGCGTGCTGTTGAGATG
PAN_BTV5_1810191_P	CACTGCGTTGTGCCTTCGTCAGC
PAN_BTV5_1810192_F	CCAGATATCGTCAGATTGCGTATT
PAN_BTV5_1810192_R	AACTCCAAATAGATTTGGATCTATCCA
PAN_BTV5_1810192_P	CTTTTACAACCCGGATGCAGCTGATGA

*Note: Probes not screened were signatures that did not pass initial gel screening.

TABLE 33. Nucleic acid extracts used to challenge the initial set of candidate signatures. These select backgrounds are used to determine which signatures will be down-selected against in the screening process.

Nucleic Acid Extract Type	Description/ID
Soil Extract	D000106
Soil Extract	D000107
Soil Extract	D000426
Soil Extract	D000054

Soil Extract	D000510
Prokaryotic DNA Extract	*Listeria monocytogenes*
Prokaryotic DNA Extract	*Pseudomonas aeruginosa*
Prokaryotic DNA Extract	*Salmonella typhimurium*
Prokaryotic DNA Extract	*Staphylococcus aureus*
Prokaryotic DNA Extract	*Streptococcus pneumoniae*
Eukaryotic DNA Extract	Monkey
Eukaryotic DNA Extract	Chicken
Eukaryotic DNA Extract	Human
Eukaryotic DNA Extract	Rabbit
Eukaryotic DNA Extract	Bovine

TABLE 34. LLNL available target and near neighbor Trizol extracted RNA used in gel screening.

EHD-1 Santa Barbara	Near Neighbor
EHD-1 New Jersey	Near Neighbor
EHD-2 Alberta	Near Neighbor
BTV-2	Target
BTV-10	Target
BTV-11	Target
BTV-13	Target
BTV-17	Target

TABLE 35. Gel screening results summary. The amplicon is the predicted target size for each signature when screened against target RNA. The results of the gel PCR screening is reported in amplicon size when tested in triplicate. Signatures in red were dropped from further screening because they failed to produce the correct product size with target or, as is the case with signature Pan_BTV10_1810197, target product size was produced when screened against soils. None of the signatures showed cross-reactions with the EHD near-neighbors.

Signature	Amplicon	Backgrounds	BTV #2	BTV # 10	BTV #11	BTV #13	BTV #17
Pan_BTV10_1810193	134	N/A	160	160	180	180	180
Pan_BTV10_1810194	115	N/A	120	N/A	N/A	N/A	N/A
Pan_BTV10_1810195	163	N/A	N/A	N/A	N/A	N/A	N/A
Pan_BTV10_1810196	199	N/A	200	200	200	200	200
Pan_BTV10_1810197	143	hit soils	Eliminated				
Pan_BTV10_1810198	143	N/A	80	80	80	80	80
Pan_BTV10_1810199	40	N/A	40	40	60	60	60
Pan_BTV10_1810200	76	N/A	80	80	80	80	100
Pan_BTV10_1810201	96	N/A	80	80	70	70	80
Pan_BTV10_1810202	46	N/A	60	60	60	60	75
Pan_BTV10_1810203	196	N/A	200	200	N/A	N/A	200
Pan_BTV10_1810204	170	N/A	170	170	170	170	170
Pan_BTV10_1810205	62	N/A	100	100	100	100	100

Pan_BTV10_1810206	82	N/A	40	40	N/A	N/A	N/A
Pan_BTV10_1810207	85	N/A	250	250	80	80	80
Pan_BTV10_1810208	51	N/A	N/A	N/A	N/A	N/A	N/A
Pan_BTV5_1810182	131	N/A	160	160	180	180	180
Pan_BTV5_1810183	87	N/A	100	100	120	140	140
Pan_BTV5_1810184	82	N/A	40	40	100	100	100
Pan_BTV5_1810185	54	N/A	50	100	120	120	120
Pan_BTV5_1810186	67	N/A	65	120	120	N/A	125
Pan_BTV5_1810187	167	N/A	N/A	N/A	N/A	170	N/A
Pan_BTV5_1810188	50	N/A	50	100	100	100	100
Pan_BTV5_1810189	42	N/A	N/A	N/A	N/A	N/A	N/A
Pan_BTV5_1810190	37	N/A	60	60	50	50	50
Pan_BTV5_1810191	39	N/A	40	60	60	60	60
Pan_BTV5_1810192	80	N/A	80	80	80	80	80

2006 Bluetongue Virus (BTV) – Real-time RT- PCR Screening Report

Background real-time RT-PCR tests were carried out in triplicate as 25ul reactions in 96 well PCR plates on BioRad's iCYCLERs. Each reaction mix consisted of 1X PCR Buffer [200mM Tris-HCL (pH 8.4), 500mM KCl], 6mM $MgCl_2$, 0.2mM each dNTP, 80ng BSA, 0.2uM each forward and reverse primers, 0.4uM Probe with a 5' Fam and a 3' BHQ modification, 1.25U Platinum *Taq* DNA polymerase, PCR water and a variable amount of extracted DNA template. A total of 5ng of soil DNA or 1ng of Eukaryotic DNA or 200pg of Prokaryotic DNA was added to each 25ul reaction mix.

Because the target is RNA, we used the Clontech Powerscript RT-PCR kit. Each reaction was performed in triplicate as per the manufacturer's suggested protocol, in a 25ul volume with a total of 200pg TRIZOL extracted RNA.

A real-time RT- PCR reaction was deemed positive if a Ct (cycle threshold) value of below 36 cycles was observed at least 2 of the 3 times the test was performed.

TABLE 36. List of signatures that were considered successful through gel screening and were brought forward to be screened in real-time RT- PCR format.

PAN_BTV10_1810193_F	GCACCCTCCCCCGTTATAC
PAN_BTV10_1810193_R	AATGACGCGGTGAGGATGA
PAN_BTV10_1810193_P	CCTACTGTCCTCAGGTTAATGGCATTTCGAA
PAN_BTV10_1810196_F	AAAGTGTCGCTGCCATGCTAT
PAN_BTV10_1810196_R	GACATCGCTTTATCCAATATTTCAAG
PAN_BTV10_1810196_P	CCACCGAGATATGCTCCGAGTGCC
PAN_BTV10_1810198_F	TAATGATGCGGTGAGGATGAG
PAN_BTV10_1810198_R	GCGCCACTCTACCTACTGATCTT
PAN_BTV10_1810198_P	CCCGCTAGATGGTTTCGAACTACCATTAACC
PAN_BTV10_1810199_F	CACATGTCGCTTAATTTGTCTTAACC
PAN_BTV10_1810199_R	GCGGAGAAGGCTGCATT

PAN_BTV10_1810199_P	ACGAAACGCTTCCGCGTACGATG
PAN_BTV10_1810200_F	TTAAGCCTCCTAGGTCACTTTTCAA
PAN_BTV10_1810200_R	AAAGCTGCATTCGCATCGT
PAN_BTV10_1810200_P	CACATCATCACGAAACGCTTCTGCG
PAN_BTV10_1810201_F	TAATGATGCGGTGAGGATGAGT
PAN_BTV10_1810201_R	CGCCACTCTACCTACTGATCTTAGG
PAN_BTV10_1810201_P	AGTCCCGCTAGATGGTTTCGAATTACCATTA
PAN_BTV10_1810202_F	GATCACTTTTCAGTTTGGGTAGAATCT
PAN_BTV10_1810202_R	CGTACGCAGAAGCGTTTCG
PAN_BTV10_1810202_P	TGGCGTTTAATTTGTCTCAACCTCACATCA
PAN_BTV10_1810204_F	ATAATGATGCGGTGAGGATGAG
PAN_BTV10_1810204_R	GCATACCCTCCCCCGTTAG
PAN_BTV10_1810204_P	TGATCTCACGATGCAGACTCCTACTGCTG
PAN_BTV10_1810205_F	TCAATTTTGGTAGAATTTGTTCATTCA
PAN_BTV10_1810205_R	GCGGAGAAGGCTGCATTC
PAN_BTV10_1810205_P	ACGAAACGCTTCCGCGTACGATG
PAN_BTV10_1810207_F	CAAACACAAAAGGCGGAGAAG
PAN_BTV10_1810207_R	GGCGTTTAATCTGTCTTAGTCTTACGT
PAN_BTV10_1810207_P	TCGCATCGTACGCAGAAGCGTTTC
PAN_BTV5_1810182_F	GATTGCTTCACGGCCTCAT
PAN_BTV5_1810182_R	TTGGCAAAGGAGGCAATGT
PAN_BTV5_1810182_P	TGCACCCCGCACCGCTTC
PAN_BTV5_1810183_F	CTTCGTCAGCTCCCATCTCA
PAN_BTV5_1810183_R	GGCCTTGATTACAACTGCGATT
PAN_BTV5_1810183_P	CGCGTCCGAGCATGAAAATACCCTC
PAN_BTV5_1810184_F	AATGGATGATTTTGCGAAGCAT
PAN_BTV5_1810184_R	CTCTTCAGCATAACCAACTCTCAGAT
PAN_BTV5_1810184_P	CAGGTACGCCTCGATGGCGATACAAC
PAN_BTV5_1810185_F	ACCTGTTGGAACCCTTCCAAA
PAN_BTV5_1810185_R	TAATAGGTATGCTTCGATGGCTATACA
PAN_BTV5_1810185_P	AGCTGCTGCACATACCTGACCTCTTCAGC
PAN_BTV5_1810188_F	TCTTTCTGAATTAGCCAGTGCAGAT
PAN_BTV5_1810188_R	AGCGGTGTGGAGTGCAACT
PAN_BTV5_1810188_P	TCACGGCCTCATCCATCATTCCACT
PAN_BTV5_1810190_F	CACCGATCCTGATGATCCAA
PAN_BTV5_1810190_R	GGCGATGAGAACTGGCAAA
PAN_BTV5_1810190_P	CCCTCACGAATGATGATCATAGGTAATAGCCA
PAN_BTV5_1810191_F	ATCCATAACACACGAAACAGAACAG
PAN_BTV5_1810191_R	GCGCGTGCTGTTGAGATG
PAN_BTV5_1810191_P	CACTGCGTTGTGCCTTCGTCAGC
PAN_BTV5_1810192_F	CCAGATATCGTCAGATTGCGTATT
PAN_BTV5_1810192_R	AACTCCAAATAGATTTGGATCTATCCA
PAN_BTV5_1810192_P	CTTTTACAACCCGGATGCAGCTGATGA

TABLE 37a-c. Real-time RT- PCR background screening consisted of an extensive list of soil, Prokaryotic and Eukaryotic extracted DNAs in addition to 3 aerosol blocks, each containing 752 samples. All signatures passed real-time RT- PCR background screening. None of the 18 signatures produced an amplicon when screened against the listed backgrounds. All signatures moved forward to be screened against the available BTV targets and near neighbors.

(a) Total of 45 soils screened

D000402	D000531	S252	S271	S280	S290	S300
D000109	D000542	S253	S272	S282	S291	S301
D000107	D000533	S254	S273	S283	S292	S303
D000500	D000561	S255	S274	S284	S295	S304
D000505	D000562	S256	S275	S286	S296	S305
D000521	D000501	S257	S276	S287	S297	S307
D000551	D000550	S259	S277	S288	S298	
D000527	S251	S260	S279	S289	S299	

(b) Total of 16 Eukaryotes screened

Bovine	Drosophila	Monkey	Rabbit
Cat	Equine	Mosquito	Rat
Chicken	Flea	Mouse	Sheep
Dog	Human	Porcine	Tick

(c) Total of 54 Prokaryotes screened

A. suis	*C. butyricum*	*L. gasseri*	*P. oleovorans*
A migulanus	*C. pseudodipthericum*	*L. monocytogenes*	*R. leguminosarum*
B. cereus	*C. marinoflava*	*L. seeligeri*	*R. rhodochrous*
B. globigii	*E. amylovora*	*M. luteus*	*S. typhimurium*
B. subtilis	*E. herbicola*	*M. lacunatica*	*S. muelleri*
B thuringiensis	*E. coli*	*O. ssp. Maris*	*Alcaligenes sp.*
B. denticum	*G.caldoxylosilyticus*	*P. napthalaenovorans*	*S. aureus*
B. burgdorferi	*H. homophile*	*P. dentifrices*	*S. pneumoniae*
B. acacia	*H. influenza*	*P sanguineus*	*S. scabiei*
C. vibriodes	*H. seropedicae*	*P. mirabillis*	*T. maceachernii*
C. michganensis	*L. garviease*	*P aeruginosa*	*V. paraheamolyticus*
			X. translucens

TABLE 38. LLNL Targets and near neighbors available for real-time RT-PCR screening consisted of:

EHD-1 Santa Barbara	Near Neighbor
EHD-1 Georgia	Near Neighbor
EHD-2 Alberta	Near Neighbor
BTV-2	Target
BTV-10	Target
BTV-11	Target
BTV-13	Target
BTV-17	Target

TABLE 39. Results of signatures tested against LLNL BTV domestic serotype targets in Real-time RT- PCR. Screenings were performed in triplicate and the average Ct value for each signature against the listed target is reported. None of the signatures tested in real-time RT-PCR against the near neighbor EHD produced amplicons. Signatures 1810193, 1810196, 1810202 and 1810191 (highlighted in red), were dropped because they failed to produce PCR product with available target in real-time RT-PCR format. Where "N" indicates no detectable PCR product. This left 14 signatures that produced PCR product in real-time RT-PCR format with at least one of our available targets and did not produce PCR products when screened against any of our available near neighbors or backgrounds. A group of 8 signatures were "hand" chosen as best candidates (based on reactivity against all domestic serotypes) from the available 14 successful signatures screened at LLNL. These signatures (highlighted in green), along with 2 additional signatures from a previous BTV assay (developed in 2005) were sent to Bill Wilson at the USDA in Wyoming for further testing against all 19 foreign BTV serotypes and additional isolates of all five domestic serotypes.

SIGNATURE	BTV2-2	BTV-10	BTV-11	BTV-13	BTV-17
Pan_BTV10_1810193	N	N	N	N	N
Pan_BTV10_1810196	N	N	N	N	N
Pan_BTV10_1810198	22.6	20	22.3	N	21.3
Pan_BTV10_1810199	30.7	22.3	23	23.3	20.4
Pan_BTV10_1810200	28.8	23.8	26	24.6	21.3
Pan_BTV10_1810201	N	27	25.7	N	22.1
Pan_BTV10_1810202	N	N	N	N	N
Pan_BTV10_1810204	N	27.3	27.9	28.4	24.4
Pan_BTV10_1810205	25.5	22.8	23.5	23.8	21
Pan_BTV10_1810207	27.7	28.3	29.4	29.8	25.4
Pan_BTV5_1810182	21.9	N	N	N	N
Pan_BTV5_1810183	23.3	25.3	26	29.4	26.7
Pan_BTV5_1810184	N	23.7	N	23.7	29.5
Pan_BTV5_1810185	25.4	N	N	N	N
Pan_BTV5_1810188	26.3	27.8	28.1	27.2	27.8
Pan_BTV5_1810190	N	23.6	24.8	24	23.3
Pan_BTV5_1810191	N	N	N	N	N
Pan_BTV5_1810192	N	24.2	28.9	28.8	25.8

2006 Pan Bluetongue Virus (BTV) Real-time RT-PCR screening at the USDA in Wyoming

Foreign BTV isolates were amplified in Baby Hamster Kidney (BHK) cells. Briefly, BHK cells were grown to a confluent monolayer, rinsed with PBS, fresh media was added and cells were inoculated with the BTV isolates and allowed to incubate for 30 minutes. Additional media was added and cells were incubated at 37ºC for 3-4 days or until cells showed 80% infection. At this point viral RNA was extracted from the cell culture using Purescript RNA Purification kit (Gentra, Minneapolis MN).

TABLE 40. Results of each signature tested with domestic and foreign BTV serotypes at Bill Wilson's lab in Wyoming (USDA). Signatures 1759932 and 1759933 (highlighted in gray), are the two previous V1.0 multiplex BTV signatures developed in 2005 whose reactivity against all 24 serotypes was never before tested. Signatures were screened in duplicate and the Ct value reported in this table is the average. "N" indicates no detectable PCR product.

	1810205	**1810207**	**1810182**	**1810183**	**1810188**	**1810199**	**1810200**	**1810201**	**1759932**	**1759933**
BTV1	20.4	19.5	18.6	17.6	23.6	20.1	19.2	19.3	23.9	N
BTV2	20.9	21	17.9	18.1	23.9	27.2	24.1	23.1	23	24.7
BTV3	22	20.4	19.3	24.3	19.6	20.7	21.6	N	23.7	N
BTV4	24.1	21.2	16.2	18.8	22	25.1	23.3	N	23.2	N
BTV5	22.5	20.2	18	18.2	22.6	23.2	23.7	35.7	24.8	N
BTV6	22.5	20.6	17.9	18.3	24.5	20.5	23.9	N	26.9	N
BTV7	15.1	18.4	13.1	15.5	16.7	18.1	14.8	15	21.4	N
BTV8	19.8	21	N	20.1	18.9	20.3	18.5	18.4	24	N
BTV9	21.9	18.9	15.9	17	21.9	18.2	23.6	N	23.3	N
BTV10	19.2	15.9	24.5	16.8	20.3	26.3	17.5	N	20.7	17.1
BTV11	16.5	24.3	24.7	15.9	20.2	15.7	14.8	16.6	16.8	14.7
BTV12	18.4	19.3	14.1	14.7	19.2	23.8	19.9	19.4	19.1	N
BTV13	19	23.5	25.4	22.5	22.9	16.9	17	20.6	23	18.1
BTV14	17.8	20.3	16.5	16.7	22.7	22.4	19.8	N	23.8	N
BTV15	25	24.7	19.8	23.5	23.6	25.5	33.3	27.2	28.6	N
BTV16	26.6	21.7	N	N	N	30.4	35.5	19.3	N	N
BTV17	18.6	26.5	N	20.2	N	15.7	16.5	18	22.7	18.5
BTV18	20.3	19.2	16.7	20.3	21.8	22.9	25.2	N	24.9	N
BTV19	27	30	23	29.9	25.9	29.3	24.4	25	26.8	N
BTV20	26.1	25.1	N	N	N	27.2	26.2	21.5	N	N
BTV21	28.4	32.5	N	N	33.6	32.1	30.1	23.9	N	N
BTV22	21.7	24.2	20	23.9	23.5	22.7	21.1	21.5	26.4	N
BTV23	24.6	26.2	N	N	31.9	30.4	27.6	22	N	34.2
BTV24	27.1	24.4	23.3	28.9	25.5	28.8	29.7	N	30.5	N

TABLE 41. Results of each signature crossed with EHD near-neighbor isolates at Bill Wilson's lab in Wyoming (USDA). Signatures were screened in duplicate and the Ct value reported in this table is the average. "N" indicates no detectable PCR product. None of the signatures screened cross-reacted with near-neighbor EHD and positive control (**BT+**) *Bacillus thuringiensis* confirmed reliability of the PCR reaction.

Isolate	**1810199**	**1810200**	**1810205**	**1810207**
EHDV-1 (SV123)	N	N	N	N
EHDV-2 (SV124)	N	N	N	N
EHDV-3	N	N	N	N
EHDV-4	N	N	N	N
EHDV-5	N	N	N	N
EHDV-6	N	N	N	N
EHDV-7	N	N	N	N
EHDV-8	N	N	N	N
BT+	23.23	15.64	21.76	16.85

2006 Pan Bluetongue Virus (BTV) TaqMan Conclusions:

From the 27 signatures generated to target Pan BTV, 14 signatures at LLNL produced repeatable predicted PCR product when performed in a TaqMan assay with domestic BTV targets and did not produce PCR product when assayed against background or near-neighbor templates.

From these 14 signatures, 8 were chosen to undergo further screening against foreign BTV targets at the USDA in Laramie, WY. Four signatures made PCR product with all foreign BTV isolates, 1810199, 1810200, 1810205 and 1810207, and were given to the MUX group to incorporate into their Ag panel. Signature 1810201 was included in the signatures passed on to the MUX group because it compliments the existing BTV signature already in the multiplex panel, 1759933.

TABLE 42. Final signatures turned over to MUX for assay development

Pan_BTV10_1810199_F	CACATGTCGCTTAATTTGTCTTAACC
Pan_BTV10_1810199_R	GCGGAGAAGGCTGCATT
Pan_BTV10_1810199_P	ACGAAACGCTTCCGCGTACGATG
Pan_BTV10_1810200_F	TTAAGCCTCCTAGGTCACTTTTCAA
Pan_BTV10_1810200_R	AAAGCTGCATTCGCATCGT
Pan_BTV10_1810200_P	CACATCATCACGAAACGCTTCTGCG
Pan_BTV10_1810201_F	TAATGATGCGGTGAGGATGAGT
Pan_BTV10_1810201_R	CGCCACTCTACCTACTGATCTTAGG
Pan_BTV10_1810201_P	AGTCCCGCTAGATGGTTTCGAATTACCATTA
Pan_BTV10_1810205_F	TCAATTTTGGTAGAATTTGTTCATTCA
Pan_BTV10_1810205_R	GCGGAGAAGGCTGCATTC
Pan_BTV10_1810205_P	ACGAAACGCTTCCGCGTACGATG
Pan_BTV10_1810207_F	CAAACACAAAAGGCGGAGAAG
Pan_BTV10_1810207_R	GGCGTTTAATCTGTCTTAGTCTTACGT
Pan_BTV10_1810207_P	TCGCATCGTACGCAGAAGCGTTTC

2006 Pan Bluetongue Virus (BTV) Limit of Detection at the USDA in Wyoming

TABLE 43. The USDA in Wyoming performed further screening to determine the relative limit of detection for each of the five successful Pan BTV signatures. The five signatures were screened against each of the 24 BTV strains using 2 pg, 0.2 pg, 20 fg and 2 fg total RNA, for relative limit of detection (qualitative comparison). The tests were performed in triplicate and values below are the average Ct value, where the limit of detection is defined as the point at which no Ct values are detected. The "score" indicates how many strains of BTV that particular signature was able to detect.

	Limit of Detection Summary (units in pg)				
	1810205 (B1)	**1810207 (B3)**	**1810199 (A7)**	**1810200 (A8)**	**1810201 (A9)**
BTV1	0.2	<0.002	0.02	<0.002	0.2
BTV2	0.02	<0.002	0.02	0.02	0.2

BTV3	0.02	<0.002	0.02	0.2	N
BTV4	0.2	<0.002	0.2	0.2	N
BTV5	0.02	<0.002	0.02	2	>2
BTV6	0.02	0.02	0.02	2	N
BTV7	<0.002	<0.002	<0.002	<0.002	<0.002
BTV8	<0.002	<0.002	<0.002	<0.002	0.02
BTV9	<0.002	<0.002	0.02	0.2	N
BTV10	<0.002	<0.002	2	<0.002	N
BTV11	0.02	0.02	<0.002	<0.002	0.02
BTV12	0.2	0.2	<0.002	2	>2
BTV13	<0.002	0.02	<0.002	<0.002	0.02
BTV14	<0.002	0.02	0.2	0.02	N
BTV15	<0.002	0.02	0.2	>2	0.02
BTV16	<0.002	0.02	>2	>2	>2
BTV17	<0.002	0.02	<0.002	<0.002	0.2
BTV18	0.02	0.02	0.02	2	N
BTV19	<0.002	0.2	0.2	0.02	0.02
BTV20	2	0.2	0.2	0.2	0.02
BTV21	>2	>2	2	2	2
BTV22	<0.002	0.02	<0.002	<0.002	<0.002
BTV23	<0.002	2	0.02	2	0.2
BTV24	2	0.02	0.02	>2	N
Score	24/24	24/24	24/24	24/24	16/24

1.7. BTV MULTIPLEXED PCR SCREENING REPORT **2005 DEVELOPMENT**

Screening and Down-selection Technical Approach:

Signatures that pass gel and real-time PCR screening are candidates for multiplexed assays. If the number of candidate signatures is large than a subset of the available signatures are selected based on measured sensitivity and specificity observed in real-time screening. In preliminary screening the background of individual signatures is measured by running "blank" (no sample added) controls and observing signature response. In some cases a primer-to-primer non-specific interference will eliminate candidate signatures. Signatures are then added iteratively to each multiplexed panel, titrated, and with each signature addition, collectively, all signatures are re-titrated to determine if the presence of one signature will interfere with the performance of another. During this process signatures are added or subtracted from the multiplexed panel depending on individual outcomes until all desirable signatures have been added.

Criterion for down-selection of multiplexed signatures

Signature specificity. In initial real-time PCR screening tests undergo target and near neighbor screening and screening against environmental confounders which assess

individual signature specificity. In multiplex format this is repeated to verify that a more complex reaction system gives the same level of specificity.

Signature sensitivity. In initial real-time PCR screening tests undergo limit of detection testing where a dilution curve is generated for each signature. This determines individual signature sensitivity. In multiplex format this is repeated to verify that a more complex reaction system gives acceptable signature sensitivity.

Optimal signal to noise ratio. In the multiplex assay system the potential for cross reactivity is larger than in single reaction testing, this may cause unwanted PCR reaction products or non-specific cross-reactivity. Signatures are preferred to have a high signal to noise ratio, where desired signal is significantly greater than the background noise of the signature when there is no target nucleic acid in the reaction.

Compatibility with other signatures in multiplex. In some cases a primer set may interfere with other primers or probes in the multiplex and there by cause limit of detection shifting for one or more assay in the multiplex. For each scenario it must be evaluated whether the shift in detection level is significant enough to remove (what could be a very desirable signature) from the panel.

TABLE 44. 2005 Nomenclature Key

Multiplex Group ID	LLNL Bioinformatics/Taqman ID
BTV_2	BTV_1759932
BTV_3	BTV_1759933

Multiplex Data Analysis -Technical approach

Disease signature thresholds

Thresholds were initially determined using all available data from known negative samples (blanks) by plotting a histogram, which showed the probability of a given sample generating a particular MFI. In the absence of cross reactivity, each signature in the multiplex assay could be treated as an individual assay result. For example, for a sample spiked with BPSV virus, data for other disease signatures could contribute towards negative data, providing that cross reaction between disease signature sets was not observed. This is a way to increase the number of samples that can be used to establish thresholds.

TABLE 45. The threshold values that were established for the assay controls and each 2005 signature in the multiplex assay panel are shown below.

Signature Multiplex Group ID	Threshold (MFI)
BTV-2	>55
BTV-3	>31

TABLE 46. List of 2005 targets screened.

Organism	Strain/ID[1]	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	Use stock titer	Titer Method
Bluetongue Virus	2	CAHFS	10^6.5 $TCID_{50}$/0.1mL	Unknown	12/2006	Trizol	6.32 x 10^5 $TCID_{50}$/0.1mL	Unknown
Bluetongue Virus	10	CAHFS	10^6.5 $TCID_{50}$/0.1mL	Unknown	12/2006	Trizol	6.32 x 10^5 $TCID_{50}$/0.1mL	Unknown
Bluetongue Virus	11	CAHFS	10^6.5 $TCID_{50}$/0.1mL	Unknown	12/2006	Trizol	6.32 x 10^5 $TCID_{50}$/0.1mL	Unknown
Bluetongue Virus	13	CAHFS	10^4.25 $TCID_{50}$/0.1mL	Unknown	12/2006	Trizol	3.56 x 10^3 $TCID_{50}$/0.1mL	Unknown
Bluetongue Virus	17	CAHFS	10^5.75 $TCID_{50}$/0.1mL	Unknown	12/2006	Trizol	1.12 x 10^5 $TCID_{50}$/0.1mL	Unknown

"2005 Pre-screening" & Target/near-neighbor screening

TABLE 47. Data source: LLNL date 20050817. "Pre-screening of extracted target nucleic acid for BTV. Each BTV serotype was challenged in RT-PCR by adding 5uL of the below described concentration of BTV virus (all 100pg/uL) for a total of 500pg per reaction. The yellow highlighted data is for a BTV-2 singleplex assay, thus only the BTV-2 column should respond, and likewise, the blue codes for the BTV-3 singleplex assay and should only respond on the BTV-3 column of data, as is observed. These results indicate that all serotypes react at approximately the same level for each signature, BTV-2 and BTV-3; however, it was noted that the BTV-3 signatures had slightly higher responses for all. Both signatures exhibited reasonably low background noise in the absence of the PCR amplification of target.

Color key:	BTV-2 Singleplex Assay BTV-3 Singleplex Assay					
Sample	**BTV-2**	**BTV-3**	**16S**	**MT7**	**bMT7**	**MT7-Cy3**
Blank_BTV-2	9	13	13	37	18987.5	3923
Blank	10	22	12	38	18838	3806
Blank	11	29	13	37	18900.5	3742
BTV_2 (100pg/ul)	1425.5	22	14	40	18141	3583
BTV_10 (100pg/ul)	1232	41.5	14.5	41	18591.5	3601
BTV_11 (100pg/ul)	1373	41	14	42	18523	3904.5
BTV_13 (100pg/ul)	1406	14	12.5	39	19085	3756
BTV_17 (100pg/ul)	1773	27	12	40	19273.5	3750
Blank_BTV-2	9	14	12	37	17648	3810
Blank	9	20	10	36	18018.5	3755
Blank	9	26	11	38	18647	3811
BTV_2 (100pg/ul)	1279	19	12	39	18527.5	3723
BTV_10 (100pg/ul)	1205	34	13	38	18518.5	3817
BTV_11 (100pg/ul)	1301	38	13	40	19013	3734
BTV_13 (100pg/ul)	1377	14	12.5	38	19566.5	3693.5

BTV_17 (100pg/ul)	1649	22	13.5	38	18193.5	3878
Blank_BTV-3	13	69	13	38	17551.5	3736
Blank	9	47	13	36	17920	3688
Blank	10	36	13	37	17811	3659
BTV_2 (100pg/ul)	15	2349	13	38	18056	3867
BTV_10 (100pg/ul)	12	4595	14	41	18493	4114
BTV_11 (100pg/ul)	11	4780	14	40	18171	3818.5
BTV_13 (100pg/ul)	11	5082	14	41	17767	3790.5
BTV_17 (100pg/ul)	12	5194.5	14	41	18478	3877
Blank_BTV-3	12	52	10.5	34	16985.5	3694
Blank	9	38	12	37.5	18560	3579
Blank	9	31	12	35	17163	3605
BTV_2 (100pg/ul)	12	2151	12	37	16040.5	3766
BTV_10 (100pg/ul)	12	4396	14	38.5	17981.5	3772
BTV_11 (100pg/ul)	11.5	4381	15	40	18958	3567
BTV_13 (100pg/ul)	10	4491	14	39	18221.5	3731
BTV_17 (100pg/ul)	11	4293	12	38	17533	3775

2005 Multiplexed PCR Assay Titrations

Upon finalization of the assay panel constituents each agent assay is characterized in multiplexed PCR format by running dose-response (titration) curves against titered virus extracted from virus infected cell culture for each available agent strain. Each sample is tested in duplicate and reported as a mean value of the median fluorescence intensity units (MFI) against agent concentration (per reaction volume, 5uL) on a double log plot. Concentration units are reported in TCID50/rxn (assumes 5uL sample volume per reaction) or pfu/rxn depending on virus type and source. In some cases where titered virus was not available data is reported as picogram units (pg). Each assay is evaluated by its ability to detect agent strains and differentiate from near neighbors (specificity) and by its range of sensitivity to each strain relative to each signature tested for that agent. Because this method is not aimed at determination of analytical sensitivities (requiring a more extensive sample set tested in clinical sample matrices which is not practical for this stage of development) it does however, provide tentative limits of detection that are used to measure the performance of each assay.

(a) **(b)**

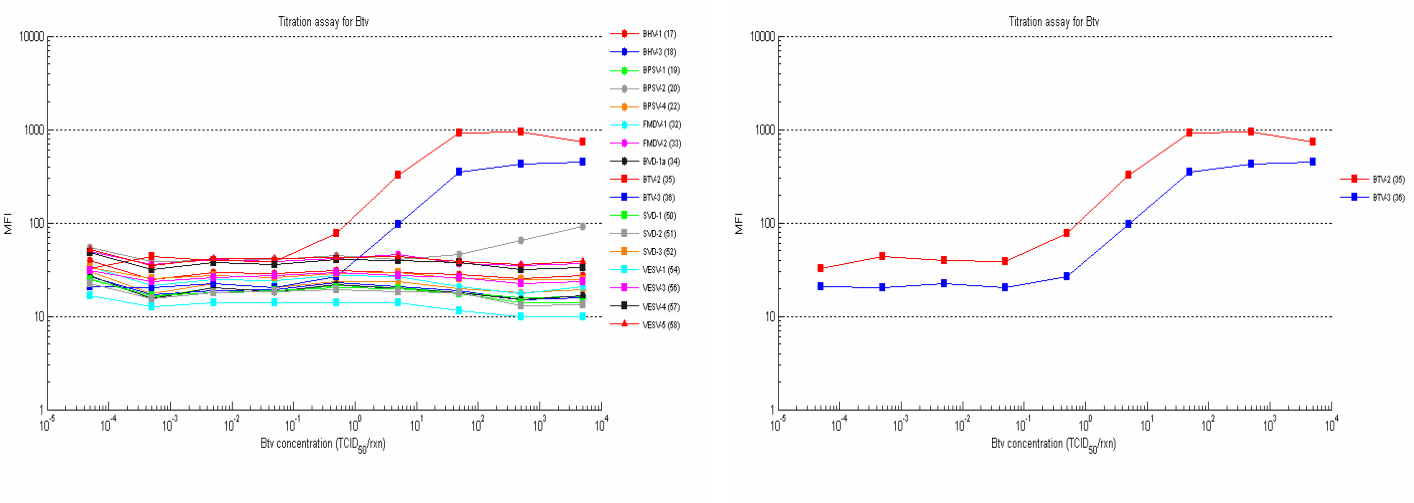

FIG. 1. Multiplexed RT-PCR titrations for BTV using RNA extracted from titered BTV-13 strain virus. (a) Plot shows all assays in the presence of BTV assay titration. Note that even at high concentration of target nucleic acid, no cross-reactivity is seen on other assay channels, with the exception of slight increase in signal intensity for BPSV-2 (below threshold level). (b) Same plot as (a), however, other assay data has been removed just to show BTV-specific assays. **Data source: LLNL date:20060117**

1.8. BTV MULTIPLEXED PCR SCREENING REPORT **2006 DEVELOPMENT**

SCREENING AND DOWN-SELECTION TECHNICAL APPROACH:

Signatures that pass gel and real-time PCR screening are candidates for multiplexed assays. If the number of candidate signatures is large than a subset of the available signatures are selected based on measured sensitivity and specificity observed in real-time screening. In preliminary screening the background of individual signatures is measured by running "blank" (no sample added) controls and observing signature response. In some cases a primer-to-primer non-specific interference will eliminate candidate signatures. Signatures are then added iteratively to each multiplexed panel, titrated, and with each assay addition, collectively, all signatures are re-titrated to determine if the presence of one signature will interfere with the performance of another. During this process signatures are added or subtracted from the multiplexed panel depending on individual outcomes until all desirable signatures have been added.

Criterion for down-selection of multiplexed signatures

Signature specificity. In initial real-time PCR screening signatures undergo target and near neighbor screening and screening against environmental confounders which assess individual signature specificity. In multiplex format this is repeated to verify that a more complex reaction system gives the same level of specificity.

Signature sensitivity. In initial real-time PCR screening signatures undergo limit of detection testing where a dilution curve is generated for each signature. This determines individual

signature sensitivity. In multiplex format this is repeated to verify that a more complex reaction system gives acceptable signature sensitivity.

Optimal signal to noise ratio. In the multiplex assay system the potential for cross reactivity is larger than in single reaction testing, this may cause unwanted PCR reaction products or non-specific cross-reactivity. Signatures are preferred to have a high signal to noise ratio, where desired signal is significantly greater than the background noise of the signatures when there is no target nucleic acid in the reaction.

Compatibility with other signatures in multiplex. In some cases a primer set may interfere with other primers or probes in the multiplex and there by cause limit of detection shifting for one or more signature in the multiplex. For each scenario it must be evaluated whether the shift in detection level is significant enough to remove (what could be a very desirable signature) from the panel.

TABLE 48. Order details for 2005 and 2006 signatures ordered for multiplexed assay screening and development.

ID	Mux ID	Modification details	Vendor
BTV_1759932 .BF BTV_1759932 .FCP BTV_1759932.R	BTV_1759932	5'-/5Bio/GCACCC/iBiodT/ATATGTT/iBiodT/CCAGACCA-3'	IDT DNA
		5'- /5AmMC6//iSp18/CTAACTCGTGGGCCAATCATCATCTTCTGT-3'	IDT DNA
		5'-CAGCTAACTCTTCAGCCACACG-3'	IDT DNA
BTV10_1810199_A7_BF BTV10_1810199_A7_FCP BTV10_1810199_A7_R	BTV10_1810199	5'-/5Bio/CACATGTCGCTTAATTTGTCTTAACC-3'	Biosearch
		5'-/5AmMC6/iSp18/ACGAAACGCTTCCGCGTACGATG-3'	Biosearch
		5'-GCGGAGAAGGCTGCATT-3'	Biosearch
BTV10_1810205_B1_BF BTV10_1810205_B1_FCP BTV10_1810205_B1_R	BTV10_1810205	5'-/5Bio/TCAATTTTGGTAGAATTTGTTCATTCA-3'	Biosearch
		5'-/5AmMC6/iSp18/ACGAAACGCTTCCGCGTACGATG-3'	Biosearch
		5'-GCGGAGAAGGCTGCATTC-3'	Biosearch
BTV10_1810207_B3_BF BTV10_1810207_B3_FCP BTV10_1810207_B3_R	BTV10_1810207	5'-/5Bio/CAAACACAAAAGGCGGAGAAG-3'	Biosearch
		5'-/5AmMC6/iSp18/GAAACGCTTCTGCGTACGATGCGA-3'	Biosearch
		5'-GGCGTTTAATCTGTCTTAGTCTTACGT-3'	Biosearch

Multiplex Data Analysis -Technical approach

Disease signature thresholds

Thresholds were initially determined using all available data from known negative samples (blanks) by plotting a histogram, which showed the probability of a given sample generating a particular MFI. In the absence of cross reactivity, each signature in the multiplex assay could be treated as an individual signature result. For example, for a sample spiked with BTV virus, data for other disease signatures could contribute towards negative data, providing that cross reaction between disease signature sets was not observed. This is a way to increase the number of samples that can be used to establish thresholds.

TABLE 49. Individual signature thresholds for BTV. For FY06, threshold determinations have not yet been made as they require a significant number of tests (>500) to generate this information.

Signature Name	Mux Assay name	Signature background in Tris NaCl Buffer (MFI +/-)	Signature background in Clinical matrices (MFI +/-)	Threshold
BTV_1759932	BTV_1759932	TBD	TBD	TBD
BTV10_1810199	BTV10_1810199	TBD	TBD	TBD
BTV10_1810205	BTV10_1810205	TBD	TBD	TBD
BTV10_1810207	BTV10_1810207	TBD	TBD	TBD

TABLE 50. List of targets screened in 2006 multiplexed PCR format. All BTV strains were extracted nucleic acids with unknown titers.

Virus	Strain[1]	Unique ID	Source	Previous Passage History	ABADRL Passages since 1983	Extraction date/ID	Extraction Method	Use stock titer	Titer Method
BTV	BTV-10	lot #001 ODV 0001 Dec 5, 2000	NVSL	(NVSL)BHK?(LLNL)BHK1	N/A	12/14/06	Trizol	$6.32x10^5$ $TCID_{50}$/0.1 mL	Reed-Meunch
BTV	BTV-11	lot#002 ODV0101 Nov 30, 2001	NVSL	(NVSL)BHK?(LLNL)BHK1	N/A	12/14/06	Trizol	$6.32x10^5$ $TCID_{50}$/0.1 mL	Reed-Meunch
BTV	BTV-13	no lot or strain given	NVSL	unknown	N/A	12/14/06	Trizol	$3.56x10^3$ $TCID_{50}$/0.1 mL	Reed-Meunch
BTV	BTV-17	lot#004 ODV 0201 Nov 28, 2002	NVSL	(NVSL)BHK?(LLNL)BHK1	N/A	12/14/06	Trizol	$1.12x10^5$ $TCID_{50}$/0.1 mL	R Reed-Meunch
BTV	BTV-2	no lot given	NVSL	(NVSL)BHK?(LLNL)BHK2	N/A	12/14/06	Trizol	$6.32x10^5$ $TCID_{50}$/0.1 mL	Reed-Meunch

TABLE 51. List of near-neighbors screened against the 2006 Bovine Panel. All near-neighbors samples were extracted nucleic acids with unknown titers.

Virus	Strain[1]	Unique ID	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	NA conc. used or stock titer	Titer Method
Border Disease virus	Coos Bay	4-6-92	NADC, Julia Ridpath	1.5×10^8 TCID50/mL	unknown	12/14/06	Trizol	1.18×10^6 TCID50/mL	Reed & Muench
Bovine Herpes Virus-1	California	NVSL 20032 111903	CAHFS	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Cooper	NVSL 86741 111903	CAHFS	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Los Angeles	Los Angeles ATCC VR188	ATCC (CAHFS)	1×10^5 TCID50/mL	(CAHFS) MDBK2(LLNL)MDBK1	5/17/07	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	RLB-106	ATCC VR793	ATCC (CAHFS)	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	A033640072	CAHFS, R. Mock, #5 Swab, 1BDFK1, 1/28/2004, A030020072	unknown	1BDFK1,	4/23/04	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	A040130066	CAHFS, R. Mock, #4 Lung,	unknown	1BFK1	4/23/04	Phenol/ Chloroform	40 pg/uL	N/A

			1BFK1, 1/28/2004, A040130066						
Bovine Herpes Virus-1	IBR	Texas A030020072	CAHFS, R. Mock, #80 Swab, 1BDFK1, 1/28/2004, A030020072	unknown	1BDFK1	4/23/2004	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	California	NVSL 200032	NVSL, CAHFS	unknown	MDBK CAHFS	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Virginia	NVSL 231221	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	California	NVSL 51619	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Minnesota	NVSL 86741	NVSL, CAHFS	unknown	MDBK CAHFS	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Minnesota	NVSL 97-10720	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Cooper	NVSL-Cooper RA 309	NVSL, CAHFS	unknown	MDBK CAHFS	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes-5	DN-599	A032540006	R. Mock, from lung tissue, 2BFK2, 2/3/2004	Unknown	CAHFS Lab 2BFK2 4/30/2004	6/3/2004	Phenol/Chloroform	40 pg/uL	N/A
Bovine Herpes-5	DN-599	A040150085	R. Mock, #2 Lung, 5BFK5, 2/3/04	Unknown	CAHFS Lab 5BFK5 4/23/2004	7/7/2004	Phenol/Chloroform	40 pg/uL	N/A
Bovine Herpes-5	D9402133	D9402133	Sonoma Co. 3/94 Severe necrotizing encephalitis, 1 week old calf	Unknown	CAHFS Lab (MDBK), (1 flasks) 11/19/2003	11/19/2003	Phenol/Chloroform	40 pg/uL	N/A
Bovine Herpes Virus	BHV 5	D9403153	CAHFS	unknown	unknown	unknown	unknown	40 pg/uL	unknown
Caprine Herpes Virus		VR462	ATCC	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Caprine Herpes Virus	D0201157	D0201157	CAHFS, Isolate D0201157 History: San Joaquin Co. 2/02 Aborted fetus-third trimester	unknown	CAHFS Lab (MDBK)	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Caprine Herpes Virus	S0201998	S0201998	CAHFS, Isolate S0201998 History: San Diego Co. 2/02 Aborted fetus-third trimester	unknown	CAHFS Lab (MDBK)	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-1	Santa Barbara	N/A	Bridget McLaughlin CAHFS	unknown	unknown	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-1	Georgia	N/A	Bridget McLaughlin CAHFS	unknown	unknown	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-2	Alberta	041 ODV 0401	NVSL	unknown	BHK	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-1 (SV123)	New Jersey (3TD) Deer, NJ 1955	041 ODV 0401	NVSL	1 x $10^{5.125}$ $TCID_{50}$/0.1 ml	+BHK-3 (12/7/83); (12/11/86); (12/18/86)	Unknown	Trizol	40 pg/uL	N/A

Equine Herpes 1	Equine Herpes 1	A011120004	CAHFS, R. Mock, from brain tissue, 2BFK2, 2/3/2004, A011120004	unknown	CAHFS, 2BFK2	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A99043047	CAHFS, R. Mock, Spleen, 1BFK1, 2/3/2004, A99043047	unknown	CAHFS, 1BFK1	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A9904309	CAHFS	unknown	CAHFS, unknown	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Bovine 1247	Bovine 1247 ATCC VR2003	ATCC	unknown	CAHFS, unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	NVSL 002	NVSL 002 1/28/04	NVSL	unknown	CAHFS, unknown	1/29/03	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	LK	LK ATCC VR701	ATCC	unknown	CAHFS, unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	D990	D990	CAHFS	unknown	CAHFS, unknown	1/29/03	Phenol/ Chloroform	40 pg/uL	N/A
Feline Herpes	C-27	ATCC VR636	ATCC	unknown	CAHFS, unknown	unknown	unknown	40 pg/uL	N/A
Fowl Pox	Vaccine strain	Poxine™	Jeffers Livestock	unknown	ECE	5/17/07	Phenol/ Chloroform	40 pg/uL	N/A
Parainfluenza Virus type 3	C-243	TC-81335	LLNL	1×10^5 TCID50/0.1 mL	(VL)MK14(LLNL)H292-1	5/17/07	Trizol	40 pg/uL	Reed and Muench
Porcine Coronavirus	AR310	ATCC VR-2384	LLNL	$1 \times 10^{4.25}$ $TCID_{50}$/0.1 ml	ST2				Reed and Muench
Porcine Herpes Pseudorabies	Shope	RA 180	CAHFS	1×10^5 TCID50/mL		5/17/07	Trizol	40 pg/uL	N/A
Pseudorabies		92-12013	NVSL 92-12013 bovine isolate Iowa December, 1991	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies		93-11745	NVSL 93-11745 Illinois December, 1992	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies	Shope	RA180	NVSL Shope (PRV) RA 180	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies Titered	Shope	N/A	NVSL	unknown		unknown	Phenol/ Chloroform		Reed & Muench
Respiratory Syncytial virus	Long	N/A		unknown	(VL)KB6L2KB2HFDL2A549-1(LLNL)HeLa3	5/17/07	Trizol	40 pg/uL	N/A
Transmissible Gastroenteritis virus of hogs	Perdue	NVSL 9801	LLNL	$1 \times 10^{4.75}$ $TCID_{50}$/0.1 ml	ST 1		Trizol	40 pg/ul	Reed and Muench

TABLE 52. Panel membership for assay. The 4 BTV signatures (3 new signatures plus 1 signature from Version 1.0 panel) were included in the **Bovine-specific multiplexed assay**

panel for FMDV rule-out diagnostics. The below table describes the constituents of that multiplexed assay panel in which these signatures were tested.

Bead ID	Signature/ Target Name	Mux Group ID	Virus	Source	Forward Primer	Reverse Primer	Luminex Probe (-strand, FCP)
113	emcf_94975.F, emcf_94976.R, emcf_94977.P	MCF_1	Malignant Catarrhal Fever	LLNL	ATGCCAGTCACTGGCTCTCA	GGGTGTTGTAGAATCCTGAAATGG	GTTGATCACGGTGGCACCCTGG
114	emcf_95059.F, emcf_95060.R, emcf_95061.P	MCF_2	Malignant Catarrhal Fever	LLNL	GTTCTGGAAACTGACCAAACAGTGT	AGTGGCACTTGAGTGTAACTTTTATTG	GCACTCTGGCAGGCATAAGGGAAATACA
115	emcf_95155.F, emcf_95156R, emcf_95157.P	MCF_3	Malignant Catarrhal Fever	LLNL	CCCTGGAAGCTGTCATACAAAA	AAACATTGGCATATCTTGCAAGGT	CAGTAGAGTCCAGGGCTGCAGTTGTCTCA
117	BVH__94666.F, BVH_94667.R, BVH_94668.P	BHV-94668	Bovine Herpes Virus	LLNL	GTGCCAGCCGCGTAAAAG	GACGACTCCGGGCTCTTTT	TCCTGGTTCCAGAGCGCTAACATGGAG
118	BVH__94738.F, BVH_94739.R, BVH94740.P	BHV-94740	Bovine Herpes Virus	LLNL	TGAGGCCTATGTATGGGCAGTT	GCGCGCCAAACATAAGTAAA	AAATAACACGGTGTGCACTTAAATAAGATTCGCG
119	BPSV_95719.F, BPSV_95720.R, BPSV_95721.P	PPOX_1	Bovine Papular Stomatitis Virus	LLNL	GCAGATGCGCTCCTGGTT	GCACCTCTGCTGCTGCAA	CCGACTCCGACGTGGAGAACGTG
120	BPSV_95722.F, BPSV_95723.R, BPSV_95724.P	PPOX_2	Bovine Papular Stomatitis Virus	LLNL	GATGGCCGTGCAGCTCTT	CGTACAAGATCACGGCCAACT	TGTACGGGCTCATGGGCTTCCG
122	BPSV_95731.F, BPSV_95732.R, BPSV_95733.P	PPOX_4	Bovine Papular Stomatitis Virus	LLNL	GCAGCAGTGCACCACGTAGT	CGCTGAACCCGTACATCCT	GACTTCGAGGCGGACAACAAGCG
132	FMDV.TC	FMDV.TC	Foot and Mouth Virus	Tetracore	ACTGGGTTTTACAAACCTGTGA	GCGAGTCCTGCCACGGA	GTCCCACGGCGTGCAAAGGA
133	FMDV.Pir	FMDV.Pir	Foot and Mouth Virus	Pirbright	CACYTYAAGRTGACAYTGRTACTGGTAC	CAGATYCCRAGTGWCICITGTTA	CCTCGGGGTACCTGAAGGGCATCC
134	BVD_1a	BVD_SIG1	Bovine Viral Diarrhea	UCD, CAHFS lab	GGTAGTCGTCAGTGGTTCGAC	CATGTGCCATGTACAGCAGAGAT	CCTCGTCCACGTGGCATCTCGAG
138	BVD_1821165.F, BVD_1821164.R, BVD_1821166.P	BVD_SIG2	Bovine Viral Diarrhea	LLNL	GGGAGTCGTCAATGGTTCGAC	TCCATGTGCCATGTACAGCAGAG	CCTCGTCCACITGGCATCTCGAG
135	BTV_1759932	BTV_1759932	Bluetongue Virus	LLNL	GCACCCTATATGTTTCCAGACCA	CAGCTAACTCTTCAGCCACACG	CTAACTCGTGGGCCAATCATCATCTTCTGT
136	BTV10_1810207	BTV10_1810207	Bluetongue Virus	LLNL	CAAACACAAAAGGCGGAGAAG	GGCGTTTAATCTGTCTTAGTCTTACGT	GAAACGCTTCTGCGTACGATGCGA
137	BTV10_1810199	BTV10_1810199	Bluetongue Virus	LLNL	CACATGTCGCTTAATTTGTCTTAACC	GCGGAGAAGGCTGCATT	ACGAAACGCTTCCGCGTACGATG
139	BTV10_1810205	BTV10_1810205	Bluetongue Virus	LLNL	TCAATTTTGGTAGAATTTGTTCATTCA	GCGGAGAAGGCTGCATTC	ACGAAACGCTTCCGCGTACGATG
163	VSV_1798943	VSV_1798943	Vesicular Stomatitis Virus	LLNL	CGCCACAAGGCAGAGATGT	TGTCAAATTCTGACTTAGCATACTTGC	GCATACTGCATCATATCAGGAGTCGGTTTTCTG
166	VSV_1798947	VSV_1798947	Vesicular Stomatitis Virus	LLNL	CCCAATCAATGCCATGATACA	CTCCAATGGAAGGGTCCAAA	TTTGAAAGTAGAACTGTGCAAGCCCGGTATC
167	VSV_1798949	VSV_1798949	Vesicular Stomatitis Virus	LLNL	GGCGCTCATTATAAAATTCGGA	ACATTTTCTCGTAGTAATGCAGCAG	GAAGTCCCTGTAATGGATTCCCATTCCATGT
169	VSV_1811408	VSV_1811408	Vesicular Stomatitis Virus	PIADC	CTCACAACATGGGTCCTGAA	TTCTTGACCTGGATACATCAT	GGCATAGYTCGTCTGCRACTTCCCT
160	RPV_1814853	RPV_1814853	Rinderpest Virus	LLNL	GGATCGCTGAAATGATCTGTGA	GGAGCCAGTTCACCCATTTG	CTGGCCAACCCTGCCTCCACTATGTA
161	RPV_1814855	RPV_1814855	Rinderpest Virus	LLNL	TGCATCTTATGTGACTTTGGTTCA	GGCTATCCGCACAGCTGAC	CAGTCCTCTCATCTGTTGTCGATCCGATGTA
162	RPV_1814856	RPV_1814856	Rinderpest Virus	LLNL	AACTCCTGACCTCATTCCTTGC	GGCTCTATAATCCCACTATGCCA	TGGCTCAGTGCATTCACAAAGACCTTGAATA
170	N/A	NC (MT-7)	Maritima	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
171	N/A	b-MT7 (FC)	N/A	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
172	N/A	MT7/Cy3 (IC)	N/A	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
173	Alien RNA	arRNA Control	None	Ambion	GACATCAAGGCTCAAACTAATTTTACC	CAAAGGCTGCCAACATAAAATG	CAAGCGTAAATGCAGCGTCCA

[1]FCP indicates that the probe is on the negative strand (Forward Compliment to the Probe).

1.8.1. BOVINE PANEL 2006 MULTIPLEX PCR DATA

Preliminary screening: Preliminary screening can be broken down into several steps. The signatures are preliminarily screened by first assessing how the signatures will perform under

multiplex PCR conditions and whether or not the addition of the new signatures will adversely effect other signatures, these preliminary screening methods are described as “signature baseline screening” and “multiplex addition screening” respectively. First signature baseline screening tests the performance of the signature in a no-target reaction (blank), a “passing” score is indicated by a low (typically less than 100) MFI. All BTV signatures passed the signature baseline screening. Next in multiplex addition screening the new signature primers are added one-by –one to the other panel signatures to determine if there would be any cross-reactions between primer pairs in the panel. Further assessment of the signatures is done through target and near-neighbor screening and the determination of relative sensitivities. The signature down-selection for the BTV signatures is further described in Table 26 below.

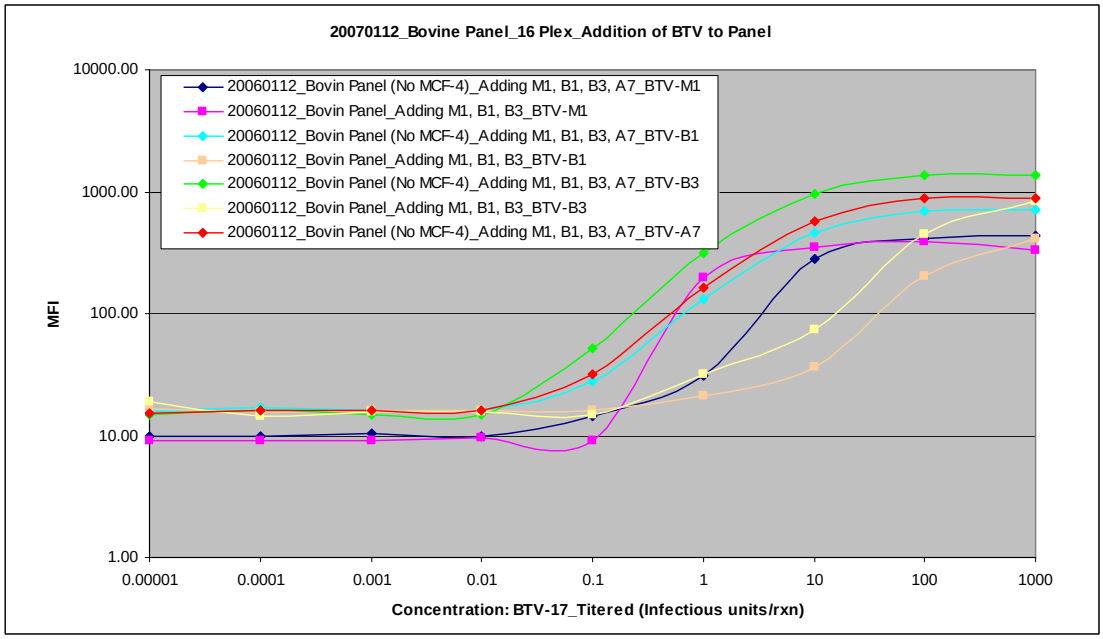

FIG. 2. The MCF-4 probe was noted for cross-reacting with BTV10_1810199 primers. In the absence of the MCF-4 signature, the BTV signatures markedly improve as seen in this plot. When BTV-1810199 (A7) is present in the mix the relative sensitivity of the assay seems to improve for BTV-1810205 (B1) and BTV-1810207 (B3) and does not have much affect on BTV-1759932 (M1). Based on this it was decided to be more optimal to have an additional BTV signature in the mix rather than the MCF-4 signature especially if it improves the other BTV signatures.

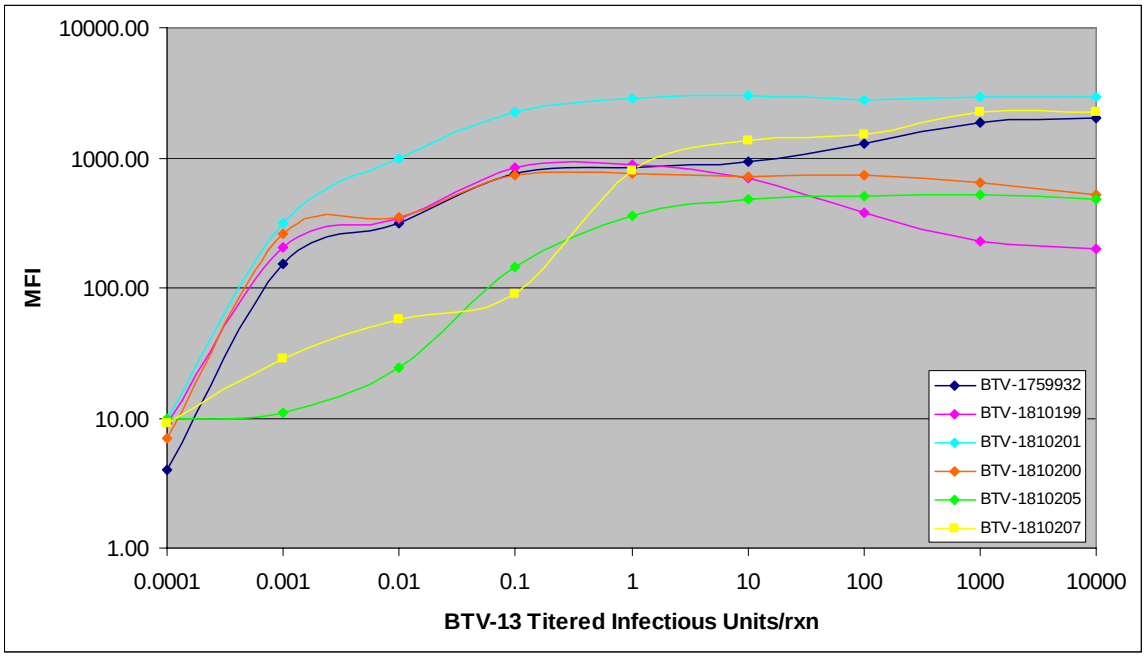

FIG. 3. BTV singleplex screening data for 6 candidate BTV signatures against extracted nucleic acids from isolate BTV-13 (LLNL,NVSL). Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. . Each point represents the mean response (n=2).

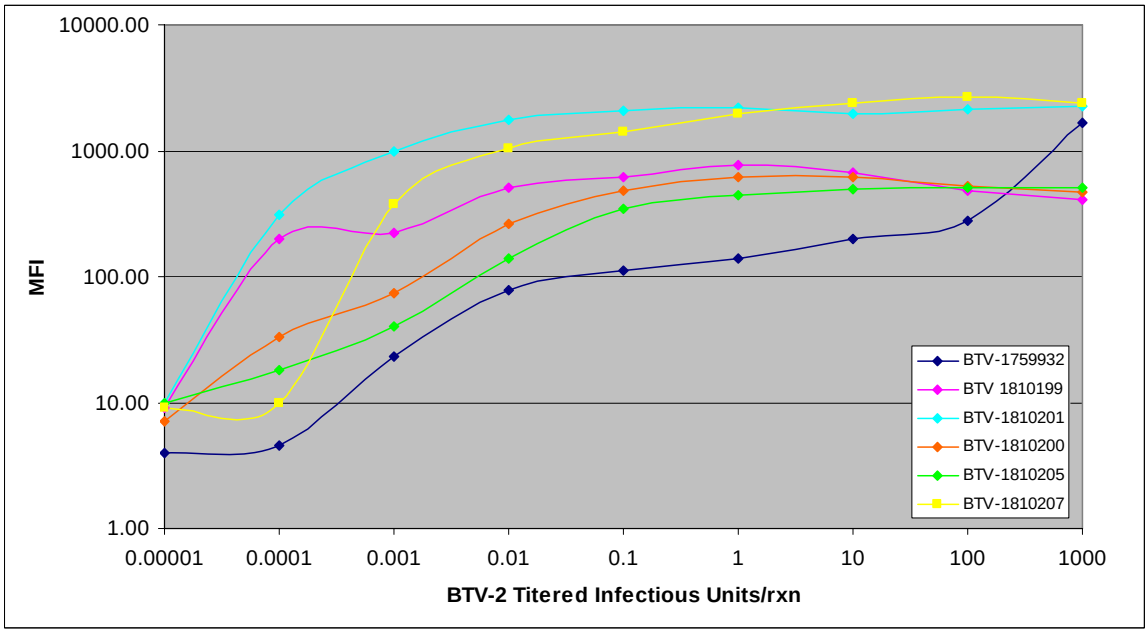

FIG. 4. BTV singleplex screening data for 6 candidate BTV signatures against extracted nucleic acids from isolate BTV-2 (LLNL, NVSL). Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2).

TABLE 53. Multiplexed assay down-selection summary. Signature baseline screening indicates the performance of the signature in a no-sample reaction (blank), a "passing" score is indicated by a low (typically less than 100) MFI. All signatures passed the baseline screening. In the multiplex addition screening the primers are added one-by –one to the other panel constituents to determine if there would be any cross-reactions between primer pairs in the panel. Signatures BTV_1759933, BTV10_1810200 and BTV10_1810201 failed screening due to an observed cross-reaction with other signatures when added to the multiplexed panel. As a result no further screening was conducted with those signatures.

Signature	Mux Screening: Assay Down Selection					
	Signature baseline screening	**Multiplex addition screening**	**Target screening**	**Near-neighbor screening**	**Limit of detection screening**	**Noted cross-reactions**
BTV_1759932 (2)	Pass	Pass	Pass	Pass	Pass	None
BTV_1759933 (3)	Pass	Fail (4-30-07): Crossreaction with PPOX-2 verified. Also it is overall the worst narrow BTV signature in Mux and TaqMan	No further testing	No further testing	No further testing	BTV_1759933 primers crossreact with PPOX-2 probe
BTV10_1810199 (A7)	Pass	Pass	Pass	Pass	Pass	None
BTV10_1810200 (A8)	Pass	Fail (12-19-06): High backgrounds in multiplex	No further testing	No further testing	No further testing	None
BTV10_1810201 (A9)	Pass	Fail (12-29-06): Cross-reacts with BVD-1a signature	No further testing	No further testing	No further testing	BTV10_1810201 primers cross-react with BVD-1a probe
BTV10_1810205 (B1)	Pass	Pass	Pass	Pass	Pass	None
BTV10_1810207 (B3)	Pass	Pass	Pass	Pass	Pass	None

Near-neighbor and Target screening: All four signatures were added to the Bovine panel. All four signatures exhibited reasonably low background response (<10 MFI, except BTV1759932 with signals 10-60MFI) in the Bovine panel.

TABLE 54. 2006 Backgrounds screening in **multiplexed** format for MCF at LLNL and PIADC.

	Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors	Targets
Mux PCR Screening	54	135	16	0	43	5

[1]-There are 752 pooled samples in each Aerosol Block.

TABLE 55. Backgrounds screening in **multiplexed** format for all BTV signatures against the below listed **eukaryotes**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. None of the signatures cross-reacted with any of the samples listed below.

Description	BTV-9932 (35)	BTV-0207 (36)	BTV-0199 (37)	BTV-0205 (39)
CHICKEN	21	6	5	7
TICK	24	4	3	5
DOG	26	4	4	7
MOUSE	29	5	5	7
RABBIT	30	6	5	7
RAT	31	3	4	6
HUMAN	32	3	3	5
PIG / PORCINE	33	6	5	7
FLEA	33	4	4	6
EQUINE	35	4	4	6
DROSOPHILA MELANOGASTER	36	5	5	6
MOSQUITO	39	6	4	7
MONKEY	51	6	5	8
CAT	54	5	5	7
SHEEP	57	4	3	5
BOVINE	62	6	5	7

TABLE 56. Backgrounds screening in **multiplexed** format for all BTV signatures against the below listed **prokaryotes**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. None of the signatures cross-reacted with any of the samples listed below.

Description	BTV-9932 (35)	BTV-0207 (36)	BTV-0199 (37)	BTV-0205 (39)
Erwinia amylovora	28	6	5	8
Actinobacillus suis	43	6	6	8
Aneurinbacillus migulanus	37	6	5	8
Bacillus cereus	40	6	5	8
Bacillus globigii	36	6	6	8
Bacillus subtilis	39	4	4	6

Bacillus thuringiensis	41	7	6	8
Bifidobacterium denticum	34	7	6	8
Borrellia burgdorferi	43	6	6	8
Burkholderia acacia	19	6	6	8
Caulobacter vibriodes	14	5	5	7
Clavibacter michganensis	19	6	5	7
Clostridium butyricum	28	6	6	7
Corynebacterium pseudodipthericum	26	7	6	9
Cytophaga marinoflava	30	6	5	7
Erwinia herbicola	49	7	6	8
Escherichia coli	60	7	7	8
Geobacillus caldoxylosilyticus	37	5	5	7
Halomonas homophile	24	5	5	7
Haemophilus influenza	31	6	5	8
Herbaspirillium seropedicae	29	7	6	9
Lactobacillus garvieae	34	5	5	7
Lactobacillus gasseri	34	6	5	7
Listeria monocytogenes	38	5	5	7
Listeria seeligeri	35	6	5	7
Micrococcus luteus	29	6	5	8
Moraxella lacunatica	23	7	6	8
Oceanospirillium ssp. Maris	25	7	7	8
Paenibacillus napthalaenovorans	28	6	5	8
Paracoccus dentifrices	30	7	6	8
Porphyrobacter sanguineus	29	7	6	8
Proteus mirabillis	19	6	5	7
Pseudomonas aeruginosa	30	6	6	7
Pseudomonas oleovorans	22	6	5	8
Rhizobium leguminosarum	34	7	6	9
Rhodococcus rhodochrous	19	5	5	7
Salmonella typhimurium	34	6	6	7
Simonsiella muelleri	37	7	5	7
Sphingomonas sp. (Alcaligenes sp)	19	7	6	8
Staphylococcus aureus	33	5	5	7
Streptococcus pneumoniae	34	6	6	8
Streptomyces scabiei	32	6	5	7
Tatlockia maceachernii	19	7	7	9
Vibrio paraheamolyticus	18	6	6	8
Xanthomonas translucens	35	6	6	8

TABLE 57. Backgrounds screening in **multiplexed** format for all MCF signatures against the below listed **soils**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. None of the signatures cross-reacted with any of the samples listed below.

Description	BTV-9932 (35)	BTV-0207 (36)	BTV-0199 (37)	BTV-0205 (39)
D 000107-49	15	8	7	9
D 000109 # 50	18	8	6	8
D 000402 # 53	29	8	7	9
D 000500 - 26 - 1	40	8	7	9

D 000501-14-1	16	8	8	10
D 000505 - 11 - 4	32	8	7	9
D 000521 - 23	26	8	7	10
D 000527 - 3	24	9	8	10
D 000531 - 21	34	7	8	10
D 000533 - 17 -1	24	7	7	9
D 000542 - 6	20	8	8	10
D 000550 - 20	33	9	7	10
D 000551 - 5	21	8	8	10
D 000561 - 8 - 6	15	8	7	9
D 000562 - 30 - 5	22	9	8	10
S 251	14	7	7	10
S 252	40	8	6	9
S 253	28	7	6	8
S 254	20	8	7	10
S 255	19	8	6	9
S 256	15	8	7	10
S 257	11	7	6	9
S 259	20	7	6	9
S 260	9	8	7	8
S 271	11	6	6	9
S 272	35	8	7	9
S 273	25	9	8	9
S 274	28	9	7	9
S 275	12	8	7	10
S 276	17	7	7	10
S 277	20	9	6	10
S 279	11	7	6	8
S 280	15	8	7	9
S 282	14	6	6	7
S 283	17	8	7	9
S 284	17	8	7	10
S 286	14	7	7	9
S 287	6	4	4	6
S 288	13	7	6	8
S 289	16	8	6	9
S 290	27	7	6	8
S 291	13	6	6	8
S 292	17	13	11	11
S 295	10	8	6	9
S 296	14	8	7	9
S 297	14	7	7	9
S 298	9	7	6	8
S 299	13	7	6	8
S 300	22	7	5	8
S 301	11	7	6	8
S 303	9	6	6	8

S 304	10	6	5	8
S 305	11	7	6	8
S 307	8	7	6	8

TABLE 58. Bovine Panel **Near-Neighbor multiplex** Screening (Data from 20070601) against BTV signatures. Values shown are Median Flourescence Intensity (MFI) units. "Blanks" are buffer-only samples (no nucleic acid added to reaction), shown below in red. Extracted nucleic acid was added to each PCR reaction in triplicate totaling 200pg/rxn. The data shows that none of the BTV signatures cross- reacted with any of the near-neighbors listed below from the Bovine panel.

Description	**BTV-9932 (35)**			**BTV-0207 (36)**			**BTV-0199 (37)**			**BTV-0205 (39)**		
replicate	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Blank	69	26	84	8	8	7	9	8	7	11	11	9
Blank	45	60	51	8	8	6	8	7	7	11	10	10
BHV A040150085	88	56	87	9	6	8	8	5	8	11	9	10
BHV (BFK)	123	83	74	9	9	7	8	8	7	12	11	10
BHV-1 A040130066	100	39	72	8	7	6	9	7	8	11	9	11
BHV-1 A033640072	116	59	33	8	6	7	9	7	6	11	9	9
BHV-1 ATCC VR 793	102	43	69	9	9	6	7	8	6	11	10	9
IBR CA 111903	99	20	56	9	2	9	8	3	9	11	4	11
IBR MN 111903	25	62	52	7	8	7	7	8	8	10	11	11
BHV-1 NVSL 231221	76	49	108	6	9	8	7	8	8	10	11	9
BHV-1 RA309	79	74	64	8	9	6	8	8	7	11	11	9
BHV-1 NVSL 97-10720	98	40	61	8	7	7	8	7	8	10	9	12
BHV-1 NVSL 51619	73	56	72	8	9	7	8	8	6	11	10	9
BHV-1 NVSL 86741	60	49	47	7	7	7	7	8	8	9	10	10
BHV-1 NVSL 200032	78	49	70	8	9	8	9	8	9	11	10	11
BHV-1 LA ATCC VR188	84	85	67	8	7	6	9	8	7	11	11	10
BHV-1 (IBR) Texas CAHFS A030020072	65	52	58	8	9	7	8	8	7	11	12	10
EHV-1 ATCC VR2003	59	38	78	9	9	7	9	8	7	11	12	10
EHV-1 A9904309	111	62	96	8	7	7	8	9	7	11	2	10
EHV-1 A011120004 CAHFS	48	32	41	9	7	7	8	7	7	12	10	9
EHV-1 NVSL 00002	86	59	40	8	7	6	8	7	7	10	10	9
EHV-2 ATCC VR701	72	77	66	9	8	7	8	7	7	10	10	10
EHV-1 A99043047 CAHFS	112	59	42	10	8	7	8	8	6	11	12	10
EHV-2 D990 CAHFS	103	57	95	7	8	7	8	7	7	12	11	10
Pseudorabies Titered	99	38	54	8	8	7	8	7	8	12	10	11
Pseudorabies NVSL 93-11745	59	37	75	9	7	6	9	7	7	11	11	10
Pseudorabies NVSL 92-12013	80	34	46	8	6	8	7	7	7	10	9	10
Pseudorabies RA180 CAHFS	107	34	84	8	7	6	7	7	7	10	10	9

Porcine Herpes Pseudorabies Shope	68	60	57	8	9	7	8	8	7	12	11	10
Feline Herpes ATCC VR636	63	59	31	8	9	7	8	8	7	11	11	9
Caprine Herpes ATCC VR462	68	43	70	9	9	7	9	8	7	11	10	9
Caprine Herpes S0201998 CAHFS	50	77	70	9	10	6	9	8	7	11	11	9
Caprine Herpes D0201157 CAHFS	55	53	47	9	7	7	9	8	7	12	9	10
BHV-5 A040150085 CAHFS	113	79	34	9	8	7	9	9	8	11	12	10
BHV-5 A032540006 CAHFS	49	64	90	11	7	6	11	7	7	11	9	10
BHV-5 D9403153 CAHFS	60	64	48	8	8	7	9	8	8	12	9	9
BHV-5 D9402133 CAHFS	30	56	39	6	9	7	6	7	7	8	10	10
BDV Coos Bay	45	31	43	8	7	7	8	7	7	12	10	9
EHD-1 Georgia	85	57	54	10	5	7	9	6	7	14	7	9
EHD-1 New Jersey	60	59	47	9	7	7	8	7	7	13	10	10
EHD-1 Santa Barbara	98	25	59	8	5	6	9	6	7	11	8	10
EHD-2 Alberta	29	76	52	9	8	7	9	8	7	11	11	10
Fowl Pox	106	100	75	8	8	6	8	8	7	11	11	10
Parainfluenza Type 3	51	103	48	7	7	6	9	7	6	10	11	9
Respiratory Syncytial	45	85	63	8	7	6	9	6	7	12	8	10

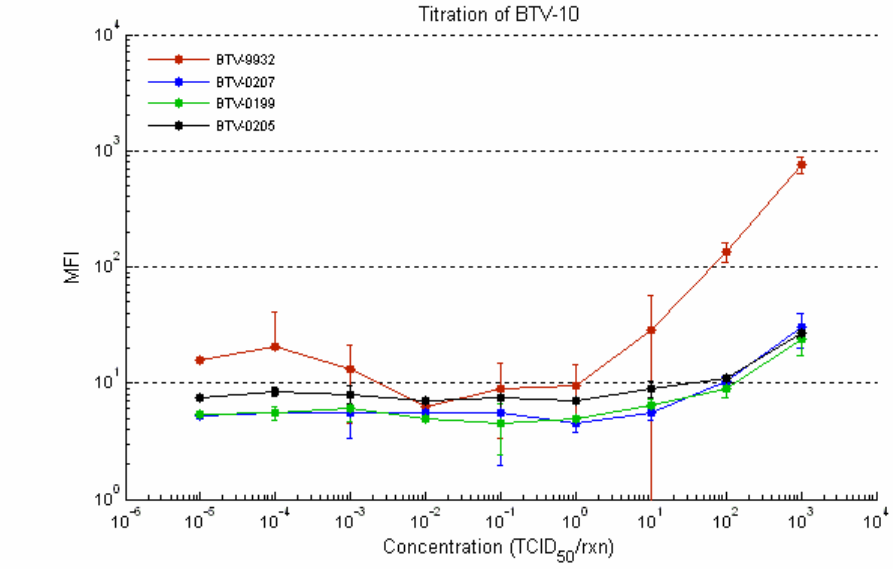

FIG. 5. Multiplex screening data for the four BTV signatures against extracted nucleic acids from isolate BTV-10 (LLNL, NVSL). Serial dilution of nucleic acid Trizol extracted from virus-

infected cell culture. Each point represents the mean response (n=2). Error bars indicate ±1 of the mean.

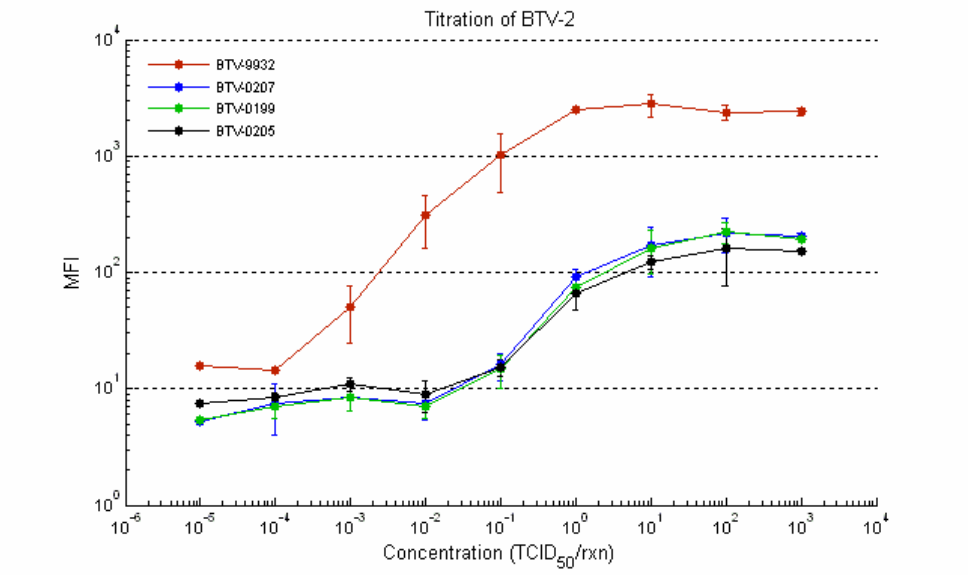

FIG. 6. Multiplex screening data for the four BTV signatures against extracted nucleic acids from isolate BTV-2 (LLNL, NVSL). Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2). Error bars indicate ±1 of the mean.

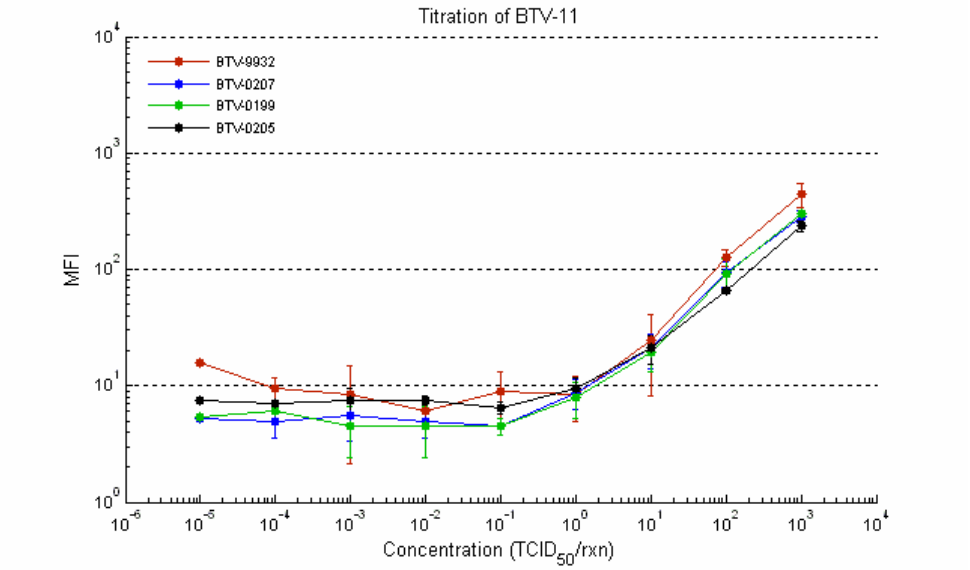

FIG. 7. Multiplex screening data for the four BTV signatures against extracted nucleic acids from isolate BTV-11 (LLNL, NVSL). Serial dilution of nucleic acid Trizol extracted from virus-

infected cell culture. Each point represents the mean response (n=2). Error bars indicate ±1 of the mean.

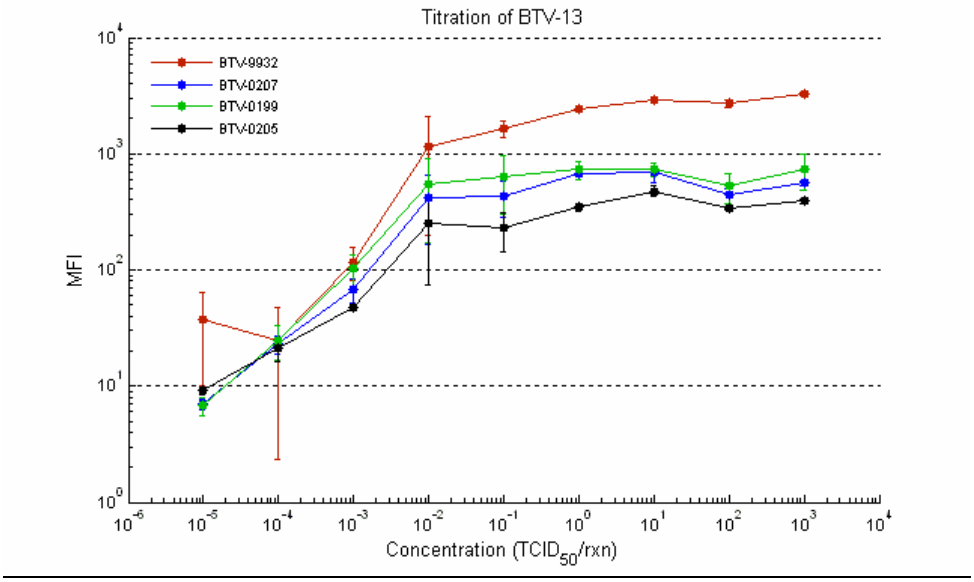

FIG. 8. Multiplex screening data for the four BTV signatures against extracted nucleic acids from isolate BTV-13 (LLNL, NVSL). Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2). Error bars indicate ±1 of the mean.

RESULTS: In 2005 we generated BTV signatures to detect all domestic serotypes of BTV which were screened in real-time and multiplexed PCR Two signatures were determined suitable for the Version 1.0 panel. In 2006 we designed additional signatures to detect the entire global serotype diversity of BTV. Using unpublished segment 5 and 10 sequence information from a collaborator combined with Genbank sequence information, we used our computational signature generation system to identify the regions of BTV that are not present in any other sequenced microbial organism in Genbank nor our internal sequence database. We mined the available genome(s) for triplet oligonucleotides that met our universal parameters set for Real-time RT- PCR. The bioinformatics group was able to generate 27 candidate signatures all of which were screened in Real-time RT-PCR testing and of these 27 signatures, only 5 of these passed in their abilities to detect a broad diversity of BTV serotypes in a real-time test. These 5 signatures were screened in the Bovine panel, and of these five, three were found useful. These three signatures plus one from 2005 have been currently added the Bovine panel and tested against domestic BTV serotypes, all 4 signatures have been determined to perform well with various levels of sensitivity. Based on this preliminary data against domestic serotypes, all four signatures would be effective candidate signatures in screening for detection of bluetongue virus.

2. BOVINE HERPES VIRUS-1 (BOVINE PANEL) 2005

OBJECTIVE: In 2005 we were tasked by the Department of Homeland Security to develop specific and reliable Real-time RT- PCR signatures for Bovine Herpes Virus 1, among other major agriculturally-impacting viruses that are clinically indistinguishable from foot and mouth disease virus [FMDV]. In 2006 the goal of these assays (collectively with other virus-specific signatures developed in parallel) is to develop a species specific (i.e. bovine) multiplexed detection assay for Foot-and-Mouth disease rule-out for use in veterinary diagnostic laboratories. The two signatures that were developed and incorporated into the Version 1.0 FMDV Rule-out panel have been re-evaluated for use in a bovine-specific FMDV rule-out panel. This document describes the historic data and information as well as the new 2006 data screening the 2 BHV signatures for inclusion into the Bovine panel.

2.1. BACKGROUND AND ETIOLOGY OF BHV

Bovine herpesvirus 1 (BHV-1) is associated with several diseases in cattle: infectious bovine rhinotracheitis (IBR), infectious pustular vulvovaginitis (IPV), balanoposthitis, conjunctivitis, abortion, encephalomyelitis, and mastitis. Only a single serotype of BHV-1 is recognized; however, three subtypes of BHV-1 have been described on the basis of endonuclease cleavage patterns of viral DNA—BHV-1.1 (respiratory subtype), BHV-1.2 (genital subtype), and BHV-1.3 (encephalitic subtype). BHV-1.3 has been reclassified as a distinct herpesvirus designated BHV-5.

BHV-1 infections are widespread in the cattle population. In feedlot cattle, the respiratory form is most common. The viral infection alone is not life-threatening but predisposes to secondary bacterial pneumonia, which may result in death. In breeding cattle, abortion or genital infections are more common. Genital infections can occur in bulls (infectious pustular balanoposthitis) and cows (IPV) within 1-3 days of mating or close contact with an infected animal. Transmission can occur in the absence of visible lesions and through artificial insemination with semen from sub clinically infected bulls. Cattle with latent BHV-1 infections generally show no clinical signs when the virus is reactivated, but they serve as a source of infection for other susceptible animals[2].

2.2. BHV-1 SIGNATURE COMPREHENSIVE ASSAY SUMMARY

Bioinformatics

Virus name: Bovine Herpes Virus 1
Signature generation reference: Single whole BHV-1 genome (gi 9629818/ref/NC_001847.1

Level of discrimination: *Species; predicted targets are all BHV Subtype 1*

[2] *Source: The Merck Veterinary Manual*
http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/121212.htm&word=IBR)

Number of Initial Signatures: 177
Number of Signatures forwarded to real-time bench-screening: 101
Total number of Genome Sequences used for alignment: 1

Real-time PCR Screening Summary

TABLE 59. Final signatures down-selected in real-time RT-PCR screening (4).

LLNL Signature Designation	Sequence
BVH__94666.F	GTGCCAGCCGCGTAAAAG
BVH__94667.R	GACGACTCCGGGCTCTTTT
BVH__94668.P	CTCCATGTTAGCGCTCTGGAACCAGGA
BVH__94678.F	GTCCCCACGGGCGTAGTA
BVH__94679.R	CCCAACCGAGACGGAAAG
BVH__94680.P	CACCGGGCGTGCTGTCCTCTG
BVH__94738.F	TGAGGCCTATGTATGGGCAGTT
BVH__94739.R	GCGCGCCAAACATAAGTAAA
BVH__94740.P	CGCGAATCTTATTTAAGTGCACACCGTGTTATTT
BVH__94855.F	CGTCCCGATTTCTCCGAAT
BVH__94856.R	TTCGTATAGGGAGTACGCGATGA
BVH__94857.P	CGCGACGAGAACGTCCACATCGT

TABLE 60. Summary of wet-bench screening in signature down-selection for gel and real-time PCR screening. Gel screening consists of a smaller subset of screening than is done in real-time screening. Cross reactions were seen in gel screening against backgrounds (soils, microbes, aerosols) but none against near neighbors. No cross reactions were observed in Real-time PCR screening.

	Soils	Eukaryotes	Prokaryotes	Aerosols[1]	Near Neighbors	Target
Gel Screening	3	4	0	0	5	5
Real-time PCR Screening	15	16	13	2 Aerosol Blocks	19	10

[1]There are 752 pooled samples in each Aerosol Block.

Multiplexed PCR Screening Summary

TABLE 61. Backgrounds screening in multiplexed format for BHV at LLNL and PIADC.

	Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors[2]	Targets
Mux PCR Screening	54	135	16	0	4	15

[1-]There are 752 pooled samples in each Aerosol Block. [2]Near-neighbors indicated (43) apply to the near-neighbor panel that is not uniquely specific for BHV, but for the other panel constituents that were screened concurrently.

TABLE 62. Signature summary for BHV multiplexed signatures in the bovine panel.

LLNL Signature Designation	MUX Group ID	Gene ID	Relative Limit of detection[1]	Targets Screened	Near Neighbors Screened
BHV_94668	BHV-1	UL43/1487394	$1x10^{-1}$ - $5x10^{3}$ $TCID_{50}$/rxn	3	4

BHV_94738	BHV-3	UL43/1487382	$5x10^{-2}$ - $1x10^{2}$ $TCID_{50}$/rxn	3	4

[1] The relative "Limit of detection" is described as a range of lowest and highest sensitivity determined by multiple target strains screened and is described further in the Multiplex results summary report section.

2.3. BHV-1 SIGNATURE BIOINFORMATICS AND GENERATION INFORMATION

Bioinformatics process:
The LLNL Bioinformatics team have developed a computational process that uses available DNA sequence information for a given target organism to generate candidate DNA signature sequences that are conserved and unique. Signatures are primarily used to underlie reliable and specific pathogen detection assays. Evidence of the success of the process is that several of the Real-time PCR signatures that are used in the national BioWatch monitoring system were generated by this process, and to date, has resulted in no false positives from a total of over 3 million assays performed.

The general approach is the following: All available complete genomes of different strains of the target species are compared using a multiple genome alignment programs. A *consensus gestalt* is formed from the alignments that contain the sequence conserved amongst all target inputs. (This step is bypassed if only one target sequence is available).

To determine that organism-conserved sequence which does not occur in any other sequenced microbial organism, the consensus gestalt is compared against our immense, continuously updated database of microbial organism. A customized algorithm accomplishes this electronic subtraction, and the result is a *uniqueness gestalt* that is mined for potential signature candidates. A final computational screening is done to verify that cross-reactions are not detected.

Target Virus Information:
Virus name: Bovine Herpes Virus 1.
Type: dsDNA virus.
Genome size: 135301 bp.

Primer/Probe Set Generation Information
Alignment and All Microbe Database subtraction.

TABLE 63. List of main parameters and settings used in generating candidate signatures using Primer 3 signature generation tool.

Primer3 Parameters	
Parameters	**Standard Settings**
PRIMER_OPT_SIZE	**20**
PRIMER_MIN_SIZE	**18**
PRIMER_MAX_SIZE	**27**
PRIMER_PRODUCT_OPT_SIZE	**100**
PRIMER_PRODUCT_SIZE_RANGE	**71-600**
PRIMER_OPT_TM	**62**

PRIMER_MIN_TM	**61**
PRIMER_MAX_TM	**63**
PRIMER_MIN_GC	**20**
PRIMER_MAX_GC	**80**
PRIMER_PICK_INTERNAL_OLIGO	**1**
PRIMER_INTERNAL_OLIGO_OPT_SIZE	**31**
PRIMER_INTERNAL_OLIGO_MIN_SIZE	**18**
PRIMER_INTERNAL_OLIGO_MAX_SIZE	**36**
PRIMER_INTERNAL_OLIGO_OPT_TM	**72**
PRIMER_INTERNAL_OLIGO_MIN_TM	**71**
PRIMER_INTERNAL_OLIGO_MAX_TM	**73**
Number of Primers/Probe Set generated:	**177**

TABLE 64. K-path run id: 13761. Total Number of Genome Sequences for alignment: 1. Reference Genome is Bovine Herpesvirus 1 gi|9629818|ref|NC_001847.1.

	Genome Description	GI Number	Sequence Length (bp)	K-path ID
1	**Bovine Herpesvirus 1**	9629818	135301	13761

Signature Information

Source: LLNL
Project name: BHV-1
Level of discrimination: Species.

Number of initial signatures: 177
Number of signatures forwarded to gel bench-screening: 101
Number of signatures forwarded to real-time bench-screening: 4
Number of final signatures forwarded to multiplex screening: 4

Signature list.

Note: For a listing of computationally predicted product sequences, please see Appendix II: "BHV Taqsim Run Data".

Taqsim description

We used a computational Real-time PCR simulator program, "Taqsim", to identify all potential targets for each signature from all sequences available in Genbank. Taqsim is a BLAST-based comparison of each signature as a triplet against all sequences in Genbank to identify the targets that are predicted to produce a Real-time PCR reaction at 57 degrees primer annealing and 67 degrees for probe annealing (these temperatures are according to Primer 3 oligo Tm calculations). The first column of the excel spreadsheets list the signatures by the name, and the following columns report the predicted Genbank targets, coordinates of signature region, amplicon size, and accession #'s for each target.

All sequences are listed in the 5'→ 3' direction.

TABLE 65a-d. Signature bioinformatics (a) BVH__94666 (b) BVH__94678 (c) BVH__94738 (d) BVH__94855

(a)

Target Virus	Bovine-BHV
Forward Primer	BVH__94666.F
FWD Primer Length (bp)	18
FWD Primer TM (°C)	62
FWD Primer GC Content (%)	61
Forward Sequence	GTGCCAGCCGCGTAAAAG
Reverse Primer	BVH__94667.R
Rev Primer Length (bp)	19
Rev Primer TM (°C)	61
Rev Primer GC Content (%)	57
Reverse Sequence	GACGACTCCGGGCTCTTTT
Probe Name	BVH__94668.P
Probe Length (bp)	27
Probe TM (°C)	72
Probe GC Content (%)	55
Probe Sequence	CTCCATGTTAGCGCTCTGGAACCAGGA
Probe strand	plus
Predicted Product Size	140

(b)

Target Virus	Bovine-BHV
Forward Primer	BVH__94678.F
FWD Primer Length (bp)	18
FWD Primer TM (°C)	61
FWD Primer GC Content (%)	66
Forward Sequence	GTCCCCACGGGCGTAGTA
Reverse Primer	BVH__94679.R
Rev Primer Length (bp)	18
Rev Primer TM (°C)	61
Rev Primer GC Content (%)	61
Reverse Sequence	CCCAACCGAGACGGAAAG
Probe Name	BVH__94680.P
Probe Length (bp)	21
Probe TM (°C)	72
Probe GC Content (%)	71
Probe Sequence	CACCGGGCGTGCTGTCCTCTG
Probe strand	plus
Predicted Product Size	114

(c)

Target Virus	Bovine-BVH

Forward Primer	BVH__94738.F
FWD Primer Length (bp)	22
FWD Primer TM (^{o}C)	62
FWD Primer GC Content (%)	50
Forward Sequence	TGAGGCCTATGTATGGGCAGTT
Reverse Primer	BVH__94739.R
Rev Primer Length (bp)	20
Rev Primer TM (^{o}C)	61
Rev Primer GC Content (%)	45
Reverse Sequence	GCGCGCCAAACATAAGTAAA
Probe Name	BVH__94740.P
Probe Length (bp)	34
Probe TM (oC)	71
Probe GC Content (%)	38
Probe Sequence	CGCGAATCTTATTTAAGTGCACACCGTGTTATTT
Probe strand	plus
Predicted Product Size	186

(d)

Target Virus	Bovine-BVH
Forward Primer	BVH__94855.F
FWD Primer Length (bp)	19
FWD Primer TM (^{o}C)	62
FWD Primer GC Content (%)	52
Forward Sequence	GCAGCAGTGCACCACGTAGT
Reverse Primer	BVH__94856.R
Rev Primer Length (bp)	23
Rev Primer TM (^{o}C)	62
Rev Primer GC Content (%)	47
Reverse Sequence	TTCGTATAGGGAGTACGCGATGA
Probe Name	BVH__94857.P
Probe Length (bp)	23
Probe TM (oC)	72
Probe GC Content (%)	60
Probe Sequence	CGCGACGAGAACGTCCACATCGT
Probe strand	plus
Predicted Product Size	199

TABLE 66a-b.(a) Reference Genomes used for Gene Information. (b) Target region gene information from annotated genomes available.

(a)

Genome Description	**GI Number**	**Sequence Length (bp)**

1	**Bovine Herpesvirus 1**	9629818/NC_001847.1	135301

(b)

				Gene Location		Target Region Location	
kpath Primer ID	**Primer**	**Gene**	**Description**	**Start**	**End**	**Start**	**End**
644761	BVH__94666.F	UL43/1487394	glycoprotein C	16613	19524	17101	17240
644766	BVH__94678.F	UL43/1487394	glycoprotein C	16613	19524	17977	18090
644797	BVH__94738.F	UL27/1487382	glycoprotein B	53058	58228	58190	58375
644869	BVH__94855.F	UL5/1487398	component of DNA helicase/primase complex	93756	96357	96154	96352
		UL41487391	virion protein	96293	96869	96154	96352

2.4. BHV-1 GEL AND REAL-TIME PCR SCREENING REPORT

Wet-lab screening process. To ensure extremely high selectivity and sensitivity, we have established a screening protocol that rigorously screens candidate DNA signatures to select the optimal sets for eventual field use. Candidate signature primer pairs that survive computational screening proceed to wet chemistry screening utilizing standard PCR with agarose gel electrophoresis. This step ensures that the primers will react across strains representative of the diversity of the pathogen (avoid false negative), but will not react with the nucleic acids of other organisms that could be present in a sample (avoid false positives). The collection of purified DNA templates used in this screening process is listed below:

1) Strain panels representative of the diversity of the target organism in nature. We have a panel of 10 Bovine Herpes Virus strains.
2) Near neighbors-organisms that are closely related at the genetic level and therefore have the greatest likelihood of cross-reaction. We screened the candidate signatures against 19 near-neighbor isolates.
3) Eukaryotic DNA that may carry over from sample collection procedures.
4) Prokaryotic DNA that would be expected to be present in environmental samples and may also be present in Bovine and Porcine oral cavities.
5) Fifteen soil samples from around the country representing diverse climates and contain a complex mixture of organisms from diverse environmental collections
6) Aerosol samples: 752 samples collected from aerosol collectors and pooled for background testing purposes.

Based on data from primer pair screening, a set of **4** specific and reliable signatures were then further tested for suitability for real-time Taqman® fluorogenic PCR detection protocols. The selected signatures showed a robust signal in all target reactions.

TABLE 67. List of targets screened.

Virus	Strain/ID[1]	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	Use stock titer	Titer Method
Bovine	Virginia NVSL	NVSL	Unknown	Unknown	CAHFS	Phenol/Chloroform	N/A	N/A

Herpes -1[2]	A231221							
Bovine Herpes-1 [2]	A030020072	CAHFS R. Mock #80 swab	Unknown	Unknown	1/28/04	Phenol/Chloroform	N/A	N/A
Bovine Herpes-1 [2]	LA ATCC VR-188	ATCC	Unknown	Unknown	CAHFS	Phenol/Chloroform	N/A	N/A
Bovine Herpes-1 [2]	RLB-106 ATCC VR-793	ATCC	Unknown	Unknown	CAHFS	Phenol/Chloroform	N/A	N/A
Bovine Herpes-1[2]	California NVSL 51619	NVSL	Unknown	Unknown	CAHFS	Phenol/Chloroform	N/A	N/A
Bovine Herpes -1[2]	Minnesota NVSL 9710720	NVSL	Unknown	Unknown	CAHFS	Phenol/Chloroform	N/A	N/A
Bovine Herpes-1[2]	California NVSL 200032	NVSL	Unknown	Unknown	CAHFS	Phenol/Chloroform	N/A	N/A
Bovine Herpes-1 [2]	Minnesota NVSL 86741	NVSL	Unknown	Unknown	CAHFS	Phenol/Chloroform	N/A	N/A
Bovine Herpes-1 [2]	A033640072	CAHFS R. Mock #5 swab	Unknown	Unknown	7/19/2004	Phenol/Chloroform	N/A	N/A
40 Bovine Herpes-1 [2]	A040130066	CAHFS R. Mock # 4 lung	Unknown	Unknown	CAHFS	Phenol/Chloroform	N/A	N/A

[1]As a disclaimer please note that some strains available for multiplexed testing were not available for gel or real-time PCR screening.
[2] Strain used in Multiplex PCR Phase I preliminary screening.
[3] Strain used for signature characterization Multiplex PCR in Phase II screening.

TABLE 68. List of 19 near-neighbors screened.

Virus	Strain[1]	Unique ID	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	Use stock titer	Titer Method
Bovine Herpes-5	D9402133	D9402133	Sonoma Co. 3/94 Severe necrotizing encephalitis, 1 week old calf	Unknown	CAHFS Lab (MDBK), (1 flasks) 11/19/2003	11/19/2003	Phenol/Chloroform	N/A	N/A
Bovine Herpes-5	DN-599	A032540006	R. Mock, from lung tissue, 2BFK2, 2/3/2004	Unknown	CAHFS Lab 4/30/2004	6/3/2004	Phenol/Chloroform	N/A	N/A
Bovine Herpes-5	DN-599	A040150085	R. Mock, #2 Lung, 5BFK5, 2/3/04	Unknown	CAHFS Lab 4/23/2004	2/3/04, 7/7/2004	Phenol/Chloroform	N/A	N/A
Rhadinovirus Caprine Herpes 2	Unknown	S0201998	Isolate S0201998 History: San Diego Co. 2/02 Aborted fetus-third trimester	Unknown	CAHFS Lab (MDBK), (3 flasks) 11/19/2003	11/19/2003	Phenol/Chloroform	N/A	N/A
Rhadinovirus Caprine Herpes 2	Unknown	ATCC VR462	ATCC	Unknown	CAHFS Lab	11/16/2004	Phenol/Chloroform	N/A	N/A
Rhadinovirus Caprine Herpes 2	Unknown	D0201157	San Joaquin Co. 2/02 Aborted fetus-	Unknown	CAHFS Lab (MDBK), (2 flasks) 3/31/2003	unknown	Phenol/Chloroform	N/A	N/A

			third trimester						
V. Pseudorabies	93-11745	Unknown	NVSL 93-11745 Illinois December, 1992	Unknown	CAHFS Lab, (1 flask) 12/3/2003	1/24/05	Phenol/Chloroform	N/A	N/A
V. Pseudorabies	93-21246	Unknown	NVSL 93-21246 Iowa February, 1993	Unknown	CAHFS Lab, (1 flask) 12/3/2003	1/24/05	Phenol/Chloroform	N/A	N/A
V. Pseudorabies	93-27020	Unknown	Unknown	Unknown	Unknown	1/24/05	Phenol/Chloroform	N/A	N/A
V. Pseudorabies	96-10866	Unknown	Unknown	Unknown	Unknown	1/24/05	Phenol/Chloroform	N/A	N/A
V. Pseudorabies	92-12013	Unknown	Unknown	Unknown	Unknown	1/24/05	Phenol/Chloroform	N/A	N/A
Pseudorabies Shope	RA 180	Unknown	Unknown	Unknown	Unknown	1/24/05	Phenol/Chloroform	N/A	N/A
Equine Herpes 1	A011120004	Unknown	Unknown	Unknown	Unknown	2/3/04	Phenol/Chloroform	N/A	N/A
Equine Herpes 1	ATCC VR2003	Unknown	ATCC	Unknown	Unknown	1/24/05	Phenol/Chloroform	N/A	N/A
Equine Herpes 1	A99043047	Unknown	Unknown	Unknown	Unknown	1/24/05	Phenol/Chloroform	N/A	N/A
Equine Herpes 2	ATCC VR701	Unknown	ATCC	Unknown	Unknown	1/24/05	Phenol/Chloroform	N/A	N/A
Equine Herpes 2	D990P49625	Unknown	Unknown	Unknown	Unknown	1/24/05	Phenol/Chloroform	N/A	N/A
Equine Herpes 2	NVSL 002	Unknown	NVSL	Unknown	Unknown	1/28/02, 1/24/05	Phenol/Chloroform	N/A	N/A
Feline Herpes	ATCC VR636	Unknown	ATCC	Unknown	Unknown	1/24/05	Phenol/Chloroform	N/A	N/A

2.4.1. Bovine Herpes Virus 1 (BHV1) - Gel Screening Report

TABLE 69. Nucleic acid extracts used to challenge the initial set of 101 candidate signatures. These backgrounds are used to determine which signatures will be down-selected in the screening process. No prokaryotic backgrounds were screened. Numerous cross reactions noted (data not shown, too large). All of the 101 signatures generated cross reacted with one or more of the above backgrounds. The majority were knocked out due to Soils. From this data five of the best signatures were chosen to continue through screening from which one was rejected due to cross reaction with a near neighbor (leaving four), although even of the remaining four signatures some of these had observed cross-reactions with backgrounds as noted in the following Table below..

Nucleic Acid Extract Type	Description/ID
Soil Extract	D000505
Soil Extract	D000532
Soil Extract	D000558
Eukaryotic DNA Extract	Tick
Eukaryotic DNA Extract	Porcine

Eukaryotic DNA Extract	Rat
Eukaryotic DNA Extract	Mosquito

TABLE 70. The cross reactions against backgrounds for the four signatures included in the final assay.

	BVH__94666.F BVH__94667.R	BVH__94678.F BVH__94679.R	BVH__94738.F BVH__94739.R	BVH__94855.F BVH__94856.R
Predicted Product Size	140bp	114bp	186bp	199bp
Soil D000505	35bp	300bp: multiple bands	1000bp	400bp: multiple bands
Soil D000532	40bp	460bp: multiple bands	0	400bp: multiple bands
Soil D000558	30bp	460bp: multiple bands	580bp: multiple bands	540bp: multiple bands
Tick	35bp	Multiple bands	480bp	Double bands
Rat	30bp	0	0	0
Porcine	40bp	0	0	0
Mosquito	40bp	Triple Bands	0	Triple Bands

List of signatures screened in gel format against available targets and near neighbors:
101 signatures were developed for detecting BHV-1.

TABLE 71. Results with predicted product size for **targets** and near neighbors screening of the remaining four signatures.

	BVH__94666.F BVH__94667.R	BVH__94678.F BVH__94679.R	BVH__94738.F BVH__94739.R	BVH__94855.F BVH__94856.R
Predicted Product Size	140bp	114bp	186bp	199bp
BHV1 California 51619	**160**	**170**	**200**	**200**
BHV1 California 200032	**160**	**160**	**200**	**200**
BHV1 Minnesota	**160**	**140**	**200**	**200**
BHV1 Minnesota Duplicate Screening	**160**	**160**	**200**	**200**
BHV1 Virginia	**160**	**160**	**200**	**200**
Rhadinovirus Caprine Herpes 2	0	0	0	0
V. Pseudorabies (93-11745)	0	0	0	0
V. Pseudorabies (93-21246)	0	0	0	0
V. Pseudorabies (93-27020)	0	0	0	0
V. Pseudorabies (96-10866)	0	0	0	0

2.4.2. Bovine Herpes Virus 1 (BHV1) - Real-time PCR Screening Report

TABLE 72. List of signatures screened against Backgrounds in Real-time PCR Format.

BVH__94666.F	GTGCCAGCCGCGTAAAAG
BVH__94667.R	GACGACTCCGGGCTCTTTT
BVH__94668.P	CTCCATGTTAGCGCTCTGGAACCAGGA
BVH__94678.F	GTCCCCACGGGCGTAGTA
BVH__94679.R	CCCAACCGAGACGGAAAG
BVH__94680.P	CACCGGGCGTGCTGTCCTCTG
BVH__94738.F	TGAGGCCTATGTATGGGCAGTT
BVH__94739.R	GCGCGCCAAACATAAGTAAA
BVH__94740.P	CGCGAATCTTATTTAAGTGCACACCGTGTTATTT
BVH__94855.F	CGTCCCGATTTCTCCGAAT
BVH__94856.R	TTCGTATAGGGAGTACGCGATGA
BVH__94857.P	CGCGACGAGAACGTCCACATCGT

TABLE 73a-d. Real-time RT- PCR background screening, all performed in triplicate against an extensive list of soil, Prokaryotic and Eukaryotic extracted DNAs in addition to 2 aerosol blocks, each containing 752 samples. All signatures passed real-time RT- PCR background screening. None of the 4 signatures produced an amplicon when screened against the listed backgrounds. All signatures moved forward to be screened against the available BHV targets and near neighbors.

(a) Total of 15 soils screened

D000505	D000553	D000541	D000019	D000558	D000036	D000528	D000054
D000527	D000530	D000557	D000028	D000521	D000051	D000532	

(b) Total of 16 Eukaryotes screened

Monkey	Sheep	Tick	Chicken	Rat	Drosophila	Rabbit	Mosquito
Human	Dog	Mouse	Cat	Porcine	Bovine	Equine	Flea

(c) Total of 13 Prokaryotes screened

E. coli	*B. cereus*	*S. typhimurium*	*B. subtilis*	*E. herbicola*
B. globigii	*S. pneumonia*	*L. monocytogenes*	*H. influenza*	
P. aeruginosa	*C. burnetti*	*B. burgdorferi*	*S. aureus*	

(d) Aerosols screened

Aerosol Block	Signatures Screened	Number of Samples in Block
4 080403	4	752
3 081903	4	752
Total:		**1504 samples**

TABLE 74. Four signatures were screened against targets (**shown in bold font**) and near-neighbors in real-time PCR format. [1]Some of the real-time PCR testing is supplemented from testing that was performed against the signatures prior to obtaining the other virus stocks of targets and near-neighbors, as a result, in some instances; a different number of replicates were performed. N/A indicates that a CT value was not detected, indicating the no reaction took place.

	BVH__94666.F **BVH__94667.R** **BVH__94668.P**	**BVH__94678.F** **BVH__94679.R** **BVH__94680.P**	**BVH__94738.F** **BVH__94739.R** **BVH__94740.P**	**BVH__94855.F** **BVH__94856.R** **BVH__94857.P**
BHV 1 (A030020072)	**27.7, 27.3**	**28.4, 28.4**	**27.7, 31.5, N/A**	**27.6, 27.6, 31, 28.6**
BHV 1	**27, 27.1**	**27.6, 27.7**	**30.3, 30.5**	**N/A, N/A**

(A033640072)				
BHV 1 (A040130066)	**26.9, 27.5**	**27.8, 27.6**	**30.6, 30.3**	**N/A, N/A**
BHV1 California Unknown	**28.9, 25.8, 23.4**	**26.5, 22.7, 23.9**	**28.7, 26.9, 26.7**	**30.9, 26.5, 25.4**
BHV1 Cooper Unknown	**22, 22.7**	**21.7, 23.4**	**26.3, 26.4**	**25.1, 21.4**
BHV1 Minnesota Unknown	**26.7, 25.9, 27.9, 27, 25.1, 25.7**	**23.7, 25.4, 25.8, 28.4, 27, 27**	**26.9, 26.9, 28.4, 29.4, 29.8, 29.3**	**N/A, 26.2, N/A, N/A, 30, 29.1**
BHV1 Virginia Unknown	**31, N/A**	**26.9, 28**	**29.6, 28.2**	**N/A, 26.1**
BHV 1 (ATCC VR188)	**24.7, 24.3, 24.3**	**25, 24.6, 26**	**25.8, 24.6, 30**	**25.8, 24.6, 30**
BHV 1 (ATCC VR793)	**26.7, 26.3, 25.5**	**26.4, 26.9, 27.1**	**26.3, 27.3, 27**	**27, 24.8, 25**
BHV 5 (D9402133)	N/A, N/A, N/A	N/A, N/A, N/A	N/A, N/A, N/A	N/A, N/A, N/A
BHV 5 (A040150085)	N/A, N/A	N/A, N/A	N/A, N/A	N/A, N/A
BHV 5 (A032540006)	N/A, N/A	N/A, N/A	N/A, N/A	N/A, N/A
Caprine Herpes 2 (ATCC VR462)	N/A, N/A, N/A	N/A, N/A, N/A	N/A, N/A, N/A	N/A, N/A, N/A
Caprine Herpes 2 (S0201998)	N/A, N/A	N/A, N/A	N/A, N/A	N/A, N/A
Caprine Herpes (D0201157)	N/A, N/A	N/A, N/A	N/A, N/A	N/A, N/A
Equine Herpes 1 (A011120004)	N/A, N/A, N/A	N/A, N/A, N/A	N/A, N/A, N/A	N/A, N/A, N/A
Equine Herpes 1 (ATCC VR2003)	N/A, N/A, N/A	N/A, N/A, N/A	N/A, N/A, N/A	N/A, N/A, N/A
Equine Herpes 2 (ATCC VR701)	N/A, N/A, N/A	N/A, N/A, N/A	N/A, N/A, N/A	N/A, N/A, N/A
Equine Herpes 1 (A99043047)	N/A, N/A	N/A, N/A	N/A, N/A	N/A, N/A
Equine Herpes 2 (D990)	N/A, N/A	N/A, N/A	N/A, N/A	N/A, N/A
Equine Herpes 2 (NVSL 002)	N/A, N/A	N/A, N/A	N/A, N/A	N/A, N/A
Feline Herpes	N/A	N/A	N/A	N/A

(ATCC VR636)				
V. Pseudorabies (93-11745)	N/A, N/A	N/A, N/A	N/A, N/A	N/A, N/A
V. Pseudorabies (93-21246)	N/A, N/A	N/A, N/A	N/A, N/A	N/A, N/A
V. Pseudorabies (92-12013)	N/A, N/A	N/A, N/A	N/A, N/A	N/A, N/A
V. Pseudorabies (93-27020)	N/A, N/A	N/A, N/A	N/A, N/A	N/A, N/A
V. Pseudorabies Shope (RA 180)	N/A, N/A	N/A, N/A	N/A, N/A	N/A, N/A
V. Pseudorabies (96-10866)	N/A, N/A	N/A, N/A	N/A, N/A	N/A, N/A

[1]The number of Ct values denotes the number of replicate screenings against the particular template. Some of the real-time PCR testing is supplemented from testing that was performed against the signatures prior to obtaining the other virus stocks of targets and near-neighbors, as a result, in some instances, a different number of replicates were performed.

Bovine Herpes Virus 1 (BHV1) - LOD Report

TABLE 75. In order to determine relative limits of detection for each Real-time PCR signature developed, a dilution series of target was made of 4 logs across the predicted linear dynamic range. The untitered viral nucleic acid extracts were diluted then tested with each signature using the standard Real-time PCR protocol in triplicate and average Ct values are reported for each dilution.

	Signature Average Ct Value			
BHV 1 (ATCC VR188) Target Dilution Factor	**BHV 94857**	**BHV 94740**	**BHV 94680**	**BHV 94668**
1:10	27.4	**33.2**	27.5	27.6
1:100	30.7	**N/A**	31.7	32
1:1K	33.7	**N/A**	**33.9**	**34**
1:10K	36.5	**N/A**	**N/A**	**N/A**

TABLE 76. Summary Table of Signature LODs on: BHV 1 (ATCC VR188)

PROBE SIGNATURE	**LOD**
BHV_94857	>1:10K
BHV_94740	1:10
BHV_94680	1:1K
BHV_94668	1:1K

2.5. BHV-1 MULTIPLEXED PCR SCREENING REPORT

SCREENING AND DOWN-SELECTION TECHNICAL APPROACH:

Signatures that pass gel and real-time PCR screening are candidates for multiplexed assays. If the number of candidate signatures is large then a subset of the available signatures are selected based on measured sensitivity and specificity observed in real-time screening. In preliminary screening the background of individual signatures is measured by running "blank" (no sample added) controls and observing signatures response. In some cases a primer-to-primer non-specific interference will eliminate candidate signatures. Signatures are then added iteratively to each multiplexed panel, titrated, and with each signature addition, collectively, all signatures are re-titrated to determine if the presence of one signature will interfere with the performance of another. During this process signatures are added or subtracted from the multiplexed panel depending on individual outcomes until all desirable signatures have been added.

Criterion for down-selection of multiplexed signatures

Signature specificity. In initial real-time PCR screening signatures undergo target and near neighbor screening and screening against environmental confounders which assess individual signature specificity. In multiplex format this is repeated to verify that a more complex reaction system gives the same level of specificity.

Signature sensitivity. In initial real-time PCR screening signatures undergo limit of detection testing where a dilution curve is generated for each signature. This determines individual signature sensitivity. In multiplex format this is repeated to verify that a more complex reaction system gives acceptable signature sensitivity.

Optimal signal to noise ratio. In the multiplex assay system the potential for cross reactivity is larger than in single reaction testing, this may cause unwanted PCR reaction products or non-specific cross-reactivity. Signatures are preferred to have a high signal to noise ratio, where desired signal is significantly greater than the background noise of the signatures when there is no target nucleic acid in the reaction.

Compatibility with other signatures in multiplex. In some cases a primer set may interfere with other primers or probes in the multiplex and there by cause limit of detection shifting for one or more signature in the multiplex. For each scenario it must be evaluated whether the shift in detection level is significant enough to remove (what could be a very desirable signature) from the panel.

TABLE 77. Order details for BHV signatures ordered for multiplexed assay screening and development.

ID	Modification details	Vendor
BVH__94666.F (BHV-1)	5'-/5Bio/G/iBiodT/GCCAGCCGCG/iBiodT/AAAAG-3'	IDT DNA
BVH__94667.R (BHV-1)	5' -GACGACTCCGGGCTCTTTT -3'	IDT DNA
BVH__94668.FCP (BHV-1)	5'- /5AmMC6//iSp18/TCCTGGTTCCAGAGCGCTAACATGGAG-3'	IDT DNA
BVH__94738.F (BHV-3)	5'-/5Bio/TGAGGCC/iBiodT/ATGTATGGGCAG/iBiodT/T-3'	IDT DNA
BVH__94739.R (BHV-3)	5' - GCGCGCCAAACATAAGTAAA -3'	IDT DNA
BVH__94740.FCP (BHV-3)	5'-	IDT DNA

	/5AmMC6//iSp18/AAATAACACGGTGTGCACTTAAATAAGATTCGCG-3'	

Multiplex Data Analysis -Technical approach

Disease signature thresholds

Thresholds were initially determined using all available data from known negative samples (blanks) by plotting a histogram, which showed the probability of a given sample generating a particular MFI. In the absence of cross reactivity, each signature in the multiplex assay could be treated as an individual signature result. For example, for a sample spiked with RPV, data for other disease signatures could contribute towards negative data, providing that cross reaction between disease signature sets was not observed. This is a way to increase the number of samples that can be used to establish thresholds.

TABLE 78. Individual signature thresholds and ranges for BHV signatures. For FY06, threshold determinations have not yet been made as they require a significant number of tests (>500) to generate this information. This information will be updated when available.

Signature Name	Mux Assay name	Panel Membership	Signature background in Tris NaCl Buffer (MFI +/-)	Signature background in Clinical matrices (MFI +/-)	Threshold
BHV_94668	BHV-1	Bovine	TBD	TBD	TBD
BHV_94738	BHV-3	Bovine	TBD	TBD	TBD

TABLE 79. List of Targets screened at LLNL.

Virus	Strain[1]	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	Use stock titer	Titer Method
Bovine Herpes-1	Colorado Vaccine	NVSL	10^8/0.1mL $TCID_{50}$	unknown	12/2005	Phenol/Chloroform	7.63 x 10^6/0.1mL $TCID_{50}$	Reed & Muench

TABLE 80. List of additional targets and near-neighbors screened against the Bovine Panel. All near-neighbors samples were extracted nucleic acids with unknown titers. Additional targets shown in red.

Virus	Strain[1]	Unique ID	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	NA conc. used or stock titer	Titer Method
Border Disease virus	Coos Bay	4-6-92	NADC, Julia Ridpath	1.5 x 10^8 TCID50/mL	unknown	12/14/06	Trizol	1.18 x 10^6 TCID50/mL	Reed & Muench
Bovine Herpes Virus-1	California	NVSL 20032 111903	CAHFS	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Cooper	NVSL 86741 111903	CAHFS	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Los Angeles	Los Angeles ATCC VR188	ATCC (CAHFS)	1 x 10^5 TCID50/mL	(CAHFS) MDBK2(LLNL)MDBK1	5/17/07	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes	RLB-106	ATCC	ATCC	unknown	unknown	unknown	Phenol/	40 pg/uL	N/A

Virus-1		VR793	(CAHFS)				Chloroform		
Bovine Herpes Virus-1	IBR	A033640072	CAHFS, R. Mock, #5 Swab, 1BDFK1, 1/28/2004, A030020072	unknown	1BDFK1,	4/23/04	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	A040130066	CAHFS, R. Mock, #4 Lung, 1BFK1, 1/28/2004, A040130066	unknown	1BFK1	4/23/04	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	Texas A030020072	CAHFS, R. Mock, #80 Swab, 1BDFK1, 1/28/2004, A030020072	unknown	1BDFK1	4/23/2004	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	California	NVSL 200032	NVSL, CAHFS	unknown	MDBK CAHFS	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Virginia	NVSL 231221	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	California	NVSL 51619	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Minnesota	NVSL 86741	NVSL, CAHFS	unknown	MDBK CAHFS	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Minnesota	NVSL 97-10720	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Cooper	NVSL-Cooper RA 309	NVSL, CAHFS	unknown	MDBK CAHFS	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes-5	DN-599	A032540006	R. Mock, from lung tissue, 2BFK2, 2/3/2004	Unknown	CAHFS Lab 2BFK2 4/30/2004	6/3/2004	Phenol/Chloroform	40 pg/uL	N/A
Bovine Herpes-5	DN-599	A040150085	R. Mock, #2 Lung, 5BFK5, 2/3/04	Unknown	CAHFS Lab 5BFK5 4/23/2004	7/7/2004	Phenol/Chloroform	40 pg/uL	N/A
Bovine Herpes-5	D9402133	D9402133	Sonoma Co. 3/94 Severe necrotizing encephalitis, 1 week old calf	Unknown	CAHFS Lab (MDBK), (1 flasks) 11/19/2003	11/19/2003	Phenol/Chloroform	40 pg/uL	N/A
Bovine Herpes Virus	BHV 5	D9403153	CAHFS	unknown	unknown	unknown	unknown	40 pg/uL	unknown
Caprine Herpes Virus		VR462	ATCC	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Caprine Herpes Virus	D0201157	D0201157	CAHFS, Isolate D0201157 History: San Joaquin Co. 2/02 Aborted fetus-third trimester	unknown	CAHFS Lab (MDBK)	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Caprine Herpes Virus	S0201998	S0201998	CAHFS, Isolate S0201998 History: San Diego Co. 2/02 Aborted fetus-third trimester	unknown	CAHFS Lab (MDBK)	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-1	Santa Barbara	N/A	Bridget McLaughlin CAHFS	unknown	unknown	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic	Georgia	N/A	Bridget McLaughlin	unknown	unknown	unknown	Trizol	40 pg/uL	N/A

Disease virus-1			CAHFS						
Epizootic Hemorrhagic Disease virus-2	Alberta	041 ODV 0401	NVSL	unknown	BHK	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-1 (SV123)	New Jersey (3TD) Deer, NJ 1955	041 ODV 0401	NVSL	1 x $10^{5.125}$ $TCID_{50}$/0.1 ml	+BHK-3 (12/7/83); (12/11/86); (12/18/86)	Unknown	Trizol	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A011120004	CAHFS, R. Mock, from brain tissue, 2BFK2, 2/3/2004, A011120004	unknown	CAHFS, 2BFK2	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A99043047	CAHFS, R. Mock, Spleen, 1BFK1, 2/3/2004, A99043047	unknown	CAHFS, 1BFK1	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A9904309	CAHFS	unknown	CAHFS, unknown	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Bovine 1247	Bovine 1247 ATCC VR2003	ATCC	unknown	CAHFS, unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	NVSL 002	NVSL 002 1/28/04	NVSL	unknown	CAHFS, unknown	1/29/03	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	LK	LK ATCC VR701	ATCC	unknown	CAHFS, unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	D990	D990	CAHFS	unknown	CAHFS, unknown	1/29/03	Phenol/ Chloroform	40 pg/uL	N/A
Feline Herpes	C-27	ATCC VR636	ATCC	unknown	CAHFS, unknown	unknown	unknown	40 pg/uL	N/A
Fowl Pox	Vaccine strain	Poxine™	Jeffers Livestock	unknown	ECE	5/17/07	Phenol/ Chloroform	40 pg/uL	N/A
Parainfluenza Virus type 3	C-243	TC-81335	LLNL	1 x 10^5 TCID50/0.1 mL	(VL)MK14(LLNL)H292-1	5/17/07	Trizol	40 pg/uL	Reed and Muench
Porcine Coronavirus	AR310	ATCC VR-2384	LLNL	1 x $10^{4.25}$ $TCID_{50}$/0.1 ml	ST2				Reed and Muench
Porcine Herpes Pseudorabies	Shope	RA 180	CAHFS	1 x 10^5 TCID50/mL		5/17/07	Trizol	40 pg/uL	N/A
Pseudorabies		92-12013	NVSL 92-12013 bovine isolate Iowa December, 1991	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies		93-11745	NVSL 93-11745 Illinois December, 1992	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies	Shope	RA180	NVSL Shope (PRV) RA 180	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies Titered	Shope	N/A	NVSL	unknown		unknown	Phenol/ Chloroform		Reed & Muench
Respiratory Syncytial virus	Long	N/A		unknown	(VL)KB6L2KB2HFDL2A549-1(LLNL)HeLa3	5/17/07	Trizol	40 pg/uL	N/A

Transmissible Gastroenteritis virus of hogs	Perdue	NVSL 9801	LLNL	1 x $10^{4.75}$ $TCID_{50}$/0.1 ml	ST 1		Trizol	40 pg/ul	Reed and Muench

TABLE 81. Panel membership for assay. The 2 BHV signatures from the Version 1.0 panel were included in the **Bovine-specific multiplexed assay panel** for FMDV rule-out diagnostics. The below table describes the constituent signatures of the bovine panel in which the 2 BHV signatures were tested.

Bead ID	Signature/ Target Name	Mux Group ID	Virus	Source	Forward Primer	Reverse Primer	Luminex Probe (-strand, FCP)
113	emcf_94975.F, emcf_94976.R, emcf_94977.P	MCF_1	Malignant Catarrhal Fever	LLNL	ATGCCAGTCACTGGCTCTCA	GGGTGTTGTAGAATCCTGAAATGG	GTTGATCACGGTGGCACCCTGG
114	emcf_95059.F, emcf_95060.R, emcf_95061.P	MCF_2	Malignant Catarrhal Fever	LLNL	GTTCTGGAAACTGACCAAACAGTGT	AGTGGCACTTGAGTGTAACTTTTATTG	GCACTCTGGCAGGCATAAGGGAAATACA
115	emcf_95155.F, emcf_95156R, emcf_95157.P	MCF_3	Malignant Catarrhal Fever	LLNL	CCCTGGAAGCTGTCATACAAAA	AAACATTGGCATATCTTGCAAGGT	CAGTAGAGTCCAGGGCTGCAGTTGTCTCA
117	BVH__94666.F, BVH_94667.R, BVH_94668.P	BHV-94668	Bovine Herpes Virus	LLNL	GTGCCAGCCGCGTAAAAG	GACGACTCCGGGCTCTTTT	TCCTGGTTCCAGAGCGCTAACATGGAG
118	BVH__94738.F, BVH_94739.R, BVH94740.P	BHV-94740	Bovine Herpes Virus	LLNL	TGAGGCCTATGTATGGGCAGTT	GCGCGCCAAACATAAGTAAA	AAATAACACGGTGTGCACTTAAATAAGATTCGCG
119	BPSV_95719.F, BPSV_95720.R, BPSV_95721.P	PPOX_1	Bovine Papular Stomatitis Virus	LLNL	GCAGATGCGCTCCTGGTT	GCACCTCTGCTGCTGCAA	CCGACTCCGACGTGGAGAACGTG
120	BPSV_95722.F, BPSV_95723.R, BPSV_95724.P	PPOX_2	Bovine Papular Stomatitis Virus	LLNL	GATGGCCGTGCAGCTCTT	CGTACAAGATCACGGCCAACT	TGTACGGGCTCATGGGCTTCCG
122	BPSV_95731.F, BPSV_95732.R, BPSV_95733.P	PPOX_4	Bovine Papular Stomatitis Virus	LLNL	GCAGCAGTGCACCACGTAGT	CGCTGAACCCGTACATCCT	GACTTCGAGGCGGACAACAAGCG
132	FMDV.TC	FMDV.TC	Foot and Mouth Virus	Tetracore	ACTGGGTTTTACAAACCTGTGA	GCGAGTCCTGCCACGGA	GTCCCACGGCGTGCAAAGGA
133	FMDV.Pir	FMDV.Pir	Foot and Mouth Virus	Pirbright	CACYTYAAGRTGACAYTGRTACTGGTAC	CAGATYCCRAGTGWCICITGTTA	CCTCGGGGTACCTGAAGGGCATCC
134	BVD_1a	BVD_SIG1	Bovine Viral Diarrhea	UCD, CAHFS lab	GGTAGTCGTCAGTGGTTCGAC	CATGTGCCATGTACAGCAGAGAT	CCTCGTCCACGTGGCATCTCGAG
138	BVD_1821165.F, BVD_1821164.R, BVD_1821166.P	BVD_SIG2	Bovine Viral Diarrhea	LLNL	GGGAGTCGTCAATGGTTCGAC	TCCATGTGCCATGTACAGCAGAG	CCTCGTCCACITGGCATCTCGAG
135	BTV_1759932	BTV_1759932	Bluetongue Virus	LLNL	GCACCCTATATGTTTCCAGACCA	CAGCTAACTCTTCAGCCACACG	CTAACTCGTGGGCCAATCATCATCTTCTGT
136	BTV10_1810207	BTV10_1810207	Bluetongue Virus	LLNL	CAAACACAAAAGGCGGAGAAG	GGCGTTTAATCTGTCTTAGTCTTACGT	GAAACGCTTCTGCGTACGATGCGA
137	BTV10_1810199	BTV10_1810199	Bluetongue Virus	LLNL	CACATGTCGCTTAATTTGTCTTAACC	GCGGAGAAGGCTGCATT	ACGAAACGCTTCCGCGTACGATG
139	BTV10_1810205	BTV10_1810205	Bluetongue Virus	LLNL	TCAATTTTGGTAGAATTTGTTCATTCA	GCGGAGAAGGCTGCATTC	ACGAAACGCTTCCGCGTACGATG
163	VSV_1798943	VSV_1798943	Vesicular Stomatitis Virus -Indiana	LLNL	CGCCACAAGGCAGAGATGT	TGTCAAATTCTGACTTAGCATACTTGC	GCATACTGCATCATATCAGGAGTCGGTTTTCTG
166	VSV_1798947	VSV_1798947	Vesicular Stomatitis Virus -Indiana	LLNL	CCCAATCAATGCCATGATACA	CTCCAATGGAAGGGTCCAAA	TTTGAAAGTAGAACTGTGCAAGCCCGGTATC
167	VSV_1798949	VSV_1798949	Vesicular Stomatitis Virus -Indiana	LLNL	GGCGCTCATTATAAAATTCGGA	ACATTTTCTCGTAGTAATGCAGCAG	GAAGTCCCTGTAATGGATTCCCATTCCATGT
169	VSV_1811408	VSV_1811408	Vesicular Stomatitis Virus –New Jersey	PIADC	CTCACAACATGGGTCCTGAA	TTCTTGACCTGGATACATCAT	GGCATAGYTCGTCTGCRACTTCCCT
160	RPV_1814853	RPV_1814853	Rinderpest Virus	LLNL	GGATCGCTGAAATGATCTGTGA	GGAGCCAGTTCACCCATTTG	CTGGCCAACCCTGCCTCCACTATGTA
161	RPV_1814855	RPV_1814855	Rinderpest Virus	LLNL	TGCATCTTATGTGACTTTGGTTCA	GGCTATCCGCACAGCTGAC	CAGTCCTCTCATCTGTTGTCGATCCGATGTA
162	RPV_1814856	RPV_1814856	Rinderpest Virus	LLNL	AACTCCTGACCTCATTCCTTGC	GGCTCTATAATCCCACTATGCCA	TGGCTCAGTGCATTCACAAAGACCTTGAATA
170	N/A	NC (MT-7)	Maritima	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
171	N/A	b-MT7 (FC)	N/A	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
172	N/A	MT7/Cy3 (IC)	N/A	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
173	Alien RNA	arRNA Control	None	Ambion	GACATCAAGGCTCAAACTAATTTTACC	CAAAGGCTGCCAACATAAAATG	CAAGCGTAAATGCAGCGTCCA

[1]FCP indicates that the probe is on the negative strand (Forward Compliment to the Probe).

2.5.1. BOVINE PANEL –MULTIPLEXED PCR DATA

Preliminary screening: Preliminary screening can be broken down into several steps. The signatures are preliminarily screened by first assessing how the signatures will perform under multiplex PCR conditions and whether or not the addition of the new signatures will adversely effect other signatures, these preliminary screening methods are described as “signature baseline screening” and “multiplex addition screening” respectively. First signature baseline screening tests the performance of the signature in a no-target reaction (blank), a “passing” score is indicated by a low (typically less than 100) MFI. All BHV signatures passed the signature baseline screening. Next in multiplex addition screening the new signature primers are added one-by –one to the other panel signatures to determine if there would be any cross-reactions between primer pairs in the panel. Further assessment of the signatures is done through target and near-neighbor screening and the determination of relative sensitivities. The signature down-selection for the BHV signatures is further described below.

Historic Data Overview: Version 1.0 Panel LLNL reference date: 20060117

(a) **(b)**

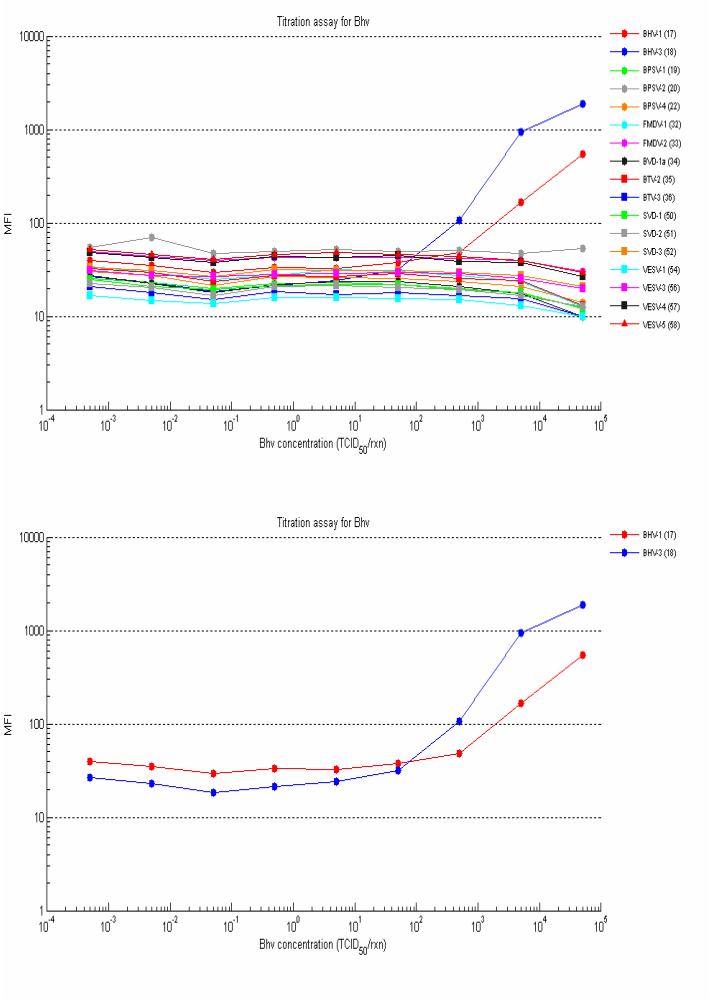

FIG. 9. Version 1.0 panel data: multiplexed RT-PCR titrations for BHV using BHV(IBR) Colorado vaccine strain. (a) Plot shows all signatures in the presence of BHV assay titration. Note that even at high concentration of target nucleic acid, no cross-reactivity is seen on other signature channels. (b) Same plot as (a), however, other signature data has been removed just to show BHV-specific signatures.

Near-neighbor and Target screening: The 2 Version 1.0 panel BHV signatures were added to the bovine multiplex panel. The signatures exhibited a reasonably low background response (<30 MFI) in the Bovine panel.

TABLE 82. Backgrounds screening in multiplexed format for BHV at LLNL and PIADC.

	Soils	Prokaryotes	Eukaryotes	Aerosols[1]	Near Neighbors	Targets
Mux PCR Screening	54	135	16	0	4	15

[1]-There are 752 pooled samples in each Aerosol Block.

TABLE 83. Bovine panel backgrounds screening in **multiplexed** format for down-selected BHV signatures against the below listed **eukaryotes**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. None of the signatures cross-reacted with any of the samples listed below.

Description	BHV-1 (17)	BHV-3 (18)
BOVINE	15	6
CAT	13	5
CHICKEN	13	6
DOG	11	4
DROSOPHILA MELANOGASTER	12	5
EQUINE	11	4
FLEA	10	4
HUMAN	7	3
MONKEY	15	6
MOSQUITO	14	5
MOUSE	12	5
PIG / PORCINE	13	5
RABBIT	14	5
RAT	9	4
SHEEP	7	3
TICK	9	4

TABLE 84. Bovine panel backgrounds screening in **multiplexed** format for the four BHV signatures against the below listed **prokaryotes**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. None of the signatures cross-reacted with any of the samples listed below.

Description	BHV-1 (17)	BHV-3 (18)
Erwinia amylovora	22	6
Actinobacillus suis	20	6
Aneurinbacillus migulanus	18	6
Bacillus cereus	21	6
Bacillus globigii	23	7
Bacillus subtilis	16	4
Bacillus thuringiensis	25	6
Bifidobacterium denticum	18	5
Borrellia burgdorferi	23	7
Burkholderia capacia	20	6
Caulobacter vibriodes	15	5
Clavibacter michganensis	17	5
Clostridium butyricum	21	6
Corynebacterium pseudodipthericum	22	6

Cytophaga marinoflava	18	5
Erwinia herbicola	22	7
Escherichia coli	28	7
Geobacillus caldoxylosilyticus	18	6
Salomon's homophile	16	5
Haemophilus influenza	20	6
Herbaspirillium seropedicae	23	7
Lactobacillus garvieae	14	5
Lactobacillus gasseri	19	6
Listeria monocytogenes	17	6
Listeria seeligeri	20	5
Micrococcus luteus	18	6
Moraxella lacunatica	21	7
Oceanospirillium ssp. Maris	25	7
Paenibacillus napthalaenovorans	21	6
Paracoccus dentrificans	20	7
Porphyrobacter sanguineus	22	6
Proteus mirabillis	19	6
Pseudomonas aeruginosa	21	6
Pseudomonas oleovorans	16	5
Rhizobium leguminosarum	24	7
Rhodococcus rhodochrous	14	5
Salmonella typhimurium	20	6
Simonsiella muelleri	18	6
Sphingomonas sp. (Alcaligenes sp)	18	6
Staphylococcus aureus	21	5
Streptococcus pneumoniae	21	5
Streptomyces scabiei	16	6
Tatlockia maceachernii	26	7
Vibrio paraheamolyticus	18	6
Xanthomonas translucens	18	6

TABLE 85. <u>Bovine panel</u> backgrounds screening in **multiplexed** format for the four BHV signatures against the below listed **soils**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. None of the signatures cross-reacted with any of the samples listed below.

Description	BHV-1 (17)	BHV-3 (18)
D 000107-49	29	7
D 000109 # 50	25	6
D 000402 # 53	27	7
D 000500 - 26 - 1	29	7
D 000501-14-1	27	7

D 000505 - 11 - 4	30	7
D 000521 - 23	29	8
D 000527 - 3	27	7
D 000531 - 21	29	7
D 000533 - 17 -1	27	6
D 000542 - 6	27	7
D 000550 - 20	26	8
D 000551 - 5	30	8
D 000561 - 8 - 6	27	7
D 000562 - 30 - 5	27	7
S 251	26	7
S 252	24	7
S 253	22	6
S 254	26	7
S 255	22	7
S 256	24	7
S 257	24	7
S 259	23	7
S 260	23	6
S 271	20	5
S 272	23	7
S 273	23	7
S 274	23	7
S 275	25	8
S 276	25	8
S 277	24	7
S 279	17	6
S 280	21	7
S 282	16	5
S 283	21	7
S 284	23	7
S 286	22	7
S 287	9	4
S 288	21	7
S 289	22	7
S 290	20	6
S 291	18	6
S 292	23	7
S 295	22	7
S 296	23	7
S 297	19	6
S 298	21	6
S 299	18	6
S 300	20	6
S 301	20	6
S 303	18	6
S 304	21	6

S 305	20	6
S 307	21	6

TABLE 86. Bovine Panel **Near-Neighbor** screening (Data from 20070601) against the 2 BHV signatures. Values shown are Median Flourescence Intensity (MFI) units. “Blanks” are buffer-only samples (no nucleic acid added to reaction), shown below in red. Extracted nucleic acid was added to each PCR reaction in triplicate totaling 200pg/rxn. The data shows that the BHV signatures but did react to all BHV-1 specific targets (shown in blue) of the Bovine panel constituents, and with the exception of a very slight cross-reaction with caprine herpes (red highlighted cells) did not cross-react with any near-neighbors.

Description	BHV-1 (17)			BHV-3 (18)		
Blank	25	22	29	6	6	6
Blank	24	22	22	7	7	6
BHV5 A040150085	35	14	42	7	5	8
BHV5 (BFK)	29	22	28	8	8	6
BHV-1 A040130066	1060	1221	627	3097	2708	2505
BHV-1 A033640072	791	875	521	2205	1956	1913
BHV-1 ATCC VR 793	1079	1092	641	3065	2251	2232
IBR CA 111903	1937	755	1662	5019	1594	4566
IBR MN 111903	901	1851	1344	2847	3545	3996
BHV-1 NVSL 231221	1007	1402	754	2801	3071	2685
BHV-1 RA309	1805	2172	1487	4262	4089	3690
BHV-1 NVSL 97-10720	1514	1822	1482	3951	3666	4359
BHV-1 NVSL 51619	1873	2159	1622	4588	4079	4725
BHV-1 NVSL 86741	1030	1440	796	3082	3341	3240
BHV-1 NVSL 200032	1612	1942	1244	3862	3823	3749
BHV-1 LA ATCC VR188	1015	1482	802	3189	3291	2869
BHV-1 (IBR) Texas CAHFS A030020072	1157	1560	844	2995	3176	2747
EHV-1 ATCC VR2003	26	23	28	7	7	7
EHV-1 A9904309	25	17	30	6	4	6
EHV-1 A011120004 CAHFS	27	20	22	7	6	6
EHV-1 NVSL 00002	27	21	22	7	6	6
EHV-2 ATCC VR701	26	24	24	7	6	6
EHV-1 A99043047 CAHFS	26	20	19	7	7	5
EHV-2 D990 CAHFS	28	22	23	7	6	6
Pseudorabies Titered	28	20	23	8	6	6
Pseudorabies NVSL 93-11745	28	20	24	8	6	6
Pseudorabies NVSL 92-12013	25	22	22	7	6	6
Pseudorabies RA180 CAHFS	26	20	22	7	6	6
Porcine Herpes Pseudorabies Shope	21	20	23	6	7	6
Feline Herpes ATCC VR636	20	19	21	7	7	7
Caprine Herpes ATCC VR462	23	21	22	8	7	6
Caprine Herpes S0201998 CAHFS	24	26	22	7	6	6
Caprine Herpes D0201157 CAHFS	33	42	28	36	85	83
BHV-5 A040150085 CAHFS	24	24	21	7	7	6
BHV-5 A032540006 CAHFS	20	21	26	7	6	6

BHV-5 D9403153 CAHFS	24	26	20	7	7	7
BHV-5 D9402133 CAHFS	20	21	19	5	7	6
BDV Coos Bay	22	22	19	7	6	6
EHD-1 Georgia	29	20	25	8	5	6
EHD-1 New Jersey	27	21	20	7	6	6
EHD-1 Santa Barbara	26	21	19	7	5	6
EHD-2 Alberta	20	35	19	7	7	6
Fowl Pox	26	35	21	7	7	5
Parainfluenza Type 3	20	31	18	6	7	6
Respiratory Syncytial	25	24	19	7	5	6

Multiplexed PCR Assay Titrations

Upon finalization of the assay panel constituents each agent assay is characterized in multiplexed PCR format by running dose-response (titration) curves against titered virus extracted from virus infected cell culture for each available agent strain. Each sample is tested in duplicate and reported as a mean value of the median fluorescence intensity units (MFI) against agent concentration (per reaction volume, 5uL) on a double log plot. Concentration units are reported in TCID50/rxn (assumes 5uL sample volume per reaction) or pfu/rxn depending on virus type and source. In some cases where titered virus was not available data is reported as picogram units (pg). Each assay is evaluated by its ability to detect agent strains and differentiate from near neighbors (specificity) and by its range of sensitivity to each strain relative to each signature tested for that agent. Because this method is not aimed at determination of analytical sensitivities (requiring a more extensive sample set tested in clinical sample matrices which is not practical for this stage of development) it does however, provide tentative limits of detection that are used to measure the performance of each assay.

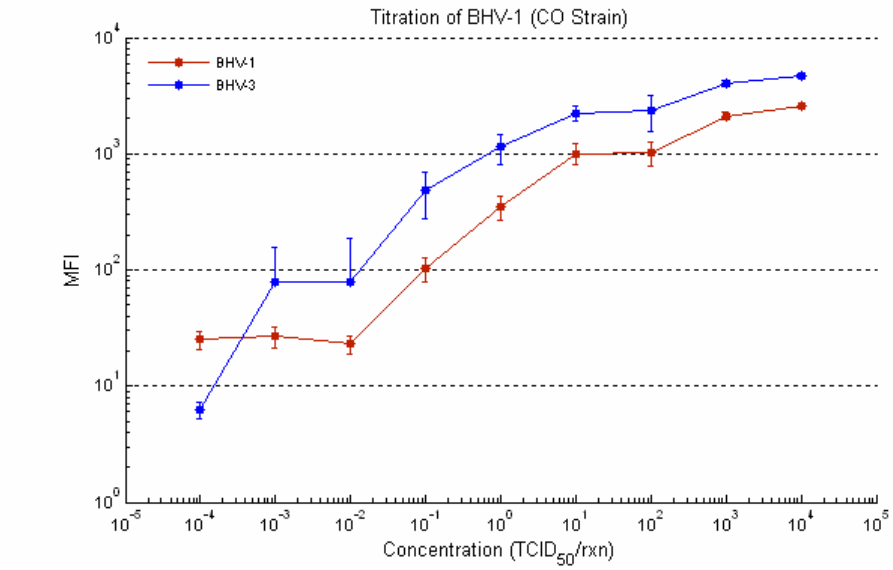

FIG. 10. Bovine multiplex screening data for the two BHV signatures against extracted nucleic acids from isolate BHV-Colorado vaccine strain. Serial dilution of nucleic acid extracted with

phenol chloroform virus-infected cell culture media then used as template. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1 of the mean.

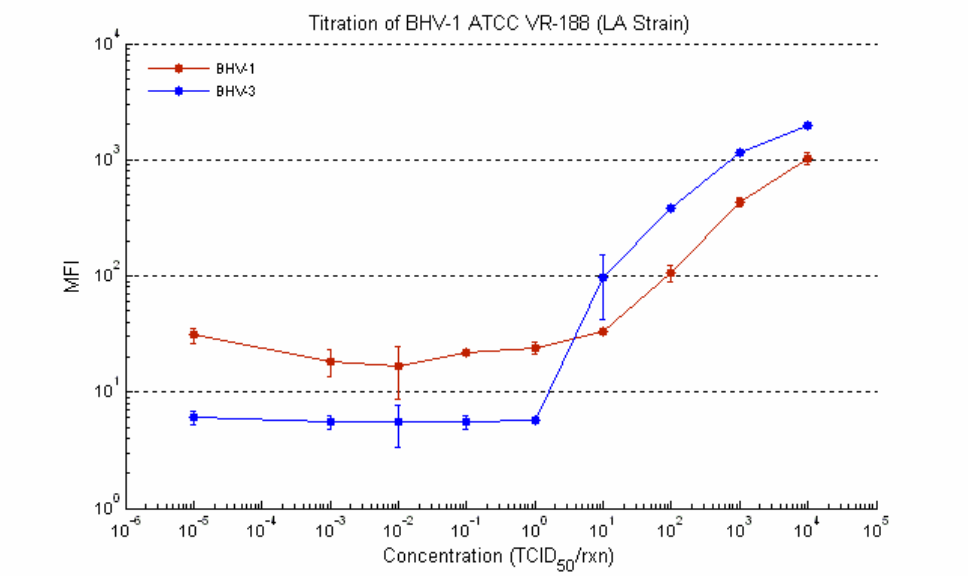

FIG. 11. Multiplex screening data for the two BHV signatures against extracted nucleic acids from isolate BHV-1 ATCC VR188 (LA strain) Serial dilution of nucleic acid extracted with phenol chloroform virus-infected cell culture media then used as template. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1 of the mean.

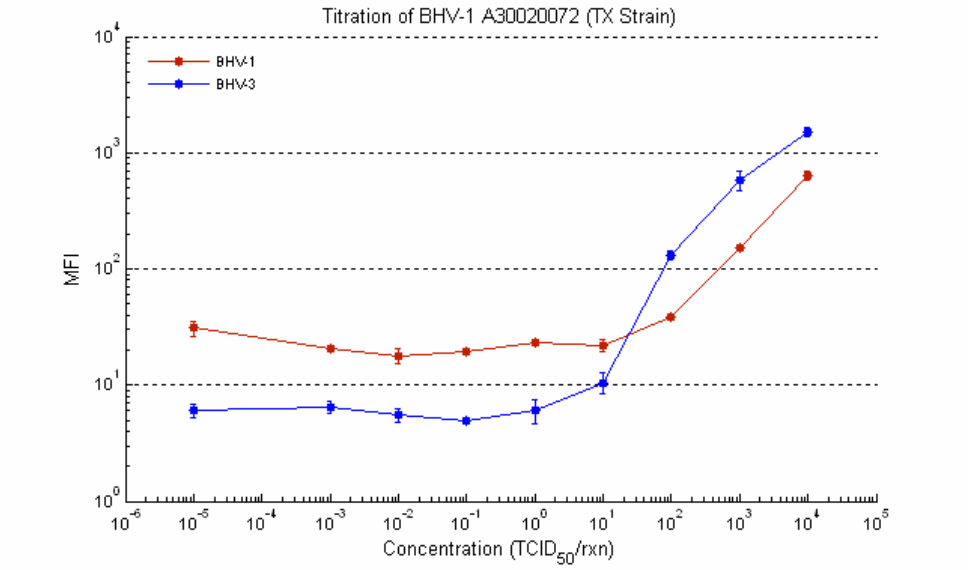

FIG. 12. Multiplex screening data for the two BHV signatures against extracted nucleic acids from isolate BHV-1 Texas strain A30020072. Serial dilution of nucleic acid extracted with

phenol chloroform virus-infected cell culture media then used as template. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1 of the mean.

RESULTS: The two Version 1.0 panel signatures were screened in the bovine panel and results indicate that these two signatures will work well with the new constituents that comprise the Bovine panel.

3. PARAPOXVIRUSES (BOVINE PANEL)

OBJECTIVE: In 2005 we were tasked by the Department of Homeland Security to develop specific and reliable Real-time RT- PCR signatures for Parapoxviruses (Bovine papular stomatitis virus, Orf, pseudocowpox), among other major agriculturally-impacting viruses that are clinically indistinguishable from foot and mouth disease virus [FMDV]. In 2006 the goal of these assays (collectively with other virus-specific signatures developed in parallel) is to develop a species specific (i.e. bovine) multiplexed detection assay for Foot-and-Mouth disease rule-out for use in veterinary diagnostic laboratories. The three signatures that were developed and incorporated into the Version 1.0 FMDV Rule-out panel have been re-evaluated for use in detection of the parapox virus BPSV and PCPV in a bovine-specific FMDV rule-out panel. This document describes both historic data for BPSV screening as well as the new 2006 data screening the 3 Parapox (called PPOX herein) signatures against the parapox virus pseudocowpox (which we were lacking titered template in 2005) and screening of the signatures for the inclusion of PCPV as a detectable multiplex target virus in the 2006 Bovine panel.

3.1. BACKGROUND AND ETIOLOGY OF POX VIRUSES

Pox viruses:
Pox diseases are acute viral diseases that affect many animals, including humans and birds, but not dogs. Typically, lesions of the skin and mucosae are widespread and progress from macules to papules, vesicles, and pustules before encrusting and healing. Most lesions contain multiple intracytoplasmic inclusions, which represent sites of virus replication in infected cells. In some poxvirus infections, vesiculation is not clinically evident, but microvesicles can be seen on histologic examination and, in some, proliferative lesions are characteristic.

Poxviruses can be classified according to their physicochemical and biologic properties. There are eleven poxvirus genera including orthopoxviruses, avipoxviruses, leporipoxviruses, suipoxviruses and parapoxviruses. Immunologically, the orthopox viruses of smallpox, cowpox, monkeypox, etc, are closely related to vaccinia virus. The avian poxviruses, the myxoma viruses, and some of the other poxviruses (e.g., swinepox) are species-specific. The viruses of orf, pseudocowpox, and bovine papular stomatitis are parapoxviruses.

Pseudocowpox: This common, mild infection of the udder and teats of cows is caused by a parapoxvirus and is widespread worldwide. The virus of pseudocowpox is related to those of contagious ecthyma (*Contagious Ecthyma: Introduction*) and bovine papular stomatitis (*Diseases of the Mouth in Large Animals: Introduction*). These parapoxviruses differ morphologically from vaccinia virus and other poxviruses. They have a limited host range and cannot be propagated in fertile eggs, and they will grow in some cell cultures although relatively poorly.

Bovine Papular Stomatitis: Viral papillomas are found around the lips and mouths of all young animals, particularly in cattle from 1 mo to 2 yr old. In some instances, the rate of occurrence may be 100%. The lesions are characteristic and usually resolve spontaneously. However, in some cases, the lesions may coalesce to form cosmetically unacceptable masses around the muzzles of young horses, and owners may request therapy. Application of cryogenetics (liquid nitrogen), use of autologous vaccines, or combinations of such therapies may be effective[3].

3.2. PARAPOX VIRUS SIGNATURE COMPREHENSIVE ASSAY SUMMARY

Bioinformatics

Virus name: Bovine Papular Stomatitis Virus and Orf
Signature generation reference: Single BPSV and contagious exanthema (Orf) genomes; intended to detect all parapox viruses.
Level of discrimination: Species-BPSV and Orf only
Number of Candidate Signatures: 8.
Number of Signatures forwarded to gel PCR bench-screening: 7.
Number of Signatures forwarded to real-time PCR bench-screening: 4.
Total number of Genome Sequences for alignment: 4.

Real-time PCR Screening Summary

TABLE 87. Final signatures down-selected in real-time RT-PCR screening (4).

LLNL Signature Designation	Sequence
none_95719.F	GCAGATGCGCTCCTGGTT
none_95720.R	GCACCTCTGCTGCTGCAA
none_95721.P	CACGTTCTCCACGTCGGAGTCGG
none_95722.F	GATGGCCGTGCAGCTCTT
none_95723.R	CGTACAAGATCACGGCCAACT
none_95724.P	CGGAAGCCCATGAGCCCGTACA

[3] *Source: The Merck Veterinary Manual*

http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/71100.htm&word=bovine%2cpapular%2cstomatitis

none_95725.F	AACAACTCCTGGCGCTTCAG
none_95726.R	AGCATGTCGCGGATGTTG
none_95727.P	CCCGCGGACTACGCCAACG
none_95731.F	GCAGCAGTGCACCACGTAGT
none_95732.R	CGCTGAACCCGTACATCCT
none_95733.P	CGCTTGTTGTCCGCCTCGAAGTC

TABLE 88. Summary of wet-bench screening in signature down-selection. Cross reactions were seen in gel screening, however no cross reactions seen in real-time PCR screening. Detailed results below in gel and real-time screening report.

	Soils	Eukaryotes	Prokaryotes	Aerosols[1]	Near Neighbors	Target
Gel Screening	4	4	none	none	7	none
Real-time PCR Screening	16	16	13	5 Aerosol Blocks	7	37

[1]There are 752 pooled samples in each Aerosol Block.

Multiplexed PCR Screening Summary

TABLE 89. ***Version 1.0 Panel Historic information.*** Final signatures from multiplexed down-selection: 3

LLNL Signature Designation	MUX Group ID	Gene ID	Reference strain[1]	Original titer of stock	Limit of detection[1]	Targets Screened[2]
none_95719.F	BPSV-1	ORF108 DNA packaging protein/ATPase	Texas A&M	10^7 $TCID_{50}$/0.1mL	1000 $TCID_{50}$/0.1mL	2
none_95722.F	BPSV-2	ORF025 DNA polymerase	Texas A&M	10^7 $TCID_{50}$/mL	10 $TCID_{50}$/0.1mL	2
none_95731.F	BPSV-4	ORF083 early transcription factor VETFL	Texas A&M	10^7 $TCID_{50}$/mL	100 $TCID_{50}$/0.1mL	2

[1] Reference strain and further information described in Multiplex results summary LOD report. [2]Only one titered virus screened, the other BPSV stock was quantified in mass (pg) units.

TABLE 90. Bovine panel backgrounds screening in multiplexed format for PPOX at LLNL and PIADC.

	Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors[2]	Targets
Mux PCR Screening	54	135	16	0	43	3

[1-]There are 752 pooled samples in each Aerosol Block. [2]Near-neighbors indicated (43) apply to the near-neighbor panel that is not uniquely specific for BHV, but for the other panel constituents that were screened concurrently.

TABLE 91. Signature summary for PPOX multiplexed signatures in the bovine panel. Nomenclature of the signatures was changed from BPSV to PPOX to provide a more accurate description of signature target specificity..

LLNL Signature Designation	MUX Group ID	Gene ID	Relative Limit of detection range[1]	Targets Screened	Near Neighbors Screened
none_95719.F	PPOX-1	ORF108 DNA packaging protein/ATPase	$5x10^{-1}$ - $1x10^{0}$ $TCID_{50}$/rxn	3	43
none_95722.F	PPOX-2	ORF025 DNA polymerase	$5x10^{-1}$ - $1x10^{0}$ $TCID_{50}$/rxn	3	43
none_95731.F	PPOX-4	ORF083 early transcription factor VETFL	$5x10^{-2}$ - $1x10^{1}$ $TCID_{50}$/rxn	3	43

[1] The relative "Limit of detection" is described as a range of lowest and highest sensitivity determined by **multiple** target strains screened and is described further in the Multiplex results summary report section.

3.3. PARAPOX SIGNATURE BIOINFORMATICS AND GENERATION INFORMATION

Bioinformatics process:
The LLNL Bioinformatics team have developed a computational process that uses available DNA sequence information for a given target organism to generate candidate DNA signature sequences that are conserved and unique. Signatures are primarily used to underlie reliable and specific pathogen detection assays. Evidence of the success of the process is that several of the Real-time PCR signatures that are used in the national BioWatch monitoring system were generated by this process, and to date, has resulted in no false positives from a total of over 3 million assays performed.

The general approach is the following: All available complete genomes of different strains of the target species are compared using a multiple genome alignment programs. A *consensus gestalt* is formed from the alignments that contain the sequence conserved amongst all target inputs. (This step is bypassed if only one target sequence is available).

To determine that organism-conserved sequence which does not occur in any other sequenced microbial organism, the consensus gestalt is compared against our immense, continuously updated database of microbial organism. A customized algorithm accomplishes this electronic subtraction, and the result is a *uniqueness gestalt* that is mined for potential signature candidates. A final computational screening is done to verify that cross-reactions are not detected.

Target Virus Information.
Virus name: Orf/Pseudocowpox/Bovine Papular Stomatitis virus.
Genome size: 139962-134431 bp.

Primer/Probe Set Generation Information
Method: Alignment and All Microbe Database subtraction
Total number of Genome Sequences for alignment: 4

TABLE 92. List of main parameters and settings used in generating candidate signatures using Primer 3 signature generation tool.

Primer3 Parameters	
Parameters	**Standard Settings**
PRIMER_OPT_SIZE	**20**

PRIMER_MIN_SIZE	**18**
PRIMER_MAX_SIZE	**27**
PRIMER_PRODUCT_OPT_SIZE	**100**
PRIMER_PRODUCT_SIZE_RANGE	**71-600**
PRIMER_OPT_TM	**62**
PRIMER_MIN_TM	**61**
PRIMER_MAX_TM	**63**
PRIMER_MIN_GC	**20**
PRIMER_MAX_GC	**80**
PRIMER_PICK_INTERNAL_OLIGO	**1**
PRIMER_INTERNAL_OLIGO_OPT_SIZE	**31**
PRIMER_INTERNAL_OLIGO_MIN_SIZE	**18**
PRIMER_INTERNAL_OLIGO_MAX_SIZE	**36**
PRIMER_INTERNAL_OLIGO_OPT_TM	**72**
PRIMER_INTERNAL_OLIGO_MIN_TM	**71**
PRIMER_INTERNAL_OLIGO_MAX_TM	**73**
Number of Primers/Probe Set generated:	**8**

TABLE 93. K-path run id: 19963. Total Number of Genome Sequences for alignment: 4. Reference Genome is Sequence 1 from Patent WO03006654 gi|32167392.

	Genome Description	GI Number	Sequence Length (bp)	K-path ID
1	Sequence 1 from Patent WO03006654	32167392	137560	19963
2	Raw sequence of Orf virus OV-IA82 from Dan Rock on 10/21/03	40019122	137241	19963
3	Raw sequence of Orf virus OV-SA00 from Dan Rock on 10/21/03	40019123	139962	19963
4	Raw sequence of BPSV BV-AR02 from Dan Rock on 10/21/03	40019124	134431	19963

Signature Information

Source: LLNL
Project name: BPSV and Orf Virus.
Level of discrimination: Species-BPSV and Orf.

Number of Candidate Signatures: 8.
Number of Signatures forwarded to gel PCR bench-screening: 7.
Number of Signatures forwarded to real-time PCR bench-screening: 7.
Number of Final Signatures forwarded to multiplex ed development: 4.

Signature list.
Note: For a listing of computationally predicted product sequences, please see attached document: "BPSV Taqsim Run Data".

Taqsim description

We used a computational TaqMan simulator program, "Taqsim", to identify all potential targets for each signature from all sequences available in genbank. Taqsim is a BLAST-based comparison of each signature as a triplet against all sequences in genbank to identify the targets that are predicted to produce a TaqMan reaction at 57 degrees primer annealing and 67 degrees for probe annealing (these temperatures are according to Primer 3 oligo Tm calculations). The first column of the excel spreadsheets list the signatures by the name, and the following columns report the predicted genbank targets, coordinates of signature region, amplicon size, and accession #'s for each target.

All sequences are listed in the 5'→ 3' direction.

TABLE 94a-d.. Signature bioinformatics for parapoxvirus bovine papular stomatitis virus BV-AR02 (a) none_95719.F (aka BPSV_95719.F) (b) none_95722.F (aka BPSV_95722.F) (c) none_95725.F (aka BPSV_95725.F) (d) none_95731.F aka BPSV_95731.F)

(a)

Target Virus	**Parapoxvirus Bovine papular stomatitis virus BV-AR02**
Forward Primer	PPOX_95719.F (aka BPSV_95719.F)
FWD Primer Length (bp)	18
FWD Primer TM (^{o}C)	62
FWD Primer GC Content (%)	61
Forward Sequence	GCAGATGCGCTCCTGGTT
Reverse Primer	PPOX_95720.R (aka BPSV_95720.R)
Rev Primer Length (bp)	18
Rev Primer TM (^{o}C)	62
Rev Primer GC Content (%)	61
Reverse Sequence	GCACCTCTGCTGCTGCAA
Probe Name	PPOX_95721.P (aka BPSV_95721.P)
Probe Length (bp)	23
Probe TM (^{o}C)	72
Probe GC Content (%)	65
Probe Sequence	CACGTTCTCCACGTCGGAGTCGG
Probe strand	plus
Predicted Product Size	178

(b)

Target Virus	**parapoxvirus Bovine papular stomatitis virus BV-AR02**
Forward Primer	PPOX_95722.F (aka BPSV_95722.F)
FWD Primer Length (bp)	18
FWD Primer TM (^{o}C)	62
FWD Primer GC Content (%)	61
Forward Sequence	GATGGCCGTGCAGCTCTT
Reverse Primer	PPOX_95723.R (aka BPSV_95723.R)
Rev Primer Length (bp)	21
Rev Primer TM (^{o}C)	62
Rev Primer GC Content (%)	52

Reverse Sequence	CGTACAAGATCACGGCCAACT
Probe Name	PPOX_95724.P (aka BPSV_95724.P)
Probe Length (bp)	22
Probe TM (^{o}C)	72
Probe GC Content (%)	63
Probe Sequence	CGGAAGCCCATGAGCCCGTACA
Probe strand	plus
Predicted Product Size	95

(c)

Target Virus	**parapoxvirus Bovine papular stomatitis virus BV-AR02**
Forward Primer	PPOX_95725.F (aka BPSV_95725.F)
FWD Primer Length (bp)	20
FWD Primer TM (^{o}C)	62
FWD Primer GC Content (%)	55
Forward Sequence	AACAACTCCTGGCGCTTCAG
Reverse Primer	PPOX_95726.R (aka BPSV_95726.R)
Rev Primer Length (bp)	18
Rev Primer TM (^{o}C)	61
Rev Primer GC Content (%)	55
Reverse Sequence	AGCATGTCGCGGATGTTG
Probe Name	PPOX_95727.P (aka BPVS_95727.P)
Probe Length (bp)	19
Probe TM (oC)	71
Probe GC Content (%)	73
Probe Sequence	CCCGCGGACTACGCCAACG
Probe strand	plus
Predicted Product Size	147

(d)

Target Virus	**parapoxvirus Bovine papular stomatitis virus BV-AR02**
Forward Primer	PPOX_95731.F aka BPSV_95731.F)
FWD Primer Length (bp)	20
FWD Primer TM (^{o}C)	62
FWD Primer GC Content (%)	60
Forward Sequence	GCAGCAGTGCACCACGTAGT
Reverse Primer	PPOX_95732.R (aka BPSV_95732.R)
Rev Primer Length (bp)	19
Rev Primer TM (^{o}C)	61
Rev Primer GC Content (%)	57
Reverse Sequence	CGCTGAACCCGTACATCCT
Probe Name	PPOX_95733.P aka BPSV_95733.P)
Probe Length (bp)	23
Probe TM (oC)	71
Probe GC Content (%)	60

Probe Sequence	CGCTTGTTGTCCGCCTCGAAGTC
Probe strand	plus
Predicted Product Size	167

Target Region Gene Information

TABLE 95a-b. (a) Reference Genomes used for Gene Information. (b) Target region gene information from annotated genomes available.

(a)

Genome Description	GI Number	Sequence Length (bp)
Orf_virus_complete_genome	41057066/NC_005336_1.	139962

(b)

				Gene Location	
kpath Primer ID	**Primer**	**Gene**	**Description**	**Start**	**End**
935544	PPOX_95719	ORF108 DNA packaging protein/ATPase	similar to Vaccinia virus strain Copenhagen A32L and Molluscum contagiosum virus MC140L	110777	111601
935545	PPOX_95722	ORF025 DNA polymerase	similar to Vaccinia virus strain Copenhagen E9L and Molluscum contagiosum virus MC039L	24637	27675
935546	PPOX_95725	ORF068 NTPase	DNA replication; similar to Vaccinia virus strain Copenhagen D5R and Molluscum contagiosum virus MC094R	69391	71754
935549	PPOX_95731	ORF083 early transcription factor VETFL	similar to Vaccinia virus strain Copenhagen A7L and Molluscum contagiosum virus MC110L	86324	88849

3.4. PARAPOX VIRUS GEL AND REAL-TIME PCR SCREENING REPORT

Wet-lab screening process. To ensure extremely high selectivity and sensitivity, we have established a screening protocol that rigorously screens candidate DNA signatures to select the optimal sets for eventual field use. Candidate signature primer pairs that survive computational screening proceed to wet chemistry screening utilizing standard PCR with agarose gel electrophoresis. This step ensures that the primers will react across strains representative of the diversity of the pathogen (avoid false negative), but will not react with the nucleic acids of other organisms that could be present in a sample (avoid false positives). The collection of purified DNA templates used in this screening process is listed below:

1) Strain panels representative of the diversity of the target organism in nature. We have a panel of 37 BPSV strains
2) Near neighbors-organisms that are closely related at the genetic level and therefore have the greatest likelihood of cross-reaction. We screened the candidate signatures against 7 near-neighbor isolates.

3) Eukaryotic DNA that may carry over from sample collection procedures.
4) Prokaryotic DNA that would be expected to be present in environmental samples and may also be present in Bovine and Porcine oral cavities.
5) Fifteen soil samples from around the country representing diverse climates and contain a complex mixture of organisms from diverse environmental collections
6) Aerosol samples: 752 samples collected from aerosol collectors and pooled for background testing purposes.

Based on data from primer pair screening, a set of **4** specific and reliable signatures were then further tested for suitability for real-time TaqMan fluorogenic PCR detection protocols. The selected signatures showed a robust signal in all target reactions.

TABLE 96. List of Near-neighbors screened. Detailed information about the history of these targets is not known. This work pre-dates current project management and data is not available.

Virus	Strain/ID[1]	Source	Original Titer	Virus History	Extraction date/ID	Extraction Method	Use stock titer	Titer Method
Goatpox	V717 Pendik 26-11-1976	PIADC	Unknown	Unknown	Unknown	Phenol/Chloroform	ng/uL	N/A
GoatpoxVirus	Held	PIADC	Unknown	LT5 05-05-1975	Unknown	Phenol/Chloroform	ng/uL	N/A
Kenyan SheepPox	Kenya Sheep/goat pox tissue pool	PIADC	Unknown	LT-1 04-18-1977	Unknown	Phenol/Chloroform	ng/uL	N/A
Kenyan Vaccine Strain SheepPox	0-180 V2164	PIADC	Unknown	15LK 1EBL 3 Vero masterseed V2164 Aug 1989 serial 8910	Unknown	Phenol/Chloroform	ng/uL	N/A
Nigerian GoatPox	G165 1683 07-17-1974	PIADC	Unknown	20% suspension made 07-17-74	Unknown	Phenol/Chloroform	ng/uL	N/A
Sheeppox	Held V2990	PIADC	Unknown	6LT, 1SCP, 1LT X-3542 Oct-04-2002	Unknown	Phenol/Chloroform	ng/uL	N/A
Sheeppox	X783 6LT	PIADC	Unknown	6LT, 1SCP, for sheep inoc. X783 03-Dec-87	Unknown	Phenol/Chloroform	ng/uL	N/A

TABLE 97. List of Targets screened. Detailed information about the history of these targets is not known. This work pre-dates current project management and data is not available.

Virus	Strain/ID[1]	Source	Original Titer	Virus History	Extraction date/ID	Extraction Method	Use stock titer	Titer Method
BPSV 1	**Illinois 721**	ATCC	Unknown	Unknown	Unknown	Phenol/Chloroform	ng/uL	N/A
orf/BPSV	(1) PIADC FADDL# 34768	PIADC	Unknown	+ 70	Unknown	Phenol/Chloroform	ng/uL	N/A
orf/BPSV	(2) PIADC FADDL#	PIADC	Unknown	Unknown	Unknown	Phenol/Chloroform	ng/uL	N/A

	34576							
orf/BPSV	(3) PIADC FADDL# 34355	PIADC	Unknown	+ IBRS2	Unknown	Phenol/Chloroform	ng/uL	N/A
orf/BPSV	(4) PIADC FADDL# 34355	PIADC	Unknown	+ LK	Unknown	Phenol/Chloroform	ng/uL	N/A
orf/BPSV	(5) PIADC FADDL# 34132	PIADC	Unknown		Unknown	Phenol/Chloroform	ng/uL	N/A
orf/BPSV	(6) PIADC FADDL# 31096	PIADC	Unknown		Unknown	Phenol/Chloroform	ng/uL	N/A
orf/BPSV	(7) PIADC FADDL# 35458	PIADC	Unknown	+ LK Passage 1	Unknown	Phenol/Chloroform	ng/uL	N/A
orf/BPSV	(8) PIADC FADDL# 35458	PIADC	Unknown	+ IBRS2	Unknown	Phenol/Chloroform	ng/uL	N/A
orf/BPSV	(9) PIADC FADDL# 3383	PIADC	Unknown		Unknown	Phenol/Chloroform	ng/uL	N/A
orf/BPSV	(10) PIADC FADDL# 3703	PIADC	Unknown	+ IBRS2	Unknown	Phenol/Chloroform	ng/uL	N/A
orf/BPSV	(11) PIADC FADDL# 3703	PIADC	Unknown	+ LK	Unknown	Phenol/Chloroform	ng/uL	N/A
orf/BPSV	(12) PIADC FADDL# 3702	PIADC	Unknown	+IBRS2	Unknown	Phenol/Chloroform	ng/uL	N/A
orf/BPSV	(13) PIADC FADDL# 3702	PIADC	Unknown	+ LK	Unknown	Phenol/Chloroform	ng/uL	N/A
orf/BPSV	(14) PIADC FADDL# 3360	PIADC	Unknown	+ IBRS2	Unknown	Phenol/Chloroform	ng/uL	N/A
orf/BPSV	(15) PIADC FADDL# 3360	PIADC	Unknown	+ LK	Unknown	Phenol/Chloroform	ng/uL	N/A
orf/BPSV	(16) PIADC FADDL# 1337	PIADC	Unknown		Unknown	Phenol/Chloroform	ng/uL	N/A
orf/BPSV	(17) PIADC FADDL# 12433	PIADC	Unknown	Homogenate	Unknown	Phenol/Chloroform	ng/uL	N/A
orf/BPSV	(18) PIADC FADDL# 3383	PIADC	Unknown	+ IBRS2	Unknown	Phenol/Chloroform	ng/uL	N/A
orf/BPSV	(19) PIADC FADDL# 9048	PIADC	Unknown	+ IBRS2 Passage 1	Unknown	Phenol/Chloroform	ng/uL	N/A
orf/BPSV	(20) PIADC FADDL# 9048	PIADC	Unknown	+ LK	Unknown	Phenol/Chloroform	ng/uL	N/A
orf/BPSV	(21) PIADC FADDL# 15740	PIADC	Unknown	homogenate	Unknown	Phenol/Chloroform	ng/uL	N/A
orf/BPSV	(22) PIADC FADDL# 17234	PIADC	Unknown	+IBRS2	Unknown	Phenol/Chloroform	ng/uL	N/A
orf/BPSV	(23) PIADC FADDL# 17234 + LK	PIADC	Unknown	+ LK	Unknown	Phenol/Chloroform	ng/uL	N/A
orf/BPSV	(24) PIADC FADDL#	PIADC	Unknown	homogenate	Unknown	Phenol/Chloroform	ng/uL	N/A

	15741 homogenate							
orf/BPSV	(25) PIADC FADDL# 17206	PIADC	Unknown	+ IBRS2	Unknown	Phenol/Chloroform	ng/uL	N/A
orf/BPSV	(26) PIADC FADDL# 17206 + LK	PIADC	Unknown	+ LK	Unknown	Phenol/Chloroform	ng/uL	N/A
orf/BPSV	(27) PIADC FADDL# 19814	PIADC	Unknown	+ LK	Unknown	Phenol/Chloroform	ng/uL	N/A
orf/BPSV	(28) PIADC FADDL# 19814	PIADC	Unknown	+ IBRS2	Unknown	Phenol/Chloroform	ng/uL	N/A
orf/BPSV	(29) PIADC FADDL# 34368	PIADC	Unknown	+ 70 + IBRS2	Unknown	Phenol/Chloroform	ng/uL	N/A
orf/BPSV	(30) PIADC FADDL# 34767	PIADC	Unknown	+ 69 + IBRS2	Unknown	Phenol/Chloroform	ng/uL	N/A
orf/BPSV	(31) PIADC FADDL# 9048	PIADC	Unknown	+ IBRS2 Passage 2	Unknown	Phenol/Chloroform	ng/uL	N/A
orf/BPSV	(32) PIADC FADDL# 34613	PIADC	Unknown	+ LK Passage 2	Unknown	Phenol/Chloroform	ng/uL	N/A
orf/BPSV	(33) PIADC FADDL# 34767	PIADC	Unknown	+ 69 + LK	Unknown	Phenol/Chloroform	ng/uL	N/A
Parapoxvirus Pseudocowpox	**TJS**	PIADC	Unknown	Unknown	Unknown	Phenol/Chloroform	ng/uL	N/A
Parapoxvirus orf or CE	**CE 79-16151**	PIADC	Unknown	Unknown	Unknown	Phenol/Chloroform	ng/uL	N/A
Parapoxvirus orf or CE	**CE D03011040**	ATCC	Unknown	Unknown	Unknown	Phenol/Chloroform	ng/uL	N/A

3.4.1. Parapox (orf.BPSV) - Gel Screening Report

TABLE 98. Nucleic acid extracts used to challenge the initial set of 8 candidate signatures. These select backgrounds are used to determine which signatures will be down-selected against in the screening process. No prokaryotes were screened. All samples were screened in duplicate.

Nucleic Acid Extract Type	Description/ID
Soil Extract	D000527
Soil Extract	D000528
Soil Extract	D000529
Soil Extract	D000532
Eukaryotic DNA Extract	Tick
Eukaryotic DNA Extract	Porcine
Eukaryotic DNA Extract	Rat
Eukaryotic DNA Extract	Bovine

TABLE 99. The cross reactions against backgrounds for the four signatures included in the final assay. Data is expressed as number of bands observed, for example 3X would indicate 3 different bands were observed. "0" indicates no bands were observed.

	95713.F 95714.R	95716.F 95717.R	95719.F 95720.R	95722.F 95723.R	95725.F 95726.R	95728.F 95729.R	95731.F 95732.R
Soil D000527	4x	Smear	Smear	3x	3x	2x	3x
Soil D000528	5x	Smear	>500	3x	Smear	>500	>500
Soil D000529	2x	3x	0	2x	0	0	0
Soil D000532	2x	>300	3x	3x	0	0	0
Bovine	>300	>500	>500	>400	>500	0	>500
Porcine	2x	3x	2x	>300	0	>300	>400
Tick	0	3x	2x	2x	0	0	0
Rat	2x	>300	0	>300	0	0	0

TABLE 100. Results of near-neighbor screening against all 7 candidate signatures. Out of the initial 7 signatures generated, four (highlited) continued to Real-time PCR screening. Data is expressed as number and size of bands observed, "0" indicates no bands were observed. Signature down-selection at this phase of testing was determined by Mr. Gordon Ward at PIADC who indicated that the others were not target specific.

	95713.F 95714.R	95716.F 95717.R	95719.F 95720.R	95722.F 95723.R	95725.F 95726.R	95728.F 95729.R	95731.F 95732.R
Predicted Product Size	78bp	189bp	178bp	95bp	147bp	156bp	167bp
Goatpox V717 Pendik 26-11-1976	0	1x>1kb	35bp	40bp	0	0	40bp
GoatpoxVirus Held LT5 05-05-1975	40bp	0	0	50bp	1kb	0	0
Kenyan SheepPox LT1 04-18-1977	>1kb	>1kb	40bp	2x>1kb	150bp 3x>1kb	0	2x<60bp
Kenyan Vaccine Strain SheepPox 0-180 15LK V2164	40bp	0	0	200bp	0	0	0
Nigerian GoatPox G165 1683 07-17-1974	0	500bp	160bp 2x>1kb	160bp 1x>1kb	150bp 1X>1kb, 40bp	0	40bp
Sheeppox Held V2990	0	0	0	0	0	0	0
SheeppoxX783 6LT	0	40bp	0	1x>1kb	0	0	40bp

Parapox (orf.BPSV) - Real-time PCR Screening Report

TABLE 101. List of signatures chosen to undergo intensive background screening in Real-time PCR Format.

PPOX_95719.F	GCAGATGCGCTCCTGGTT
PPOX_95720.R	GCACCTCTGCTGCTGCAA
PPOX_95721.P	CACGTTCTCCACGTCGGAGTCGG
PPOX_95722.F	GATGGCCGTGCAGCTCTT
PPOX_95723.R	CGTACAAGATCACGGCCAACT
PPOX_95724.P	CGGAAGCCCATGAGCCCGTACA
PPOX_95725.F	AACAACTCCTGGCGCTTCAG
PPOX_95726.R	AGCATGTCGCGGATGTTG
PPOX_95727.P	CCCGCGGACTACGCCAACG

PPOX_95731.F	GCAGCAGTGCACCACGTAGT
PPOX_95732.R	CGCTGAACCCGTACATCCT
PPOX_95733.P	CGCTTGTTGTCCGCCTCGAAGTC

TABLE 102a-d. Real-time RT- PCR background screening, all performed in duplicate against an extensive list of soil, Prokaryotic and Eukaryotic extracted DNAs in addition to 3 aerosol blocks, each containing 752 samples. All signatures passed real-time RT- PCR background screening. None of the 4 signatures produced an amplicon when screened against the listed backgrounds. All signatures moved forward to be screened against the available BPSV targets and near neighbors.

(a) Total of 16 soils screened

D000521	D000052	D000527	D000086	D000532	D000117	D000562	D000407
D000528	D000101	D000529	D000102	D000561	D000402	D000019	D000564

(b) Total of 16 Eukaryotes screened

Bovine	Cat	Chicken	Dog	Drosophila	Equine	Flea	Human
Monkey	Mosquito	Mouse	Pig	Rabbit	Rat	Sheep	Tick

(c) Total of 13 Prokaryotes screened

E. herbicola	*B. cereus*	*B. globigii*	*S. aureus*	*S. pneumonia*
E. coli	*C. burnetti*	*S. typhimurium*	*B. subtilis*	
P. aeruginosa	*B. burgdorferi*	*L. monocytogenes*	*H. influenza*	

(d)

Aerosol Block	Signatures Screened	Number of Samples in Block
4 080403	4	752
5 072003	2	752
18 062804-061204	2	752
Total:	**4**	**1504 samples**

TABLE 103. List of signatures screened in Real-time PCR format against available targets and near neighbors.

PPOX_95719.F	GCAGATGCGCTCCTGGTT
PPOX_95720.R	GCACCTCTGCTGCTGCAA
PPOX_95721.P	CACGTTCTCCACGTCGGAGTCGG
PPOX_95722.F	GATGGCCGTGCAGCTCTT
PPOX_95723.R	CGTACAAGATCACGGCCAACT
PPOX_95724.P	CGGAAGCCCATGAGCCCGTACA
PPOX_95725.F	AACAACTCCTGGCGCTTCAG
PPOX_95726.R	AGCATGTCGCGGATGTTG
PPOX_95727.P	CCCGCGGACTACGCCAACG
PPOX_95731.F	GCAGCAGTGCACCACGTAGT
PPOX_95732.R	CGCTGAACCCGTACATCCT
PPOX_95733.P	CGCTTGTTGTCCGCCTCGAAGTC

TABLE 104. Four signatures were screened against 200pg per reaction of targets (shown in bold font) and near-neighbors in real-time PCR format. [1]Some of the real-time PCR testing is supplemented from testing that was performed against the signatures prior to obtaining the other virus stocks of targets and near-neighbors, as a result, in some instances; a different number of replicates were performed. N/A indicates that no CT value was recorded for that sample, indicating that when tested, no reaction took place.

Template	95719.F 95720.R 95721.P	95722.F 95723.R 95724.P	95725.F 95726.R 95727.P	95731.F 95732.R 95733.P
BPSV 1 ATCC #VR-801	**19.1, 20.5,19.7**	**0, 21.9, 22**	**20.1, 24, 20.5**	**18.8, 18.2, 18**
Parapox orf.BPSV 3702 (12) Plum Island	**23.99**	**34.18: weak**	**25.32**	**25.14**
Parapox orf.BPSV (11) Plum Island	**20.57**	**22.17**	**21.27**	**21.55**
Parapox orf.BPSV (14) Plum Island	**21.32**	**22.74**	**21.3**	**22.35**
Parapox orf.BPSV (15) Plum Island	**21.15**	**22.49**	**20.62**	**21.7**
Parapox orf.BPSV (16) Plum Island	**22.08**	**22.94**	**21.96**	**22.64**
Parapox orf.BPSV (17) Plum Island	**34.85: weak**	**N/A**	**N/A**	**34.77**
Parapox orf.BPSV (18) Plum Island	**22.86**	**25.48**	**23.72**	**23.99**
Parapox orf.BPSV (19) Plum Island	**22.55**	**24.81**	**23.43**	**23.88**
Parapox orf.BPSV (20) Plum Island	**21.9**	**23.43**	**22.34**	**23.65**
Parapox orf.BPSV (21) Plum Island	**17.32**	**19.78**	**18.15**	**18.64**
Parapox orf.BPSV (22) Plum Island	**21.62**	**22.7**	**21.91**	**23**
Parapox orf.BPSV (23) Plum Island	**21.84**	**23.08**	**22.09**	**23.07**
Parapox orf.BPSV (24) Plum Island	**18.92**	**20.37**	**19.06**	**19.83**
Parapox orf.BPSV (25) Plum Island	**17.4**	**20.26**	**17.97**	**18.43**
Parapox orf.BPSV (27) Plum Island	**22.95**	**25.04**	**23.2**	**23.37**
Parapox orf.BPSV (28) Plum Island	**22.73**	**24.84**	**23.02**	**23.04**
Parapox orf.BPSV (29) Plum Island	**24.32**	**26.73**	**25.12**	**25.41**
Parapox orf.BPSV (3) Plum Island	**23.66**	**26.76**	**23.44**	**24.43**
Parapox orf.BPSV (30) Plum Island	**20.8**	**25.17**	**23.66**	**24.16**
Parapox orf.BPSV (31) Plum Island	**26.69**	**N/A**	**28.05**	**28.21**
Parapox orf.BPSV (32) Plum Island	**28.2**	**N/A**	**28.89**	**29.13**
Parapox orf.BPSV (33) Plum Island	**22.81**	**25.01**	**23.82**	**28.87**
Parapox orf.BPSV (8) Plum Island	**22.81**	**21.61**	**21.59**	**23.93**
Parapox orf.BPSV 213104 (13) Plum Island	**24.9**	**N/A**	**25.55**	**25.43**
Parapox orf.BPSV 26 Plum Island	**17.14, 18.26**	**19.2, 20.36**	**17.37, 18.59**	**18.1 19.17**
Parapox orf.BPSV 31090 (6) Plum Island	**25.33**	**32.72: weak**	**25.45**	**25.72**
Parapox orf.BPSV 3383 (9) Plum Island	**22.47**	**26.14**	**23.11**	**23.44**
Parapox orf.BPSV 34132 (5) Plum Island	**27.02**	**N/A**	**27.25**	**27.24**
Parapox orf.BPSV 34355 (4) Plum Island	**23.43**	**25.5**	**23.73**	**24.95**
Parapox orf.BPSV 34576 (2) Plum Island	**N/A**	**N/A**	**N/A**	**37**
Parapox orf.BPSV 34768(1) Plum Island	**22.43**	**26.01**	**23.62**	**23.32**
Parapox orf.BPSV 35458(7) Plum Island	**23.98**	**24.36**	**23.09**	**24.28**
Parapox orf.BPSV 3703 (10) Plum Island	**20.87**	**23.39**	**22.01**	**22.05**
Parapoxvirus Pseudocowpox ATCC VR-634	**24.3, 23.9 24.2**	**28, 27.9, 28**	**27.9, 27.8, 27.7**	**28.1, 28.1, 28.4**
Parapoxvirus orf or CE CE 79-16151	**24.3**	**24.7**	**26.8**	**30.5**

Parapoxvirus orf or CE CE D03011040	**25, 20.1, 20, 20.9**	**24.2, 25,25.1, 25.3**	**27.9, 26.1, 26.1, 26**	**29.3, 21.1, 20, 21**
Parapox Parapox Goatpox V717 Pendik 26-11-1976	N/A	N/A	N/A	N/A
Parapox Parapox GoatpoxVirus Held LT5 05-05-1975	N/A, N/A	N/A, N/A	N/A, N/A	N/A, N/A
Parapox Parapox Kenyan SheepPox LT1 04-18-1977	N/A	N/A	N/A	N/A
Parapox Parapox Kenyan Vaccine Strain SheepPox 0-180 15LK V2164	N/A	N/A	N/A	N/A
Parapox Parapox Nigerian GoatPox G165 1683 07-17-1974	N/A	N/A	N/A	N/A
Parapox Parapox Sheeppox Held V2990	N/A	N/A	N/A	N/A
Parapox Parapox Sheeppox X783 6LT	N/A	N/A	N/A	N/A

*Note the number of Ct values denotes the number of replicate screenings against the particular template.
** For greater detail on screening data refer to gel pictures and iCycler data files on LiveLink

TABLE 105. In order to determine relative limits of detection for each Real-time PCR signature developed, a dilution series of nucleic acid extract from non-titered virus was made of 4 logs across the predicted linear dynamic range. The diluted nucleic acid extracts were then tested with each signature using the standard Real-time PCR protocol in triplicate and average Ct values are reported for each dilution. [Note: The LODs reported here are not absolute LODs. Rather, they are relative LODs comparing one signature with another]. CT value at the LOD is highlited in green.

	95721	**95724**	**95727**	**95733**
Pseudo cowpox VR634 1:10	**29.8**	32.3	**32.5**	**36.5**
Pseudo cowpox VR634 1:100	N/A	**35.6**	N/A	N/A
Pseudo cowpox VR634 1:1K	N/A	N/A	N/A	N/A
Pseudo cowpox VR634 1:10K	N/A	N/A	N/A	N/A
Orf CE 79-16151 1:10	28.2	30.9	32.6	32.1
Orf CE 79-16151 1:100	**31.5**	**35.6**	**35.8**	**36**
Orf CE 79-16151 1:1K	N/A	N/A	N/A	N/A
Orf CE 79-16151 1:10K	N/A	N/A	N/A	N/A
BPSV VR 801 1:10	23.3	25.4	28.1	23.1
BPSV VR 801 1:100	26.7	29.8	31.3	26.9
BPSV VR 801 1:1K	**29.9**	**32.6**	**35.3**	**29.4**
BPSV VR 801 1:10K	N/A	N/A	N/A	N/A

TABLE 106. Summary Table of Signature LODs on: BPSV

	95721	**95724**	**95727**	**95733**
Pseudo cowpox VR634	1:10	1:100	1:10	1:10
Orf CE 79-16151	1:100	1:100	1:100	1:100
BPSV VR 801	1:1K	1:1K	1:1K	1:1K

3.5. PARAPOX VIRUS MULTIPLEXED PCR SCREENING REPORT

SCREENING AND DOWN-SELECTION TECHNICAL APPROACH:

Signatures that pass gel and real-time PCR screening are candidates for multiplexed assays. If the number of candidate signatures is large than a subset of the available signatures are selected

based on measured sensitivity and specificity observed in real-time screening. In preliminary screening the background of individual signatures is measured by running "blank" (no sample added) controls and observing signature response. In some cases a primer-to-primer non-specific interference will eliminate candidate signatures. Signatures are then added iteratively to each multiplexed panel, titrated, and with each signature addition, collectively, all signatures are re-titrated to determine if the presence of one signature will interfere with the performance of another. During this process signatures are added or subtracted from the multiplexed panel depending on individual outcomes until all desirable signatures have been added.

Criterion for down-selection of multiplexed signatures

Signature specificity. In initial real-time PCR screening signatures undergo target and near neighbor screening and screening against environmental confounders which assess individual signature specificity. In multiplex format this is repeated to verify that a more complex reaction system gives the same level of specificity.

Signature sensitivity. In initial real-time PCR screening signatures undergo limit of detection testing where a dilution curve is generated for each signature. This determines individual signature sensitivity. In multiplex format this is repeated to verify that a more complex reaction system gives acceptable signature sensitivity.

Optimal signal to noise ratio. In the multiplex assay system the potential for cross reactivity is larger than in single reaction testing, this may cause unwanted PCR reaction products or non-specific cross-reactivity. Signatures are preferred to have a high signal to noise ratio, where desired signal is significantly greater than the background noise of the signature when there is no target nucleic acid in the reaction.

Compatibility with other signatures in multiplex. In some cases a primer set may interfere with other primers or probes in the multiplex and there by cause limit of detection shifting for one or more signature in the multiplex. For each scenario it must be evaluated whether the shift in detection level is significant enough to remove (what could be a very desirable signature) from the panel.

TABLE 107. Order details for BHV signatures ordered for multiplexed assay screening and development.

ID	Modification details	Vendor
BPSV_95719.F	5'-/5Bio/GCAGA/iBiodT/GCGCTCC/iBiodT/GGTT-3'	IDT DNA
BPSV_95720.R	5' -GCACCTCTGCTGCTGCAA -3'	IDT DNA
BPSV_95721.FCP	5'- /5AmMC6//iSp18/CCGACTCCGACGTGGAGAACGTG-3'	IDT DNA
BPSV_95722.F	5'-/5Bio/GATGGCCG/iBiodT/GCAGC/iBiodT/CTT-3'	IDT DNA
BPSV_95723.R	5' - CGTACAAGATCACGGCCAACT-3'	IDT DNA
BPSV_95724.FCP	5'- /5AmMC6//iSp18/TGTACGGGCTCATGGGCTTCCG-3'	IDT DNA
BPSV_95731.F	5'-/5Bio/GCAGCAG/iBiodT/GCACCACG/iBiodT/AGT-3'	IDT DNA
BPSV_95732.R	5' -CGCTGAACCCGTACATCCT -3'	IDT DNA
BPSV_95733.FCP	5'- /5AmMC6//iSp18/GACTTCGAGGCGGACAACAAGCG-3'	IDT DNA

Multiplex Data Analysis -Technical approach

Disease signature thresholds

Thresholds were initially determined using all available data from known negative samples (blanks) by plotting a histogram, which showed the probability of a given sample generating a particular MFI. In the absence of cross reactivity, each signature in the multiplex assay could be treated as an individual signature result. For example, for a sample spiked with RPV, data for other disease signatures could contribute towards negative data, providing that cross reaction between disease signature sets was not observed. This is a way to increase the number of samples that can be used to establish thresholds.

TABLE 108. Individual signature thresholds and ranges for PPOX signatures. For FY06, threshold determinations have not yet been made and require a significant number of tests (>500) to generate this information. This information will be updated when it is available.

Signature Name	Mux Assay name	Panel Membership	Signature background in Tris NaCl Buffer (MFI +/-)	Signature background in Clinical matrices (MFI +/-)	Threshold
PP0X_95719.F	PPOX-1	Bovine	TBD	TBD	TBD
PPOX_95722.F	PPOX-2	Bovine	TBD	TBD	TBD
PPOX_95731.F	PPOX-4	Bovine	TBD	TBD	TBD

TABLE 109. List of targets screened in multiplex. The below reference virus for BPSV was acquired after real-time screening had been completed and as such was not previously tested in that format. Virus shown in red was used as the reference standard for signature characterization.

Virus	Serotype	Strain/ID[1]	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	Use stock titer	Titer Method
BPSV	N/A	Texas A&M	NVSL	10^7TCID$_{50}$/0.1 mL	Unknown	12/2005	Phenol/Chloroform	3.49x10^6 TCID$_{50}$/0.1mL	Reed-Meunch
BPSV 1	N/A	Illinois 721	ATCC	N/A	Unknown	Unknown	Unknown	N/A total NA	N/A (pg/uL)
Orf	N/A	vaccine strain	NVSL	N/A	Unknown	Unknown	Unknown	N/A total NA	N/A (pg/uL)
Pseudocowpox	N/A	VR634	NVSL	N/A	Unknown	Unknown	Unknown	N/A total NA	N/A (pg/uL)

TABLE 110. List of additional targets and near-neighbors screened against the Bovine Panel. All near-neighbors samples were extracted nucleic acids with unknown titers.

Virus	Strain[1]	Unique ID	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	NA conc. used or stock titer	Titer Method
Border Disease virus	Coos Bay	4-6-92	NADC, Julia Ridpath	1.5 x 10^8 TCID50/mL	unknown	12/14/06	Trizol	1.18 x 10^6 TCID50/mL	Reed & Muench
Bovine Herpes Virus-1	California	NVSL 20032 111903	CAHFS	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A

Bovine Herpes Virus-1	Cooper	NVSL 86741 111903	CAHFS	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Los Angeles	Los Angeles ATCC VR188	ATCC (CAHFS)	1 x 10^5 TCID50/mL	(CAHFS) MDBK2(LLNL)MDBK1	5/17/07	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	RLB-106	ATCC VR793	ATCC (CAHFS)	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	A033640072	CAHFS, R. Mock, #5 Swab, 1BDFK1, 1/28/2004, A030020072	unknown	1BDFK1,	4/23/04	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	A040130066	CAHFS, R. Mock, #4 Lung, 1BFK1, 1/28/2004, A040130066	unknown	1BFK1	4/23/04	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	Texas A030020072	CAHFS, R. Mock, #80 Swab, 1BDFK1, 1/28/2004, A030020072	unknown	1BDFK1	4/23/2004	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	California	NVSL 200032	NVSL, CAHFS	unknown	MDBK CAHFS	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Virginia	NVSL 231221	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	California	NVSL 51619	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Minnesota	NVSL 86741	NVSL, CAHFS	unknown	MDBK CAHFS	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Minnesota	NVSL 97-10720	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Cooper	NVSL-Cooper RA 309	NVSL, CAHFS	unknown	MDBK CAHFS	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes-5	DN-599	A032540006	R. Mock, from lung tissue, 2BFK2, 2/3/2004	Unknown	CAHFS Lab 2BFK2 4/30/2004	6/3/2004	Phenol/Chloroform	40 pg/uL	N/A
Bovine Herpes-5	DN-599	A040150085	R. Mock, #2 Lung, 5BFK5, 2/3/04	Unknown	CAHFS Lab 5BFK5 4/23/2004	7/7/2004	Phenol/Chloroform	40 pg/uL	N/A
Bovine Herpes-5	D9402133	D9402133	Sonoma Co. 3/94 Severe necrotizing encephalitis, 1 week old calf	Unknown	CAHFS Lab (MDBK), (1 flasks) 11/19/2003	11/19/2003	Phenol/Chloroform	40 pg/uL	N/A
Bovine Herpes Virus	BHV 5	D9403153	CAHFS	unknown	unknown	unknown	unknown	40 pg/uL	unknown
Caprine Herpes Virus		VR462	ATCC	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Caprine Herpes Virus	D0201157	D0201157	CAHFS, Isolate D0201157 History: San Joaquin Co. 2/02 Aborted fetus-third trimester	unknown	CAHFS Lab (MDBK)	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Caprine Herpes Virus	S0201998	S0201998	CAHFS, Isolate S0201998 History: San	unknown	CAHFS Lab (MDBK)	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A

			Diego Co. 2/02 Aborted fetus-third trimester						
Epizootic Hemorrhagic Disease virus-1	Santa Barbara	N/A	Bridget McLaughlin CAHFS	unknown	unknown	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-1	Georgia	N/A	Bridget McLaughlin CAHFS	unknown	unknown	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-2	Alberta	041 ODV 0401	NVSL	unknown	BHK	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-1 (SV123)	New Jersey (3TD) Deer, NJ 1955	041 ODV 0401	NVSL	1 x $10^{5.125}$ $TCID_{50}$/0.1 ml	+BHK-3 (12/7/83); (12/11/86); (12/18/86)	Unknown	Trizol	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A011120004	CAHFS, R. Mock, from brain tissue, 2BFK2, 2/3/2004, A011120004	unknown	CAHFS, 2BFK2	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A99043047	CAHFS, R. Mock, Spleen, 1BFK1, 2/3/2004, A99043047	unknown	CAHFS, 1BFK1	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A9904309	CAHFS	unknown	CAHFS, unknown	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Bovine 1247	Bovine 1247 ATCC VR2003	ATCC	unknown	CAHFS, unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	NVSL 002	NVSL 002 1/28/04	NVSL	unknown	CAHFS, unknown	1/29/03	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	LK	LK ATCC VR701	ATCC	unknown	CAHFS, unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	D990	D990	CAHFS	unknown	CAHFS, unknown	1/29/03	Phenol/ Chloroform	40 pg/uL	N/A
Feline Herpes	C-27	ATCC VR636	ATCC	unknown	CAHFS, unknown	unknown	unknown	40 pg/uL	N/A
Fowl Pox	Vaccine strain	Poxine™	Jeffers Livestock	unknown	ECE	5/17/07	Phenol/ Chloroform	40 pg/uL	N/A
Parainfluenza Virus type 3	C-243	TC-81335	LLNL	1 x 10^{5} TCID50/0.1 mL	(VL)MK14(LLNL)H292-1	5/17/07	Trizol	40 pg/uL	Reed and Muench
Porcine Coronavirus	AR310	ATCC VR-2384	LLNL	1 x $10^{4.25}$ $TCID_{50}$/0.1 ml	ST2				Reed and Muench
Porcine Herpes Pseudorabies	Shope	RA 180	CAHFS	1 x 10^{5} TCID50/mL		5/17/07	Trizol	40 pg/uL	N/A
Pseudorabies		92-12013	NVSL 92-12013 bovine isolate Iowa December, 1991	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies		93-11745	NVSL 93-11745 Illinois December, 1992	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies	Shope	RA180	NVSL Shope	unknown	CAHFS,	12/3/03	Phenol/	40 pg/uL	N/A

			(PRV) RA 180		unknown		Chloroform		
Pseudorabies Titered	Shope	N/A	NVSL	unknown		unknown	Phenol/ Chloroform		Reed & Muench
Respiratory Syncytial virus	Long	N/A		unknown	(VL)KB6 L2KB2HF DL2A549-1(LLNL)H eLa3	5/17/07	Trizol	40 pg/uL	N/A
Transmissible Gastroenteritis virus of hogs	Perdue	NVSL 9801	LLNL	1 x $10^{4.75}$ $TCID_{50}$/0.1 ml	ST 1		Trizol	40 pg/ul	Reed and Muench

TABLE 111. Panel membership for assay. The 3 PPox (BPSV) signatures from the Version 1.0 panel were included in the **Bovine-specific multiplexed assay panel** for FMDV rule-out diagnostics. The below table describes the constituents of that multiplexed assay panel in which these signatures were tested.

Bead ID	Signature/ Target Name	Mux Group ID	Virus	Source	Forward Primer	Reverse Primer	Luminex Probe (-strand, FCP)
113	emcf_94975.F, emcf_94976.R, emcf_94977.P	MCF_1	Malignant Catarrhal Fever	LLNL	ATGCCAGTCACTGGCTCTCA	GGGTGTTGTAGAATCCTGAAATGG	GTTGATCACGGTGGCACCCTGG
114	emcf_95059.F, emcf_95060.R, emcf_95061.P	MCF_2	Malignant Catarrhal Fever	LLNL	GTTCTGGAAACTGACCAAACAGTGT	AGTGGCACTTGAGTGTAACTTTTATTG	GCACTCTGGCAGGCATAAGGGAAATACA
115	emcf_95155.F, emcf_95156R, emcf_95157.P	MCF_3	Malignant Catarrhal Fever	LLNL	CCCTGGAAGCTGTCATACAAAA	AAACATTGGCATATCTTGCAAGGT	CAGTAGAGTCCAGGGCTGCAGTTGTCTCA
117	BVH__94666.F, BVH_94667.R, BVH_94668.P	BHV-94668	Bovine Herpes Virus	LLNL	GTGCCAGCCGCGTAAAAG	GACGACTCCGGGCTCTTTT	TCCTGGTTCCAGAGCGCTAACATGGAG
118	BVH__94738.F, BVH_94739.R, BVH94740.P	BHV-94740	Bovine Herpes Virus	LLNL	TGAGGCCTATGTATGGGCAGTT	GCGCGCCAAACATAAGTAAA	AAATAACACGGTGTGCACTTAAATAAGATTCGCG
119	BPSV_95719.F, BPSV_95720.R, BPSV_95721.P	PPOX_1	Bovine Papular Stomatitis Virus	LLNL	GCAGATGCGCTCCTGGTT	GCACCTCTGCTGCTGCAA	CCGACTCCGACGTGGAGAACGTG
120	BPSV_95722.F, BPSV_95723.R, BPSV_95724.P	PPOX_2	Bovine Papular Stomatitis Virus	LLNL	GATGGCCGTGCAGCTCTT	CGTACAAGATCACGGCCAACT	TGTACGGGCTCATGGGCTTCCG
122	BPSV_95731.F, BPSV_95732.R, BPSV_95733.P	PPOX_4	Bovine Papular Stomatitis Virus	LLNL	GCAGCAGTGCACCACGTAGT	CGCTGAACCCGTACATCCT	GACTTCGAGGCGGACAACAAGCG
132	FMDV.TC	FMDV.TC	Foot and Mouth Virus	Tetracore	ACTGGGTTTTACAAACCTGTGA	GCGAGTCCTGCCACGGA	GTCCCACGGCGTGCAAAGGA
133	FMDV.Pir	FMDV.Pir	Foot and Mouth Virus	Pirbright	CACYTYAAGRTGACAYTGRTACTGGTAC	CAGATYCCRAGTGWCICITGTTA	CCTCGGGGTACCTGAAGGGCATCC
134	BVD_1a	BVD_SIG1	Bovine Viral Diarrhea	UCD, CAHFS lab	GGTAGTCGTCAGTGGTTCGAC	CATGTGCCATGTACAGCAGAGAT	CCTCGTCCACGTGGCATCTCGAG
138	BVD_1821165.F, BVD_1821164.R, BVD_1821166.P	BVD_SIG2	Bovine Viral Diarrhea	LLNL	GGGAGTCGTCAATGGTTCGAC	TCCATGTGCCATGTACAGCAGAG	CCTCGTCCACITGGCATCTCGAG
135	BTV_1759932	BTV_1759932	Bluetongue Virus	LLNL	GCACCCTATATGTTTCCAGACCA	CAGCTAACTCTTCAGCCACACG	CTAACTCGTGGGCCAATCATCATCTTCTGT
136	BTV10_1810207	BTV10_1810207	Bluetongue Virus	LLNL	CAAACACAAAAGGCGGAGAAG	GGCGTTTAATCTGTCTTAGTCTTACGT	GAAACGCTTCTGCGTACGATGCGA
137	BTV10_1810199	BTV10_1810199	Bluetongue Virus	LLNL	CACATGTCGCTTAATTTGTCTTAACC	GCGGAGAAGGCTGCATT	ACGAAACGCTTCCGCGTACGATG
139	BTV10_1810205	BTV10_1810205	Bluetongue Virus	LLNL	TCAATTTTGGTAGAATTTGTTCATTCA	GCGGAGAAGGCTGCATTC	ACGAAACGCTTCCGCGTACGATG
163	VSV_1798943	VSV_1798943	Vesicular Stomatitis Virus -Indiana	LLNL	CGCCACAAGGCAGAGATGT	TGTCAAATTCTGACTTAGCATACTTGC	GCATACTGCATCATATCAGGAGTCGGTTTTCTG
166	VSV_1798947	VSV_1798947	Vesicular Stomatitis Virus -Indiana	LLNL	CCCAATCAATGCCATGATACA	CTCCAATGGAAGGGTCCAAA	TTTGAAAGTAGAACTGTGCAAGCCCGGTATC
167	VSV_1798949	VSV_1798949	Vesicular Stomatitis Virus -Indiana	LLNL	GGCGCTCATTATAAAATTCGGA	ACATTTTCTCGTAGTAATGCAGCAG	GAAGTCCCTGTAATGGATTCCCATTCCATGT
169	VSV_1811408	VSV_1811408	Vesicular Stomatitis Virus –New Jersey	PIADC	CTCACAACATGGGTCCTGAA	TTCTTGACCTGGATACATCAT	GGCATAGYTCGTCTGCRACTTCCCT
160	RPV_1814853	RPV_1814853	Rinderpest Virus	LLNL	GGATCGCTGAAATGATCTGTGA	GGAGCCAGTTCACCCATTTG	CTGGCCAACCCTGCCTCCACTATGTA
161	RPV_1814855	RPV_1814855	Rinderpest Virus	LLNL	TGCATCTTATGTGACTTTGGTTCA	GGCTATCCGCACAGCTGAC	CAGTCCTCTCATCTGTTGTCGATCCGATGTA
162	RPV_1814856	RPV_1814856	Rinderpest Virus	LLNL	AACTCCTGACCTCATTCCTTGC	GGCTCTATAATCCCACTATGCCA	TGGCTCAGTGCATTCACAAAGACCTTGAATA

170	N/A	NC (MT-7)	Maritima	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
171	N/A	b-MT7 (FC)	N/A	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
172	N/A	MT7/Cy3 (IC)	N/A	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
173	Alien RNA	arRNA Control	None	Ambion	GACATCAAGGCTCAAACTAATTTTACC	CAAAGGCTGCCAACATAAAATG	CAAGCGTAAATGCAGCGTCCA

[1]FCP indicates that the probe is on the negative strand (Forward Compliment to the Probe).

3.5.1. BOVINE PANEL MULTIPLEXED PCR DATA

Preliminary screening: Preliminary screening can be broken down into several steps. The signatures are preliminarily screened by first assessing how the signatures will perform under multiplex PCR conditions and whether or not the addition of the new signatures will adversely effect other signatures, these preliminary screening methods are described as "signature baseline screening" and "multiplex addition screening" respectively. First signature baseline screening tests the performance of the signature in a no-target reaction (blank), a "passing" score is indicated by a low (typically less than 100) MFI. All Ppox signatures passed the signature baseline screening. Next in multiplex addition screening the new signature primers are added one-by –one to the other panel signatures to determine if there would be any cross-reactions between primer pairs in the panel. Further assessment of the signatures is done through target and near-neighbor screening and the determination of relative sensitivities. The signature down-selection for the Ppox signatures is further described below.

Historic Data Overview: Version 1.0 Panel LLNL reference date: 20060117

(a) **(b)**

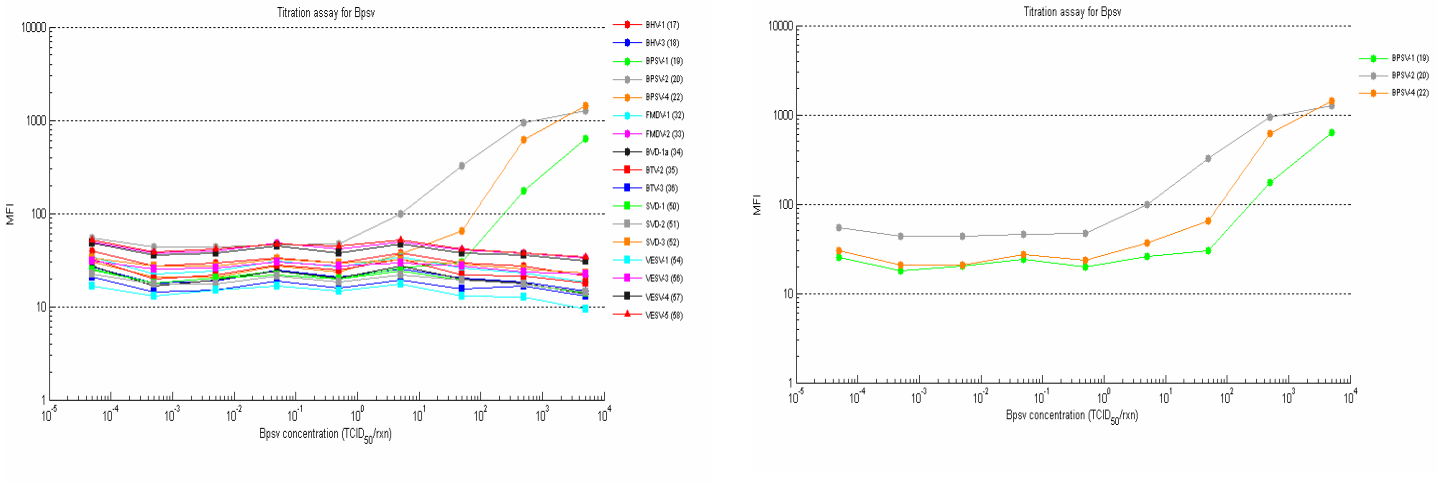

FIG. 13. Multiplexed RT-PCR titrations for BPSV using DNA extracted from a known amount of BPSV Texas A&M strain virus. (a Plot shows all signatures in the presence of BPSV assay titration. Note that even at high concentration of target nucleic acid, no cross-reactivity is seen

on other signature channels. (b) Same plot as (a), however, other signature data has been removed just to show BPSV-specific signatures.

Near-neighbor and Target screening: The three Version 1.0 panel PPOX signatures were added to the Bovine panel. The signatures exhibited a reasonably low background response (<30 MFI) in the Bovine panel.

TABLE 112. Backgrounds screening in multiplexed format for PPOX at LLNL and PIADC.

	Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors[2]	Targets
Mux PCR Screening	54	135	16	0	43	4

[1]-There are 752 pooled samples in each Aerosol Block. [2]Near-neighbors indicated (43) apply to the near-neighbor panel that is not uniquely specific for BHV, but for the other panel constituents that were screened concurrently.

TABLE 113. Bovine panel backgrounds screening in **multiplexed** format for down-selected PPOX signatures against the below listed **eukaryotes**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. None of the signatures cross-reacted with any of the samples listed below.

Description	PPOX-1 (19)	PPOX-2 (20)	PPOX-4 (22)
BOVINE	11	10	10
CAT	15	9	9
CHICKEN	8	6	8
DOG	5	9	8
DROSOPHILA MELANOGASTER	6	7	8
EQUINE	15	7	7
FLEA	5	7	7
HUMAN	6	5	6
MONKEY	26	8	10
MOSQUITO	7	7	8
MOUSE	11	5	14
PIG / PORCINE	7	7	17
RABBIT	10	18	9
RAT	4	5	6
SHEEP	5	13	5
TICK	14	6	8

TABLE 114. Bovine panel backgrounds screening in **multiplexed** format for the four PPOX signatures against the below listed **prokaryotes**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. None of the signatures cross-reacted with any of the samples listed below.

Description	PPOX-1 (19)	PPOX-2 (20)	PPOX-4 (22)
Erwinia amylovora	9	10	12
Actinobacillus suis	8	10	12
Aneurinbacillus migulanus	8	8	14
Bacillus cereus	12	12	13
Bacillus globigii	17	10	15
Bacillus subtilis	8	7	12
Bacillus thuringiensis	15	10	14
Bifidobacterium denticum	10	9	12
Borrellia burgdorferi	19	12	14
Burkholderia capacia	9	9	13
Caulobacter vibriodes	7	7	11
Clavibacter michganensis	7	8	10
Clostridium butyricum	10	10	13
Corynebacterium pseudodipthericum	9	8	13
Cytophaga marinoflava	8	7	13
Erwinia herbicola	14	10	13
Escherichia coli	17	10	14
Geobacillus caldoxylosilyticus	9	6	11
Salomon's homophile	7	8	10
Haemophilus influenza	10	22	12
Herbaspirillium seropedicae	12	10	13
Lactobacillus garvieae	9	8	10
Lactobacillus gasseri	10	7	12
Listeria monocytogenes	11	11	11
Listeria seeligeri	10	8	15
Micrococcus luteus	11	7	11
Moraxella lacunatica	12	6	12
Oceanospirillium ssp. Maris	10	10	14
Paenibacillus napthalaenovorans	9	7	12
Paracoccus dentrificans	8	6	12
Porphyrobacter sanguineus	10	9	11
Proteus mirabillis	11	8	11
Pseudomonas aeruginosa	11	10	12
Pseudomonas oleovorans	7	7	10
Rhizobium leguminosarum	15	8	14
Rhodococcus rhodochrous	10	18	9
Salmonella typhimurium	10	8	12
Simonsiella muelleri	15	8	11
Sphingomonas sp. (Alcaligenes sp)	9	9	12
Staphylococcus aureus	10	9	13
Streptococcus pneumoniae	13	8	13

Streptomyces scabiei	8	7	10
Tatlockia maceachernii	16	9	33
Vibrio paraheamolyticus	12	12	12
Xanthomonas translucens	9	8	12

TABLE 115. <u>Bovine panel</u> backgrounds screening in **multiplexed** format for the four PPOX signatures against the below listed **soils**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. No significant cross reactions were observed.

Description	PPOX-1 (19)	PPOX-2 (20)	PPOX-4 (22)
D 000107-49	13	7	16
D 000109 # 50	11	10	16
D 000402 # 53	27	10	17
D 000500 - 26 - 1	16	8	18
D 000501-14-1	12	15	46
D 000505 - 11 - 4	12	9	19
D 000521 - 23	12	8	17
D 000527 - 3	12	7	17
D 000531 - 21	13	8	16
D 000533 - 17 -1	10	5	14
D 000542 - 6	10	8	19
D 000550 - 20	10	6	15
D 000551 - 5	16	8	20
D 000561 - 8 - 6	13	5	16
D 000562 - 30 - 5	9	8	16
S 251	10	4	15
S 252	12	6	14
S 253	7	6	13
S 254	12	6	16
S 255	8	6	13
S 256	8	5	15
S 257	8	9	13
S 259	11	4	13
S 260	7	5	15
S 271	8	5	12
S 272	8	4	13
S 273	8	5	14
S 274	10	6	17
S 275	7	7	16
S 276	8	6	14
S 277	8	5	16
S 279	6	4	12
S 280	7	5	12
S 282	6	4	12
S 283	6	5	12

S 284	7	5	16
S 286	9	5	14
S 287	3	2	8
S 288	7	5	14
S 289	7	5	13
S 290	9	6	11
S 291	6	5	10
S 292	15	5	13
S 295	7	3	12
S 296	14	3	13
S 297	6	3	12
S 298	10	3	11
S 299	7	3	10
S 300	7	3	12
S 301	7	3	14
S 303	7	3	13
S 304	7	3	14
S 305	7	3	13
S 307	6	2	12

TABLE 116. Bovine Panel Near-Neighbor screening (Data from 20070601) against the 2 PPOX signatures. Values shown are Median Flourescence Intensity (MFI) units. “Blanks” are buffer-only samples (no nucleic acid added to reaction), shown below in red. Extracted nucleic acid was added to each PCR reaction in triplicate totaling 200pg/rxn. The data shows that the PPOX signatures didn’t react to any of the near-neighbors from the Bovine panel.

Description	**PPOX-1 (19)**			**PPOX-2 (20)**			**PPOX-4 (22)**		
replicate	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Blank	15	20	12	27	16	37	15	13	14
Blank	19	11	9	27	15	29	15	14	11
BDV Coos Bay	16	29	22	24	17	24	15	14	13
BHV (BFK)	11	28	16	40	23	40	17	21	19
BHV A040150085	35	5	23	54	18	47	28	10	22
BHV-1 (IBR) Texas CAHFS A030020072	17	18	13	35	24	30	20	23	17
BHV-1 A033640072	32	7	17	39	22	36	21	16	15
BHV-1 A040130066	35	12	22	38	22	34	24	20	17
BHV-1 ATCC VR 793	16	16	16	43	19	33	28	15	17
BHV-1 LA ATCC VR188	19	15	12	27	19	35	29	19	16
BHV-1 NVSL 200032	13	7	15	26	15	30	24	21	21
BHV-1 NVSL 231221	26	20	18	37	26	41	18	25	17
BHV-1 NVSL 51619	10	13	21	28	16	38	24	19	23
BHV-1 NVSL 86741	16	17	13	31	20	33	19	19	18
BHV-1 NVSL 97-10720	17	16	31	29	17	39	21	19	28
BHV-1 RA309	20	22	23	31	20	29	24	21	18
BHV-5 A032540006 CAHFS	19	15	18	24	19	34	14	17	14
BHV-5 A040150085 CAHFS	21	16	9	32	23	24	15	18	12

BHV-5 D9402133 CAHFS	21	15	12	24	18	23	14	14	10
BHV-5 D9403153 CAHFS	14	18	19	31	19	26	15	16	11
Caprine Herpes ATCC VR462	18	13	11	31	19	32	20	13	12
Caprine Herpes D0201157 CAHFS	19	16	10	31	21	27	17	15	12
Caprine Herpes S0201998 CAHFS	19	8	32	28	20	33	15	16	12
EHD-1 Georgia	20	5	9	33	23	38	20	14	39
EHD-1 New Jersey	26	18	13	34	18	28	16	14	13
EHD-1 Santa Barbara	35	17	9	29	65	22	25	14	11
EHD-2 Alberta	9	32	9	20	46	18	14	17	10
EHV-1 A011120004 CAHFS	10	13	11	30	21	29	21	18	16
EHV-1 A99043047 CAHFS	12	13	14	28	19	30	20	13	13
EHV-1 A9904309	17	13	12	35	22	34	18	19	17
EHV-1 ATCC VR2003	28	25	19	37	24	37	17	19	14
EHV-1 NVSL 00002	17	17	9	31	19	26	16	15	14
EHV-2 ATCC VR701	12	14	21	26	15	29	18	15	16
EHV-2 D990 CAHFS	15	15	15	35	25	35	20	16	14
Feline Herpes ATCC VR636	12	18	14	21	16	26	19	18	16
Fowl Pox	19	20	16	27	52	25	17	17	11
IBR CA 111903	13	4	26	36	4	30	26	8	25
IBR MN 111903	12	16	16	55	20	40	17	18	21
Parainfluenza Type 3	11	31	8	21	45	19	13	15	11
Porcine Herpes Pseudorabies Shope	9	17	13	20	15	27	13	13	11
Pseudorabies NVSL 92-12013	18	12	11	28	15	27	15	14	12
Pseudorabies NVSL 93-11745	10	13	20	30	17	35	18	13	12
Pseudorabies RA180 CAHFS	14	11	11	28	17	26	15	11	11
Pseudorabies Titered	15	21	29	38	18	34	19	13	12
Respiratory Syncytial	20	17	18	21	41	23	15	15	10

Multiplexed PCR Assay Titrations

Upon finalization of the assay panel constituents each agent assay is characterized in multiplexed PCR format by running dose-response (titration) curves against titered virus extracted from virus infected cell culture for each available agent strain. Each sample is tested in duplicate and reported as a mean value of the median fluorescence intensity units (MFI) against agent concentration (per reaction volume, 5uL) on a double log plot. Concentration units are reported in TCID50/rxn (assumes 5uL sample volume per reaction) or pfu/rxn depending on virus type and source. In some cases where titered virus was not available data is reported as picogram units (pg). Each assay is evaluated by its ability to detect agent strains and differentiate from near neighbors (specificity) and by its range of sensitivity to each strain relative to each signature tested for that agent. Because this method is not aimed at determination of analytical sensitivities (requiring a more extensive sample set tested in clinical sample matrices which is not practical for this stage of development) it does however, provide tentative limits of detection that are used to measure the performance of each assay.

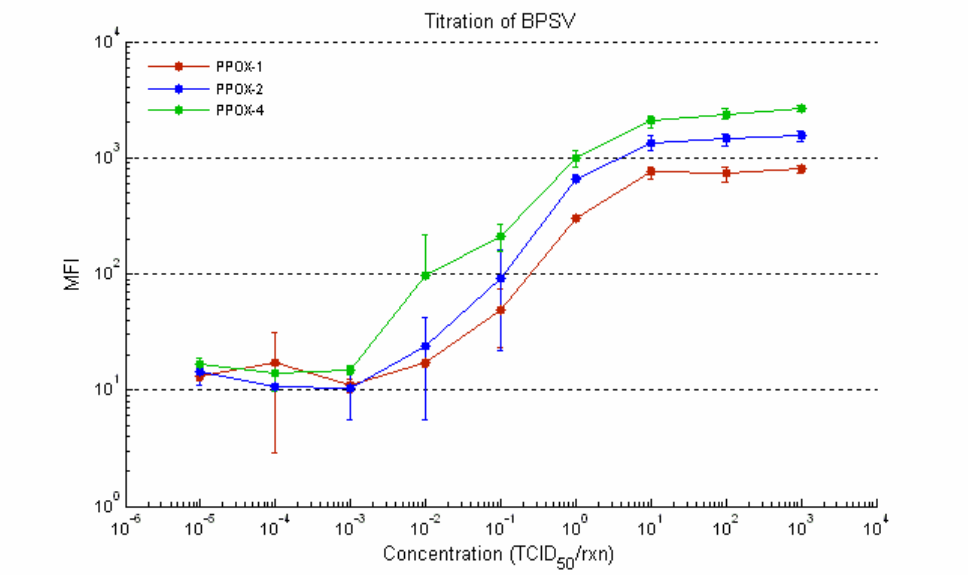

FIG. 14. Multiplex screening data for the three PPOX signatures against extracted nucleic acids from isolate BPSV Texas A&M strain. Serial dilution of nucleic acid extracted with phenol chloroform virus-infected cell culture media then used as template. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1 of the mean.

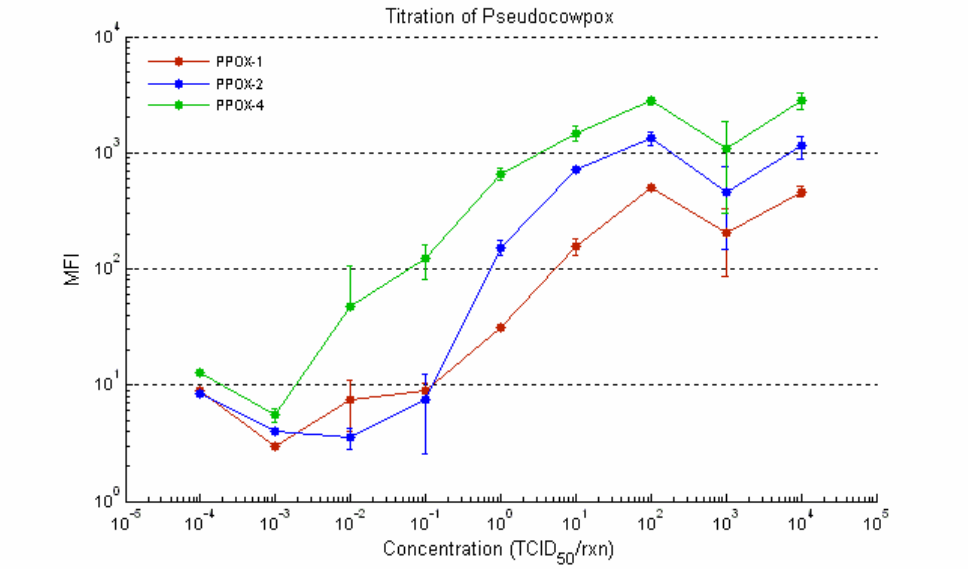

FIG. 15. Multiplex screening data for the three PPOX signatures against extracted nucleic acids from isolate Pseudocowpox VR634. Serial dilution of nucleic acid extracted with phenol chloroform virus-infected cell culture media then used as template. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1 of the mean.

RESULTS: The three Version 1.0 panel signatures were screened in the bovine panel and results indicate that these signatures will work well with the new signatures that comprise the Bovine panel. Additional screening of the PPOX signatures against various parapoxviruses (pseucowpox), provided further information to characterize the performance of these signatures. These three signatures have been currently added the Bovine panel and tested against various strains of parapox. Both signatures have been determined to be effective at detecting BPSV, and pseudocowpox as described above.

4. BOVINE VIRAL DIARRHEA VIRUS (BOVINE PANEL)

OBJECTIVE: We were tasked by the Department of Homeland Security to develop specific and reliable Real-time RT- PCR signatures for Bovine Viral Diarrhea Virus [BVDV], among other major agriculturally-impacting viruses that are clinically indistinguishable from foot and mouth disease virus [FMDV]. The purpose of these assays (collectively with other virus-specific signatures developed in parallel) is to develop a species specific (i.e. bovine) multiplexed detection assay for Foot-and-Mouth disease rule-out for use in veterinary diagnostic laboratories. This work uses one previously designed BVDV signature and one newly designed signature. The signatures were developed to meet two major criteria. First, they would be able to discriminate among multiple viral agents whose disease symptoms mimic those of Foot and Mouth Disease Virus [FMDV]. Secondly, these signatures would be uniquely capable of detecting both BVDV-1 as well as BVDV-2 genotypes. This document describes the development of one optimal Real-time RT-PCR and multiplexed [MUX] PCR signatures to detect these genotypes of BVDV.

4.1. BACKGROUND AND ETIOLOGY OF BVDV

Bovine viral diarrhea virus (BVDV) is an RNA virus classified as a Pestivirus in the family Flaviviridae (*Bovine Viral Diarrhea and Mucosal Disease Complex*). The role of BVDV in Bovine Respiratory Disease (BRD) has been controversial, but appears to be that of a virus capable of inducing immunosuppression, which allows for the development of secondary bacterial pneumonia. Seroconversion to BVDV has been reported to be predictive of the occurrence of respiratory disease in feedlot calves, and BVDV has been reported to be the virus most frequently associated with multiple viral infections of the respiratory tract of calves[4].

4.2. BVD VIRUS COMPREHENSIVE ASSAY SUMMARY

Bioinformatics

[4] *Source: The Merck Veterinary Manual*

http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/121213.htm&word=bovine%2cviral%2cdiarrhea

Virus name: Bovine Viral Diarrhea Virus
Project name: *BVD Signature Variations*
Level of discrimination: *species*
Total number of Genome Sequences used for alignment: 48
Number of Initial Signatures: 3
Number of Signatures forwarded to PCR gel screening: 0
Number of Signatures forwarded to Real-time RT-PCR screening: 3

Real-time PCR Screening Summary

TABLE 117. Final signatures down-selected in real-time screening (3).

#	LLNL Signature Designation	Sequence	#	LLNL Signature Designation	Sequence
1	BVDV_1821164_F	GGTAGTCGTCAGTGGTTCGAC	3	BVDV_1821166_F	GGGAGTCGTCAATGGTTCGAC
	BVDV_1821164_R	TCCATGTGCCATGTACAGCAGAG		BVDV_1821166_R	TCCATGTGCCATGTACAGCAGAG
	BVDV_1821164_P	CTCGAGATGCCACGTGGACGAGG		BVDV_1821166_P	CTCGAGATGCCAIGTGGACGAGG
2	BVDV_1821165_F	GGGAGTCGTCAATGGTTCGAC			
	BVDV_1821165_R	TCCATGTGCCATGTACAGCAGAG			
	BVDV_1821165_P	CTCGAGATGCCACGTGGACGAGG			

TABLE 118. Summary of wet-bench screening in signature down-selection.

Method	Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors	Target
Gel Screening	Not done	Not done	Not done	Not done	Not done	Not done
Real-time PCR Screening	54	45	16	3 Aerosol Blocks	1	7

Note: There are 752 pooled samples in each Aerosol Block

Multiplexed PCR Screening Summary

TABLE 119. Backgrounds screening in multiplexed format for BVDV signatures.

	Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors	Targets
Mux PCR Screening	54	135	16	0	44	8

[1]-There are 752 pooled samples in each Aerosol Block.

TABLE 120. Signature summary for BVD multiplexed signatures in the bovine panel.

LLNL Signature Designation	MUX Group ID	Gene ID	Limit of detection[1]	Targets Screened	Near Neighbors Screened
BVD-1a(mod)[2]	BVD-Sig1	PestiV1gp1	$5x10^{-3}$ - $1x10^{1}$ $TCID_{50}$/rxn	8	44
BVD_1821164_R	BVD-Sig2	PestiV1gp1/1489735	$5x10^{-3}$ - $1x10^{1}$	8	44

BVD_1821165_BF BVD_1821166_FCP			$TCID_{50}$/rxn		

[1] The relative "Limit of detection" is described as a range of lowest and highest sensitivity determined by **multiple** target strains screened and is described further in the Multiplex results summary report section. [2]Signature carried forward from Version 1.0 FMDV Panel.

4.3. BVDV SIGNATURE BIOINFORMATICS AND GENERATION INFORMATION

Bioinformatics process:
The LLNL Bioinformatics team have developed a computational process that uses available DNA sequence information for a given target organism to generate candidate DNA signature sequences that are conserved and unique. Signatures are primarily used to underlie reliable and specific pathogen detection assays. Evidence of the success of the process is that several of the Real-time PCR signatures that are used in the national BioWatch monitoring system were generated by this process, and to date, has resulted in no false positives from a total of over 5 million assays performed.

The general approach is the following: All available complete genomes of different strains of the target species are compared using a multiple genome alignment programs. A *consensus gestalt* is formed from the alignments that contain the sequence conserved amongst all target inputs. (This step is bypassed if only one target sequence is available).

To determine that organism-conserved sequence which does not occur in any other sequenced microbial organism, the consensus gestalt is compared against our immense, continuously updated database of microbial organism. A customized algorithm accomplishes this electronic subtraction, and the result is a *uniqueness gestalt* that is mined for potential signature candidates. A final computational screening is done to verify that cross-reactions are not detected.

Target Virus Information:
Virus name: *Bovine Viral Diarrhea* Virus
Type: ssRNA positive-strand virus
Genome size: bp.

Primer/Probe Set Generation Information

Method: Alignment and All Microbe Database subtraction.

TABLE 121. K-path run id: 104061**.** List of reference genomes used for alignment.

	Genome Description	GI Number	Sequence Length (bp)
1	Classical swine fever virus strain cF114, complete genome	13383931	12297
2	Hog cholera virus, complete genome	10518491	12310
3	Hog cholera virus complete genome	5733833	12297
4	Hog cholera virus strain Shimen, complete genome	5332357	12298

5	Hog cholera virus strain HCLV, complete genome	4092070	12310
6	Hog cholera virus strain Brescia, complete genome	3676778	12297
7	Hog cholera virus strain Glentorf, complete genome	1181835	12278
8	Hog cholera virus strain Riems, complete genome	1181833	12298
9	Classical swine fever virus strain HCLV, complete genome	22347661	12310
10	Classical swine fever virus, strain Alfort/187 complete genome	871250	12298
11	Classical swine fever virus, complete genome	37038269	12310
12	Classical swine fever virus strain GXWZ02, complete genome	34559761	12296
13	Classical swine fever virus strain Riems, complete genome	30144653	12289
14	Classical swine fever virus, complete genome	50882381	12310
15	Classical swine fever virus, complete genome	12657941	12301
16	Classical swine fever virus strain SWH, complete genome	71084281	12296
17	Classical swine fever virus isolate RUCSFPLUM, complete genome	50882742	12308
18	Classical swine fever virus strain C/HVRI, complete genome	55793860	12310
19	Classical swine fever virus strain 0406/CH/01/TWN, complete genome	49481850	12296
20	Classical swine fever virus isolate BRESCIAX, complete genome	50882740	12285
21	Classical swine fever virus strain Shimen/HVRI, complete genome	55925805	12297
22	Pestivirus type 2 strain 94.4/IL/94/TWN, complete genome	56566023	12296
23	Pestivirus type 2 strain Alfort A19, complete genome	1906629	12298
24	Classical swine fever virus 39, complete genome	15488012	12297
25	Classical swine fever virus 96TD, complete genome	49860681	12296
26	Pestivirus type 2 strain Alfort A19, complete genome	1906629	12298
27	Pestivirus type 1, complete genome	9049956	12734
28	Pestivirus type 1 noncytopathic genomic RNA, complete genome	2149468	12267
29	Pestivirus type 1 cytopathic genomic RNA, complete genome	2149466	15521
30	Bovine viral diarrhea virus 1, complete genome	9626649	12573
31	BVI133738 Bovine viral diarrhea virus complete RNA genome, isolate NADL	7960753	12578
32	BVDPOLYPRO Bovine viral diarrhea virus polyprotein RNA, complete cds	289507	12308
33	Bovine viral diarrhea virus 1 strain Singer_Arg, complete genome	71727706	12236
34	Bovine viral diarrhea virus-1, complete genome	9836967	12294
35	Bovine viral diarrhea virus strain Oregon C24V, complete genome	3661565	12310
36	Bovine viral diarrhea virus VEDEVAC ORF1 for polyprotein, complete genome, genomic RNA, strain VEDEVAC	37693100	12308
37	Bovine viral diarrhea virus 1 strain ZM-95, complete genome	76781922	12220
38	Bovine viral diarrhea virus strain Oregon C24V, complete genome	3661565	12310
39	Bovine viral diarrhea virus VEDEVAC ORF1 for polyprotein, complete genome, genomic RNA, strain VEDEVAC	37693100	12308
40	Bovine viral diarrhea virus 1 strain ZM-95, complete genome	76781922	12220
41	Bovine viral diarrhea virus genotype 2 strain New York'93, complete genome	22094502	12332
42	Bovine viral diarrhea virus genotype 2, complete genome	9629506	12255
43	Bovine viral diarrhea virus genotype 2 strain p11Q polyprotein gene, complete cds	37722299	12271
44	Bovine viral diarrhea virus genotype 2 strain p24515 polyprotein gene, complete cds	37722301	12320
45	Pestivirus Giraffe-1, complete genome	20178632	12602
46	Border disease virus 1, complete genome	20198945	12333
47	Pestivirus Reindeer-1, complete genome	20178630	12318
48	Pestivirus Reindeer-1, complete genome	20178630	12318

TABLE 122. List of parameters and settings used in generating candidate signatures using Primer 3 signature generation tool.

Primer3 Main Parameters	
Parameters	**Standard Settings**
PRIMER_OPT_SIZE	20
PRIMER_MIN_SIZE	18
PRIMER_MAX_SIZE	27
PRIMER_PRODUCT_OPT_SIZE	100
PRIMER_PRODUCT_SIZE_RANGE	71-600
PRIMER_OPT_TM	62
PRIMER_MIN_TM	61
PRIMER_MAX_TM	63
PRIMER_MIN_GC	20
PRIMER_MAX_GC	80
PRIMER_PICK_INTERNAL_OLIGO	1
PRIMER_INTERNAL_OLIGO_OPT_SIZE	31
PRIMER_INTERNAL_OLIGO_MIN_SIZE	18
PRIMER_INTERNAL_OLIGO_MAX_SIZE	36
PRIMER_INTERNAL_OLIGO_OPT_TM	72
PRIMER_INTERNAL_OLIGO_MIN_TM	71
PRIMER_INTERNAL_OLIGO_MAX_TM	73
Number of Primers/Probe Set generated:	**3**

Signature Information

Source: LLNL
Project name: BVD Signature Variations
Level of discrimination: Species.
Number of Initial Signatures: 3
Number of Signatures forwarded to gel bench-screening: 0
Number of Signatures forwarded to real-time TaqMan screening: 3
Number of Final real-time TaqMan Signatures: 3

Taqsim description
We used a computational Real-time PCR simulator program, "Taqsim", to identify all potential targets for each signature from all sequences available in genbank. Taqsim is a BLAST-based comparison of each signature as a triplet against all sequences in genbank to identify the targets that are predicted to produce a Real-time PCR reaction at 57 degrees primer annealing and 67 degrees for probe annealing (these temperatures are according to Primer 3 oligo Tm calculations). The first column of the excel spreadsheets list the signatures by the name, and the following columns report the predicted genbank targets, coordinates of signature region, amplicon size, and accession #'s for each target.

Note: For a listing of computationally predicted product sequences and potential cross reactions for each signature, please see Appendix II, Taqsim Run Data.

Signature bioinformatics

TABLE 123. Bioinformatics summary from the "final" set of down-selected signatures.

Signature	Size		Sequence	Length	T_m	GC
1821164	204bp	F	GGTAGTCGTCAGTGGTTCGAC	21bp	61.50°	57.14%
		R	TCCATGTGCCATGTACAGCAGAG	23bp	64.22°	52.17%
		P(+)	CTCGAGATGCCACGTGGACGAGG	23bp	68.18°	65.22%
1821165	204bp	F	GGGAGTCGTCAATGGTTCGAC	21bp	62.17°	57.14%
		R	TCCATGTGCCATGTACAGCAGAG	23bp	64.22°	52.17%
		P(+)	CTCGAGATGCCACGTGGACGAGG	23bp	68.18°	65.22%
1821166	204bp	F	GGGAGTCGTCAATGGTTCGAC	21bp	62.17°	57.14%
		R	TCCATGTGCCATGTACAGCAGAG	23bp	64.22°	52.17%
		P(+)	CTCGAGATGCCAIGTGGACGAGG	23bp	64.92°	60.87%

Target Region Gene Information

TABLE 124a-b. (a) Reference Genomes used for Gene Information. (b) Gene information for each signature. The information shown is red is for the Version 1.0 panel BVD signature and is for reference only.

(a)

Genome Description	GI Number	Sequence Length (bp)
Bovine viral diarrhea virus 1, complete genome	gi|9626649|ref|NC_001461.1	12573

(b)

			Gene Location		Target Region Location	
Kpath Signature ID	Gene/ID	Description	Start	End	Start	End
1821164	PestiV1gp1/1489735	polyprotein	386	12352	187	390
1821165	PestiV1gp1/1489735	polyprotein	386	12352	187	390
1821166	PestiV1gp1/1489735	polyprotein	386	12352	187	390**
BVD_1a_F	PestiV1gp1	polyprotein	1	504	187	388

**Note that the 3 new signatures target the exact same gene region, and differ by 2-3 base pair mismatches from each other. The goal is to pair up the one of the new signatures with the previous one for optimal detection capabilities.

4.4. BVDV GEL AND REAL-TIME PCR SCREENING REPORT

Wet-lab screening process. To ensure extremely high selectivity and sensitivity, we have established a screening protocol that rigorously screens candidate DNA signatures to select the optimal sets for eventual field use. Candidate signature primer pairs that survive computational screening proceed to wet chemistry screening utilizing standard PCR with agarose gel electrophoresis. This step ensures that the primers will react across strains representative of the diversity of the pathogen (avoid false negative), but will not react with the nucleic acids of other

organisms that could be present in a sample (avoid false positives). The collection of purified DNA templates used in this screening process is listed below:

1) Strain panels representative of the diversity of the target organism in nature.
2) Near neighbors-organisms that are closely related at the genetic level and therefore have the greatest likelihood of cross-reaction.
3) Eukaryotic DNA that may carry over from sample collection procedures.
4) Prokaryotic DNA that would be expected to be present in environmental samples and may also be present in Bovine and Porcine oral cavities.
5) Forty-five soil samples from around the country representing diverse climates and contain a complex mixture of organisms from diverse environmental collections
6) Aerosol samples: 752 samples collected from aerosol collectors and pooled for background testing purposes.

Note BVDV signature from Version 1.0 panel (BVD-1a) was not re-screened in gel or real-time; this data was covered in detail in the 2005 Agricultural Assay Development Report.

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TABLE 125. List of targets screened (RNAs extracted with TRIZOL)

Agent	Genotype/ subgenotype	Isolate	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	Use stock titer	Titer Method
BVDV	1a	NADL-BVDV	Julia Ridpath	6.8 x 10^6 $TCID_{50}$/mL	Unknown	12/2005	Trizol	2.9 x 10^4 $TCID_{50}$/mL	Reed & Muench
BVDV	1b	TGAN-BVDV	Julia Ridpath	1.5 x 10^9 $TCID_{50}$/mL	Unknown	12/14/06	Trizol	1.32 x 10^7 $TCID_{50}$/mL	Reed & Muench
BVDV	2a	2850805 - BVDV	Julia Ridpath	4.2 x 10^6 $TCID_{50}$/mL	Unknown	12/14/06	Trizol	3.36 x 10^4 $TCID_{50}$/mL	Reed & Muench
BVDV	2b	AU-501 (1-6-2006) BVDV	Julia Ridpath	1.47 x 10^7 $TCID_{50}$/mL	Unknown	12/14/06	Trizol	8.64 x 10^4 $TCID_{50}$/mL	Reed & Muench
BVDV	1b	NY-(5/18/02) BVDV	Julia Ridpath	6.8 x 10^7 $TCID_{50}$/mL	Unknown	12/14/06	Trizol	8.38 x 10^5 $TCID_{50}$/mL	Reed & Muench
BVDV	2b	BVD-FBS B69519 - BVDV	Julia Ridpath	2.4 x 10^5 $TCID_{50}$/mL	Unknown	12/14/06	Trizol	1.7 x 10^3 $TCID_{50}$/mL	Reed & Muench
BVDV	2	334165 = BVD2-BVD2	Julia Ridpath	2.4 x 10^5 $TCID_{50}$/mL	Unknown	12/14/06	Trizol	1.1 x 10^3 $TCID_{50}$/mL	Reed & Muench

TABLE 126. List of near-neighbors screened (RNA extracted with TRIZOL).

Agent[1]	Isolate	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	Use stock titer	Titer Method
Border Disease Virus	Coos Bay #4 (4-6-92)	Julie Ridpath	1.5×10^8 $TCID_{50}$/mL	Unknown	12/14/06	Trizol	1.18×10^6 $TCID_{50}$/mL	Reed & Muench

[1]Additional near-neighbor screening for BVD will be done in July 2007 for Border Disease Virus (BDV) and Classical Swine Fever (CSF) at PIADC.

TABLE 127. Real-time screening summary for BVDV.

	Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors	Targets
Real-time_PCR Screening	54	45	16	3_Aerosol Blocks[1]	1	7

[1]-There are 752 pooled samples in each Aerosol Block.

TABLE 128. List of 3 additional signatures developed for BVDV multiplex assay group.

SIGNATURE	FORWARD PRIMER
BVDV_1821166_F	GGGAGTCGTCAATGGTTCGAC
BVDV_1821166_R	TCCATGTGCCATGTACAGCAGAG
BVDV_1821166_P	CTCGAGATGCCAIGTGGACGAGG
BVDV_1821164_F	GGTAGTCGTCAGTGGTTCGAC
BVDV_1821164_R	TCCATGTGCCATGTACAGCAGAG
BVDV_1821164_P	CTCGAGATGCCACGTGGACGAGG
BVDV_1821165_F	GGGAGTCGTCAATGGTTCGAC
BVDV_1821165_R	TCCATGTGCCATGTACAGCAGAG
BVDV_1821165_P	CTCGAGATGCCACGTGGACGAGG

Real-time PCR materials and methods

Background real-time tests were carried out in triplicate as 25ul reactions in 96 well PCR plates on BioRad's iCYCLERs. Each reaction mix consisted of 1X PCR Buffer [200mM Tris-HCL (pH 8.4), 500mM KCl], 6mM $MgCl_2$, 0.2mM each dNTP, 80ng BSA, 0.2uM each forward and reverse primers, 0.4uM Probe with a 5' Fam and a 3' BHQ modification, 1.25U Platinum *Taq* DNA polymerase, PCR water and a variable amount of extracted DNA template. A total of 5ng of soil DNA or 1ng of Eukaryotic DNA or 200pg of Prokaryotic, target or near-neighbor DNA was added to each 25ul reaction mix.

A real-time PCR reaction was deemed positive if a Ct (cycle threshold) value of below 36 cycles was observed at least 2 of the 3 times the reaction was performed.

TABLE 129a-c. Real-time PCR background screening consisted of an extensive list of soil, prokaryotic and eukaryotic extracted DNAs in addition to 3 aerosol blocks of 784 samples each. None of the BVDV signatures produced an amplicon when screened against the listed backgrounds. All signatures passed real-time PCR background screening and moved forward to be screened against the available BVDV targets and near-neighbor.

(a) Total of 54 soils screened

D000402	D000531	S252	S271	S280	S290	S300
D000109	D000542	S253	S272	S282	S291	S301
D000107	D000533	S254	S273	S283	S292	S303
D000500	D000561	S255	S274	S284	S295	S304
D000505	D000562	S256	S275	S286	S296	S305
D000521	D000501	S257	S276	S287	S297	S307
D000551	D000550	S259	S277	S288	S298	
D000527	S251	S260	S279	S289	S299	

(b) Total of 16 Eukaryotes screened

Bovine	Drosophila	Monkey	Rabbit
Cat	Equine	Mosquito	Rat
Chicken	Flea	Mouse	Sheep
Dog	Human	Porcine	Tick

(c) Total of 45 Prokaryotes screened

A. suis	*C. butyricum*	*L. gasseri*	*P. oleovorans*
A migulanus	*C. pseudodipthericum*	*L. monocytogenes*	*R. leguminosarum*
B. cereus	*C. marinoflava*	*L. seeligeri*	*R. rhodochrous*
B. globigii	*E. amylovora*	*M. luteus*	*S. typhimurium*
B. subtilis	*E. herbicola*	*M. lacunatica*	*S. muelleri*
B thuringiensis	*E. coli*	*O. ssp. Maris*	*Alcaligenes sp.*
B. denticum	*G. caldoxylosilyticus*	*P. napthalaenovorans*	*S. aureus*
B. burgdorferi	*H. halmophila*	*P. dentrificans*	*S. pneumoniae*
B. capacia	*H. influenza*	*P sanguineus*	*S. scabiei*
C. vibriodes	*H. seropedicae*	*P. mirabillis*	*T. maceachernii*
C. michganensis	*L. garviease*	*P aeruginosae*	*V. paraheamolyticus*
			X. translucens

TABLE 130. Each BVDV signature was tested with the available target and near-neighbor strains, shown here as column headings. Each screening set was performed in triplicate indicated below as an average Ct value. The Ct value in red indicates a PCR product with a near-neighbor.However the typical cutoff Ct value for cross reactions is 35. "N" indicates no detectable PCR product after 35 cycles. *Note that the BVDV multiplex signature is not an optimal TaqMan assay due to probe placement.

	TARGETS							NEAR-NEIGHBOR
SIGNATURE	NADL	TGAN	28508-5	AU-501	NY-1	BVD-FBS	334165	Coos Bay
1821164	32.5	31.8	34.4	34.5	32.1	35.4	33.9	37
1821165	32.1	31.2	35.4	33.7	31.3	35.3	34.9	N
1821166	32.1	32.2	33.8	32	31.8	33.5	32.8	N

BVDV Real-Time Screening Conclusions: All three of the BVDV signatures passed screening requirements; having not cross reacted with backgrounds and exhibited desirable reactivity with target strains for both BVDV-1 and BVDV-2. Signature 1821164 showed very weak cross-reaction with near-neighbor; so it may be decidedly less desirable than the other 2 signatures.

Lowest level of detection data:

**This test was not performed in real time on these signatures as the probe was in an optimal configuration for multiplex but not real time testing (ie the TaqMan probe was at the 3' end of the amplicon from the primer).

4.5. BVDV MULTIPLEXED PCR SCREENING REPORT

Screening and Down-selection Technical Approach:

Signatures that pass gel and real-time PCR screening are candidates for multiplexed assays. If the number of candidate signatures is large than a subset of the available signatures are selected based on measured sensitivity and specificity observed in real-time screening. In preliminary screening the background of individual signatures is measured by running "blank" (no sample added) controls and observing signature response. In some cases a primer-to-primer non-specific interference will eliminate candidate signatures. Signatures are then added iteratively to each multiplexed panel, titrated, and with each signature addition, collectively, all signatures are re-titrated to determine if the presence of one signature will interfere with the performance of another. During this process signatures are added or subtracted from the multiplexed panel depending on individual outcomes until all desirable signatures have been added.

Criterion for down-selection of multiplexed signatures

Signature specificity. In initial real-time PCR screening tests undergo target and near neighbor screening and screening against environmental confounders which assess individual signature specificity. In multiplex format this is repeated to verify that a more complex reaction system gives the same level of specificity.

Signature sensitivity. In initial real-time PCR screening tests undergo limit of detection testing where a dilution curve is generated for each signature. This determines individual signature sensitivity. In multiplex format this is repeated to verify that a more complex reaction system gives acceptable signature sensitivity.

Optimal signal to noise ratio. In the multiplex assay system the potential for cross reactivity is larger than in single reaction testing, this may cause unwanted PCR reaction products or non-specific cross-reactivity. Signatures are preferred to have a high signal to noise ratio, where desired signal is significantly greater than the background noise of the signatures when there is no target nucleic acid in the reaction.

Compatibility with other signatures in multiplex. In some cases a primer set may interfere with other primers or probes in the multiplex and there by cause limit of detection shifting for one or more signature in the multiplex. For each scenario it must be evaluated whether the shift in detection level is significant enough to remove (what could be a very desirable signature) from the panel.

TABLE 131. Order details for signatures ordered for multiplexed assay screening and development.

ID	Modification Details	Vendor
BVD_1821164_R	5'-TCCATGTGCCATGTACAGCAGAG-3'	Biosearch
BVD_1821165_BF	5'-/5Bio/GGGAGTCGTCAATGGTTCGAC-3'	Biosearch
BVD_1821166_FCP	5'-/5AmMC6/iSp18/CCTCGTCCACITGGCATCTCGAG-3'	Biosearch
Version 1.0 Panel	5'- /5AmMC6//iSp18/CCTCGTCCACGTGGCATCTCGAG -3'	IDT DNA
BVDa.LLNLmod.FCP	5'- /5Bio/GGTAGTCG/iBiodT/CAGTGGT/iBiodT/CGAC -3'	IDT DNA
BVD-1a(mod)		IDT DNA
BVD-1a(mod)	5'-CATGTGCCATGTACAGCAGAGAT -3'	

Multiplex Data Analysis -Technical approach

Disease signature thresholds

Thresholds were initially determined using all available data from known negative samples (blanks) by plotting a histogram which showed the probability of a given sample generating a particular MFI. In the absence of cross reactivity, each signature in the multiplex assay could be treated as an individual signature result. For example, for a sample spiked with BVD virus, data for other disease signatures could contribute towards negative data, providing that cross reaction between disease signature sets was not observed. This is a way to increase the number of samples that can be used to establish thresholds.

TABLE 132. Individual signature thresholds for BVDV signatures. For FY06, threshold determinations have not yet been made as they require a significant number of tests (>500) to generate this information. This information will be updated when available.

Signature Name	Mux Assay name	Signature background in Tris NaCl Buffer (MFI +/-)	Signature background in Clinical matrices (MFI +/-)	Threshold
BVD_1821164_R BVD_1821165_BF BVD_1821166_FCP	BVD-SIG2	TBD	TBD	TBD
Version 1.0 Panel BVDa.LLNLmod.FCP BVD-1a(mod) BVD-1a(mod)	BVD-SIG1	TBD	TBD	TBD

TABLE 133. List of targets screened (RNAs extracted with TRIZOL) in multiplexed PCR format against both BVDV signatures.

Agent	Genotype/ Subgenotype	Isolate	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	Use stock titer	Titer Method
Bovine Viral Diarrhea	1 (cytopathic)	Singer	NVSL	10^7TCID$_{50}$/ 0.1mL	Unknown	12/2005	Trizol	1.98 x 10^6 TCID$_{50}$/0.1 mL	Reed & Muench
BVDV	1a	NADL-BVDV	Julia Ridpath	6.8 x 10^6 TCID$_{50}$/mL	Unknown	12/14/06	Trizol	2.9 x 10^4 TCID$_{50}$/mL	Reed & Muench
BVDV	1b	TGAN-BVDV	Julia Ridpath	1.5 x 10^9 TCID$_{50}$/mL	Unknown	12/14/06	Trizol	1.32 x 10^7 TCID$_{50}$/mL	Reed & Muench
BVDV	2a	2850805 -	Julia	4.2 x 10^6	Unknown	12/14/06	Trizol	3.36 x 10^4	Reed &

		BVDV	Ridpath	$TCID_{50}$/mL				$TCID_{50}$/mL	Muench
BVDV	2b	AU-501 (1-6-2006) BVDV	Julia Ridpath	1.47×10^7 $TCID_{50}$/mL	Unknown	12/14/06	Trizol	8.64×10^4 $TCID_{50}$/mL	Reed & Muench
BVDV	1b	NY-(5/18/02) BVDV	Julia Ridpath	6.8×10^7 $TCID_{50}$/mL	Unknown	12/14/06	Trizol	8.38×10^5 $TCID_{50}$/mL	Reed & Muench
BVDV	2b	BVD-FBS B69519 - BVDV	Julia Ridpath	2.4×10^5 $TCID_{50}$/mL	Unknown	12/14/06	Trizol	1.7×10^3 $TCID_{50}$/mL	Reed & Muench
BVDV	2	334165 = BVD2-BVD2	Julia Ridpath	2.4×10^5 $TCID_{50}$/mL	Unknown	12/14/06	Trizol	1.1×10^3 $TCID_{50}$/mL	Reed & Muench

TABLE 134. List of near-neighbors screened (RNA extracted with TRIZOL).

Virus	Isolate	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	Use stock titer	Titer Method
Border Disease Virus	Coos Bay #4 (4-6-92)	Julia Ridpath	1.5×10^8 $TCID_{50}$/mL	Unknown	12/14/06	Trizol	1.18×10^6 $TCID_{50}$/mL	Reed & Muench

TABLE 135. List of additional near-neighbors screened against the Bovine Panel. All near-neighbors samples were extracted nucleic acids with unknown titers.

Virus[1]	Strain[1]	Unique ID	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	NA conc. used or stock titer	Titer Method
Border Disease virus	Coos Bay	4-6-92	NADC, Julia Ridpath	1.5×10^8 TCID50/mL	unknown	12/14/06	Trizol	1.18×10^6 TCID50/mL	Reed & Muench
Bovine Herpes Virus-1	California	NVSL 20032 111903	CAHFS	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Cooper	NVSL 86741 111903	CAHFS	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Los Angeles	Los Angeles ATCC VR188	ATCC (CAHFS)	1×10^5 TCID50/mL	(CAHFS) MDBK2(LLNL)MDBK1	5/17/07	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	RLB-106	ATCC VR793	ATCC (CAHFS)	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	A033640072	CAHFS, R. Mock, #5 Swab, 1BDFK1, 1/28/2004, A030020072	unknown	1BDFK1,	4/23/04	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	A040130066	CAHFS, R. Mock, #4 Lung, 1BFK1, 1/28/2004, A040130066	unknown	1BFK1	4/23/04	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	Texas A030020072	CAHFS, R. Mock, #80 Swab, 1BDFK1, 1/28/2004, A030020072	unknown	1BDFK1	4/23/2004	Phenol/ Chloroform	40 pg/uL	N/A

Bovine Herpes Virus-1	California	NVSL 200032	NVSL, CAHFS	unknown	MDBK CAHFS	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Virginia	NVSL 231221	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	California	NVSL 51619	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Minnesota	NVSL 86741	NVSL, CAHFS	unknown	MDBK CAHFS	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Minnesota	NVSL 97-10720	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Cooper	NVSL-Cooper RA 309	NVSL, CAHFS	unknown	MDBK CAHFS	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes-5	DN-599	A032540006	R. Mock, from lung tissue, 2BFK2, 2/3/2004	Unknown	CAHFS Lab 2BFK2 4/30/2004	6/3/2004	Phenol/Chloroform	40 pg/uL	N/A
Bovine Herpes-5	DN-599	A040150085	R. Mock, #2 Lung, 5BFK5, 2/3/04	Unknown	CAHFS Lab 5BFK5 4/23/2004	7/7/2004	Phenol/Chloroform	40 pg/uL	N/A
Bovine Herpes-5	D9402133	D9402133	Sonoma Co. 3/94 Severe necrotizing encephalitis, 1 week old calf	Unknown	CAHFS Lab (MDBK), (1 flasks) 11/19/2003	11/19/2003	Phenol/Chloroform	40 pg/uL	N/A
Bovine Herpes Virus	BHV 5	D9403153	CAHFS	unknown	unknown	unknown	unknown	40 pg/uL	unknown
Caprine Herpes Virus		VR462	ATCC	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Caprine Herpes Virus	D0201157	D0201157	CAHFS, Isolate D0201157 History: San Joaquin Co. 2/02 Aborted fetus-third trimester	unknown	CAHFS Lab (MDBK)	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Caprine Herpes Virus	S0201998	S0201998	CAHFS, Isolate S0201998 History: San Diego Co. 2/02 Aborted fetus-third trimester	unknown	CAHFS Lab (MDBK)	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-1	Santa Barbara	N/A	Bridget McLaughlin CAHFS	unknown	unknown	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-1	Georgia	N/A	Bridget McLaughlin CAHFS	unknown	unknown	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-2	Alberta	041 ODV 0401	NVSL	unknown	BHK	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-1 (SV123)	New Jersey (3TD) Deer, NJ 1955	041 ODV 0401	NVSL	1 x $10^{5.125}$ $TCID_{50}$/0.1 ml	+BHK-3 (12/7/83); (12/11/86); (12/18/86)	Unknown	Trizol	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A011120004	CAHFS, R. Mock, from brain tissue, 2BFK2, 2/3/2004, A011120004	unknown	CAHFS, 2BFK2	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A99043047	CAHFS, R. Mock, Spleen, 1BFK1,	unknown	CAHFS, 1BFK1	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A

			2/3/2004, A99043047						
Equine Herpes 1	Equine Herpes 1	A9904309	CAHFS	unknown	CAHFS, unknown	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Bovine 1247	Bovine 1247 ATCC VR2003	ATCC	unknown	CAHFS, unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	NVSL 002	NVSL 002 1/28/04	NVSL	unknown	CAHFS, unknown	1/29/03	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	LK	LK ATCC VR701	ATCC	unknown	CAHFS, unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	D990	D990	CAHFS	unknown	CAHFS, unknown	1/29/03	Phenol/ Chloroform	40 pg/uL	N/A
Feline Herpes	C-27	ATCC VR636	ATCC	unknown	CAHFS, unknown	unknown	unknown	40 pg/uL	N/A
Fowl Pox	Vaccine strain	Poxine™	Jeffers Livestock	unknown	ECE	5/17/07	Phenol/ Chloroform	40 pg/uL	N/A
Parainfluenza Virus type 3	C-243	TC-81335	LLNL	1×10^5 TCID50/0.1 mL	(VL)MK14(LLNL)H292-1	5/17/07	Trizol	40 pg/uL	Reed and Muench
Porcine Coronavirus	AR310	ATCC VR-2384	LLNL	$1 \times 10^{4.25}$ $TCID_{50}$/0.1 ml	ST2				Reed and Muench
Porcine Herpes Pseudorabies	Shope	RA 180	CAHFS	1×10^5 TCID50/mL		5/17/07	Trizol	40 pg/uL	N/A
Pseudorabies		92-12013	NVSL 92-12013 bovine isolate Iowa December, 1991	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies		93-11745	NVSL 93-11745 Illinois December, 1992	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies	Shope	RA180	NVSL Shope (PRV) RA 180	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies Titered	Shope	N/A	NVSL	unknown		unknown	Phenol/ Chloroform		Reed & Muench
Respiratory Syncytial virus	Long	N/A		unknown	(VL)KB6L2KB2HFDL2A549-1(LLNL)HeLa3	5/17/07	Trizol	40 pg/uL	N/A
Transmissible Gastroenteritis virus of hogs	Perdue	NVSL 9801	LLNL	$1 \times 10^{4.75}$ $TCID_{50}$/0.1 ml	ST 1		Trizol	40 pg/ul	Reed and Muench

[1]Additional near-neighbor screening for BVD will be done in Aug 2007 for Border Disease Virus (BDV) and Classical Swine Fever (CSF) at PIADC.

TABLE 136. Panel membership for assay. The 2 BVDV signatures were included in the **Bovine-specific multiplexed assay panel** for FMDV rule-out diagnostics. The below table describes the constituents of that multiplexed assay panel in which these signatures were tested.

Bead ID	Signature/ Target Name	Mux Group ID	Virus	Source	Forward Primer	Reverse Primer	Luminex Probe (-strand, FCP)
113	emcf_94975.F, emcf_94976.R, emcf_94977.P	MCF_1	Malignant Catarrhal Fever	LLNL	ATGCCAGTCACTGGCTCTCA	GGGTGTTGTAGAATCCTGAAATGG	GTTGATCACGGTGGCACCCTGG
114	emcf_95059.F,	MCF_2	Malignant	LLNL	GTTCTGGAAACTGACCA	AGTGGCACTTGAGTGTAA	GCACTCTGGCAGGCATAA

	emcf_95060.R, emcf_95061.P		Catarrhal Fever		AACAGTGT	CTTTTATTG	GGGAAATACA
115	emcf_95155.F, emcf_95156R, emcf_95157.P	MCF_3	Malignant Catarrhal Fever	LLNL	CCCTGGAAGCTGTCATACAAAA	AAACATTGGCATATCTTGCAAGGT	CAGTAGAGTCCAGGGCTGCAGTTGTCTCA
117	BVH__94666.F, BVH_94667.R, BVH_94668.P	BHV-94668	Bovine Herpes Virus	LLNL	GTGCCAGCCGCGTAAAAG	GACGACTCCGGGCTCTTTT	TCCTGGTTCCAGAGCGCTAACATGGAG
118	BVH__94738.F, BVH_94739.R, BVH94740.P	BHV-94740	Bovine Herpes Virus	LLNL	TGAGGCCTATGTATGGGCAGTT	GCGCGCCAAACATAAGTAAA	AAATAACACGGTGTGCACTTAAATAAGATTCGCG
119	BPSV_95719.F, BPSV_95720.R, BPSV_95721.P	PPOX_1	Bovine Papular Stomatitis Virus	LLNL	GCAGATGCGCTCCTGGTT	GCACCTCTGCTGCTGCAA	CCGACTCCGACGTGGAGAACGTG
120	BPSV_95722.F, BPSV_95723.R, BPSV_95724.P	PPOX_2	Bovine Papular Stomatitis Virus	LLNL	GATGGCCGTGCAGCTCTT	CGTACAAGATCACGGCCAACT	TGTACGGGCTCATGGGCTTCCG
122	BPSV_95731.F, BPSV_95732.R, BPSV_95733.P	PPOX_4	Bovine Papular Stomatitis Virus	LLNL	GCAGCAGTGCACCACGTAGT	CGCTGAACCCGTACATCCT	GACTTCGAGGCGGACAACAAGCG
132	FMDV.TC	FMDV.TC	Foot and Mouth Virus	Tetracore	ACTGGGTTTTACAAACCTGTGA	GCGAGTCCTGCCACGGA	GTCCCACGGCGTGCAAAGGA
133	FMDV.Pir	FMDV.Pir	Foot and Mouth Virus	Pirbright	CACYTYAAGRTGACAYTGRTACTGGTAC	CAGATYCCRAGTGWCICITGTTA	CCTCGGGGTACCTGAAGGGCATCC
134	BVD_1a	BVD_SIG1	Bovine Viral Diarrhea	UCD, CAHFS lab	GGTAGTCGTCAGTGGTTCGAC	CATGTGCCATGTACAGCAGAGAT	CCTCGTCCACGTGGCATCTCGAG
138	BVD_1821165.F, BVD_1821164.R, BVD_1821166.P	BVD_SIG2	Bovine Viral Diarrhea	LLNL	GGGAGTCGTCAATGGTTCGAC	TCCATGTGCCATGTACAGCAGAG	CCTCGTCCACITGGCATCTCGAG
135	BTV_1759932	BTV_1759932	Bluetongue Virus	LLNL	GCACCCTATATGTTTCCAGACCA	CAGCTAACTCTTCAGCCACACG	CTAACTCGTGGGCCAATCATCATCTTCTGT
136	BTV10_1810207	BTV10_1810207	Bluetongue Virus	LLNL	CAAACACAAAAGGCGGAGAAG	GGCGTTTAATCTGTCTTAGTCTTACGT	GAAACGCTTCTGCGTACGATGCGA
137	BTV10_1810199	BTV10_1810199	Bluetongue Virus	LLNL	CACATGTCGCTTAATTTGTCTTAACC	GCGGAGAAGGCTGCATT	ACGAAACGCTTCCGCGTACGATG
139	BTV10_1810205	BTV10_1810205	Bluetongue Virus	LLNL	TCAATTTTGGTAGAATTTGTTCATTCA	GCGGAGAAGGCTGCATTC	ACGAAACGCTTCCGCGTACGATG
163	VSV_1798943	VSV_1798943	Vesicular Stomatitis Virus	LLNL	CGCCACAAGGCAGAGATGT	TGTCAAATTCTGACTTAGCATACTTGC	GCATACTGCATCATATCAGGAGTCGGTTTTCTG
166	VSV_1798947	VSV_1798947	Vesicular Stomatitis Virus	LLNL	CCCAATCAATGCCATGATACA	CTCCAATGGAAGGGTCCAAA	TTTGAAAGTAGAACTGTGCAAGCCCGGTATC
167	VSV_1798949	VSV_1798949	Vesicular Stomatitis Virus	LLNL	GGCGCTCATTATAAAATTCGGA	ACATTTTCTCGTAGTAATGCAGCAG	GAAGTCCCTGTAATGGATTCCCATTCCATGT
169	VSV_1811408	VSV_1811408	Vesicular Stomatitis Virus	PIADC	CTCACAACATGGGTCCTGAA	TTCTTGACCTGGATACATCAT	GGCATAGYTCGTCTGCRACTTCCCT
160	RPV_1814853	RPV_1814853	Rinderpest Virus	LLNL	GGATCGCTGAAATGATCTGTGA	GGAGCCAGTTCACCCATTTG	CTGGCCAACCCTGCCTCCACTATGTA
161	RPV_1814855	RPV_1814855	Rinderpest Virus	LLNL	TGCATCTTATGTGACTTTGGTTCA	GGCTATCCGCACAGCTGAC	CAGTCCTCTCATCTGTTGTCGATCCGATGTA
162	RPV_1814856	RPV_1814856	Rinderpest Virus	LLNL	AACTCCTGACCTCATTCCTTGC	GGCTCTATAATCCCACTATGCCA	TGGCTCAGTGCATTCACAAAGACCTTGAATA
170	N/A	NC (MT-7)	Maritima	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
171	N/A	b-MT7 (FC)	N/A	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
172	N/A	MT7/Cy3 (IC)	N/A	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
173	Alien RNA	arRNA Control	None	Ambion	GACATCAAGGCTCAAACTAATTTTACC	CAAAGGCTGCCAACATAAAATG	CAAGCGTAAATGCAGCGTCCA

[1]FCP indicates that the probe is on the negative strand (Forward Compliment to the Probe).

4.5.1. BOVINE PANEL MULTIPLEXED PCR DATA

Preliminary screening: Preliminary screening can be broken down into several steps. The signatures are preliminarily screened by first assessing how the signatures will perform under multiplex PCR conditions and whether or not the addition of the new signatures will adversely effect other signatures, these preliminary screening methods are described as “signature baseline screening” and “multiplex addition screening” respectively. First signature baseline screening tests the performance of the signature in a no-target reaction (blank), a “passing” score is

indicated by a low (typically less than 100) MFI. All BVDV signatures passed the signature baseline screening. Next in multiplex addition screening the new signature primers are added one-by –one to the other panel signatures to determine if there would be any cross-reactions between primer pairs in the panel. Further assessment of the signatures is done through target and near-neighbor screening and the determination of relative sensitivities. The signature down-selection for the BVDV signatures is further described in the table below.

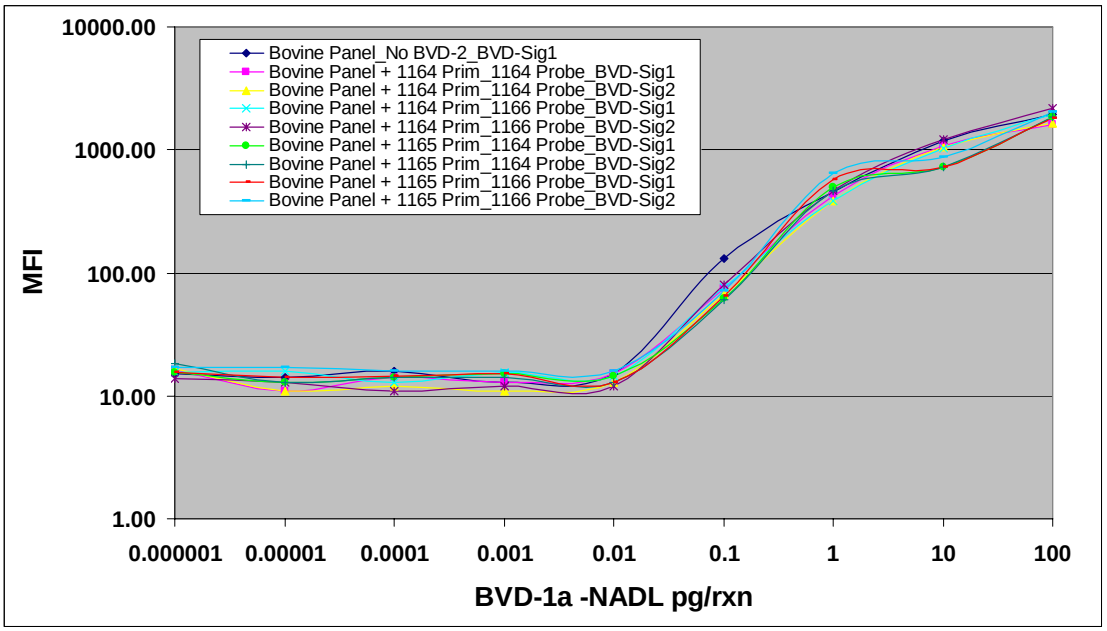


FIG. 16. Results of screening the 3 candidate signatures in multiple configurations to determine the optimal primer/probe pairing for the most sensitive signature when screened against BVD isolate BVD-1a NADL. The BVD from the Version 1.0 panel is shown for reference on each plot (BVDV-1a mod, called BVD-Sig1). The new version of the BVD probe, is called BVD-Sig2. From these results an signature is picked from the combination that is shown to perform the best. This is determined to be primers: BVD_1821164_R, BVD_1821165_BF with probe: BVD_1821166_FCP (Herein called BVD-Sig-2). Serial dilution of nucleic acid Trizol extracted from untitered virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC.

TABLE 137. Multiplexed signature down-selection summary. Signature baseline screening indicates the performance of the signature in a no-sample reaction (blank), a "passing" score is indicated by a low MFI. All signatures passed the signature baseline screening. In the multiplex addition screening the primers are added one-by –one to the other panel constituents to determine if there would be any cross-reactions between primer pairs in the panel. Both signatures passed all phases of screening.

	Mux Screening: Assay Down Selection					
Signature	**Signature baseline screening**	**Multiplex addition screening**	**Target screening**	**Near-neighbor screening**	**Limit of detection screening**	**Noted cross-reactions**

BVD-SIG1	Pass	Pass	Pass	Pass	Pass	None
BVD-SIG2	Pass	Pass	Pass	Pass	Pass	None

Near-neighbor and Target screening: Both signatures were added to the Bovine panel. Both signatures exhibited a very low background response (<10 MFI) in the Bovine panel. Initially only one titered strain of BVD was available for testing (BVD genotype 1 Singer). Through a collaboration with Julia Ridpath, a pestivirus expert from the ARS National Animal Disease Center, IA we were able to obtain 7 other genotypes of BVDV for signature screening.

TABLE 138. Backgrounds screening in multiplexed format for MCF at LLNL and PIADC.

	Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors	Targets
Mux PCR Screening	54	135	16	0	44	8

[1]-There are 752 pooled samples in each Aerosol Block.

TABLE 139. Backgrounds screening in **multiplexed** format for both BVDV signatures against the below listed **eukaryotes**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. None of the signatures cross-reacted with any of the samples listed below.

Description	BVD-1a (34)	BVD-2 (38)
BOVINE	4	8
CAT	6	10
CHICKEN	7	13
DOG	3	6
DROSOPHILA MELANOGASTER	3	7
EQUINE	3	7
FLEA	3	5
HUMAN	2	4
MONKEY	4	7
MOSQUITO	3	7
MOUSE	3	7
PIG / PORCINE	3	7
RABBIT	4	8
RAT	2	5
SHEEP	3	5
TICK	3	6

TABLE 140. Backgrounds screening in **multiplexed** format for both BVDV signatures against the below listed **prokaryotes.** 200pg per reaction of extracted total nucleic acid is added to each

PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. None of the signatures cross-reacted with any of the samples listed below.

Description	BVD-1a (34)	BVD-2 (38)
Erwinia amylovora	4	8
Actinobacillus suis	4	7
Aneurinbacillus migulanus	4	8
Bacillus cereus	4	8
Bacillus globigii	4	9
Bacillus subtilis	3	6
Bacillus thuringiensis	4	8
Bifidobacterium denticum	4	7
Borrellia burgdorferi	4	8
Burkholderia capacia	4	8
Caulobacter vibriodes	3	7
Clavibacter michganensis	4	7
Clostridium butyricum	4	8
Corynebacterium pseudodipthericum	4	8
Cytophaga marinoflava	3	7
Erwinia herbicola	3	9
Escherichia coli	4	9
Geobacillus caldoxylosilyticus	3	8
Halomonas halmophila	4	7
Heamophilus influenza	4	7
Herbaspirillium seropedicae	4	9
Lactobacillus garvieae	3	7
Lactobacillus gasseri	4	8
Listeria monocytogenes	10	14
Listeria seeligeri	4	8
Micrococcus luteus	4	7
Moraxella lacunatica	5	11
Oceanospirillium ssp. Maris	4	9
Paenibacillus napthalaenovorans	4	8
Paracoccus dentrificans	4	9
Porphyrobacter sanguineus	4	8
Proteus mirabillis	4	8
Pseudomonas aeruginosae	4	8
Pseudomonas oleovorans	4	7
Rhizobium leguminosarum	4	9
Rhodococcus rhodochrous	3	7
Salmonella typhimurium	4	8
Simonsiella muelleri	4	8
Sphingomonas sp. (Alcaligenes sp)	4	9
Staphylococcus aureus	14	22
Streptococcus pneumoniae	11	19
Streptomyces scabiei	4	8
Tatlockia maceachernii	5	9
Vibrio paraheamolyticus	3	8
Xanthomonas translucens	4	8

TABLE 141. Backgrounds screening in **multiplexed** format for both BVDV signatures against the below listed **soils**. 200pg per reaction of extracted total nucleic acid is added to each PCR in

triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. None of the signatures cross-reacted with any of the samples listed below.

Description	BVD-1a (34)	BVD-2 (38)
D 000107-49	5	9
D 000109 # 50	5	9
D 000402 # 53	5	10
D 000500 - 26 - 1	5	9
D 000501-14-1	5	10
D 000505 - 11 - 4	5	9
D 000521 - 23	5	10
D 000527 - 3	5	10
D 000531 - 21	4	9
D 000533 - 17 -1	5	9
D 000542 - 6	5	10
D 000550 - 20	4	10
D 000551 - 5	5	10
D 000561 - 8 - 6	4	11
D 000562 - 30 - 5	5	10
S 251	4	10
S 252	5	9
S 253	4	8
S 254	5	10
S 255	5	9
S 256	5	10
S 257	5	10
S 259	4	9
S 260	5	9
S 271	4	8
S 272	4	9
S 273	5	9
S 274	5	9
S 275	5	9
S 276	5	9
S 277	5	9
S 279	4	9
S 280	4	9
S 282	4	7
S 283	5	9
S 284	4	10
S 286	4	10
S 287	3	5
S 288	5	9
S 289	5	9
S 290	5	9
S 291	4	8
S 292	4	9
S 295	5	9

S 296	4	9
S 297	4	8
S 298	5	9
S 299	4	8
S 300	3	8
S 301	3	9
S 303	4	8
S 304	3	8
S 305	4	8
S 307	4	8

TABLE 142. Bovine Panel Near-Neighbor Screening (Data from 20070601) against BVDV signatures. Values shown are Median Flourescence Intensity (MFI) units. “Blanks” are buffer-only samples (no nucleic acid added to reaction), shown below in red. Extracted nucleic acid was added to each PCR reaction in triplicate totaling 200pg/rxn. The data shows that the BVDV signatures cross- reacted with several listed near-neighbors from the Bovine panel constituents. Contamination of BVD from FBS used to culture virus in other virus stocks has been documented in our work; thus this is not considered a cross-reaction, but rather a BVD-specific reaction to BVDV target in a contaminated viral stock. We can explore the acquisition of additional BVDV free stocks of EHV to confirm this if further characterization is funded.

Description	**BVD-SIG1 (34)**			**BVD-SIG2 (38)**		
replicate	**1**	**2**	**3**	**1**	**2**	**3**
Blank	4	4	5	9	10	9
Blank	4	4	4	10	9	8
BHV A040150085	23	3	16	34	6	23
BHV (BFK)	5	11	4	11	21	8
BHV-1 A040130066	5	5	9	9	8	16
BHV-1 A033640072	5	4	5	9	8	8
BHV-1 ATCC VR 793	5	4	4	10	9	9
IBR CA 111903	5	2	5	9	3	10
IBR MN 111903	4	5	5	9	9	8
BHV-1 NVSL 231221	4	5	5	9	9	8
BHV-1 RA309	5	5	4	10	8	8
BHV-1 NVSL 97-10720	5	4	5	10	9	10
BHV-1 NVSL 51619	4	5	4	8	9	8
BHV-1 NVSL 86741	4	4	4	9	9	9
BHV-1 NVSL 200032	5	4	5	10	8	11
BHV-1 LA ATCC VR188	4	39	43	9	55	58
BHV-1 (IBR) Texas CAHFS A030020072	4	6	4	10	9	9
EHV-1 ATCC VR2003	412	402	526	528	497	662
EHV-1 A9904309	550	520	564	702	768	718
EHV-1 A011120004 CAHFS	1207	1180	1088	1448	1514	1378
EHV-1 NVSL 00002	4	4	4	9	8	8
EHV-2 ATCC VR701	244	161	180	317	218	233
EHV-1 A99043047 CAHFS	154	144	94	210	181	141

EHV-2 D990 CAHFS	5	5	4	10	9	8
Pseudorabies Titered	5	4	4	10	9	9
Pseudorabies NVSL 93-11745	5	5	4	8	9	8
Pseudorabies NVSL 92-12013	5	4	4	9	9	9
Pseudorabies RA180 CAHFS	4	4	4	9	8	8
Porcine Herpes Pseudorabies Shope	4	4	4	9	8	8
Feline Herpes ATCC VR636	1775	1889	1685	2321	2351	2317
Caprine Herpes ATCC VR462	5	5	4	10	8	8
Caprine Herpes S0201998 CAHFS	5	5	4	9	9	8
Caprine Herpes D0201157 CAHFS	5	5	5	11	8	8
BHV-5 A040150085 CAHFS	178	107	44	243	141	67
BHV-5 A032540006 CAHFS	5	9	5	9	16	8
BHV-5 D9403153 CAHFS	4	4	5	9	10	8
BHV-5 D9402133 CAHFS	4	4	4	7	8	8
BDV Coos Bay	6	19	7	11	23	11
EHD-1 Georgia	6	4	27	9	8	45
EHD-1 New Jersey	24	4	4	36	7	8
EHD-1 Santa Barbara	28	5	18	40	9	29
EHD-2 Alberta	5	5	5	9	9	8
Fowl Pox	4	5	3	9	9	7
Parainfluenza Type 3	5	5	4	10	8	8
Respiratory Syncytial	5	6	4	9	9	8

Multiplexed PCR Assay Titrations

Upon finalization of the assay panel constituents each agent assay is characterized in multiplexed PCR format by running dose-response (titration) curves against titered virus extracted from virus infected cell culture for each available agent strain. Each sample is tested in duplicate and reported as a mean value of the median fluorescence intensity units (MFI) against agent concentration (per reaction volume, 5uL) on a double log plot. Concentration units are reported in TCID50/rxn (assumes 5uL sample volume per reaction) or pfu/rxn depending on virus type and source. In some cases where titered virus was not available data is reported as picogram units (pg). Each assay is evaluated by its ability to detect agent strains and differentiate from near neighbors (specificity) and by its range of sensitivity to each strain relative to each signature tested for that agent. Because this method is not aimed at determination of analytical sensitivities (requiring a more extensive sample set tested in clinical sample matrices which is not practical for this stage of development) it does however, provide tentative limits of detection that are used to measure the performance of each assay.

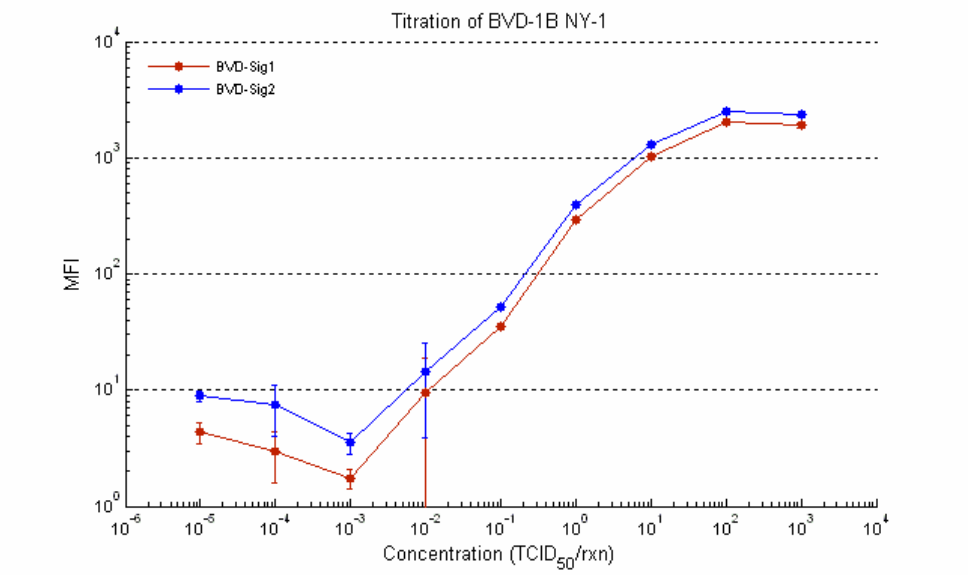


FIG. 17. Multiplex screening data for the two BVDV signatures against extracted nucleic acids from isolate BVD-1B (NY-1). Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1 of the mean.

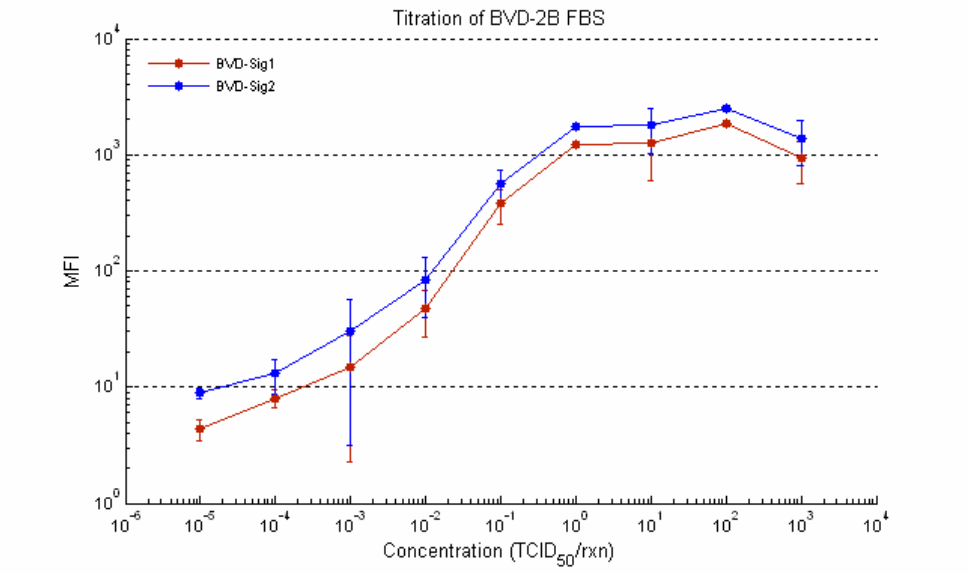


FIG. 18. Multiplex screening data for the two BVDV signatures against extracted nucleic acids from isolate BVD-2B (FBS). Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1 of the mean.

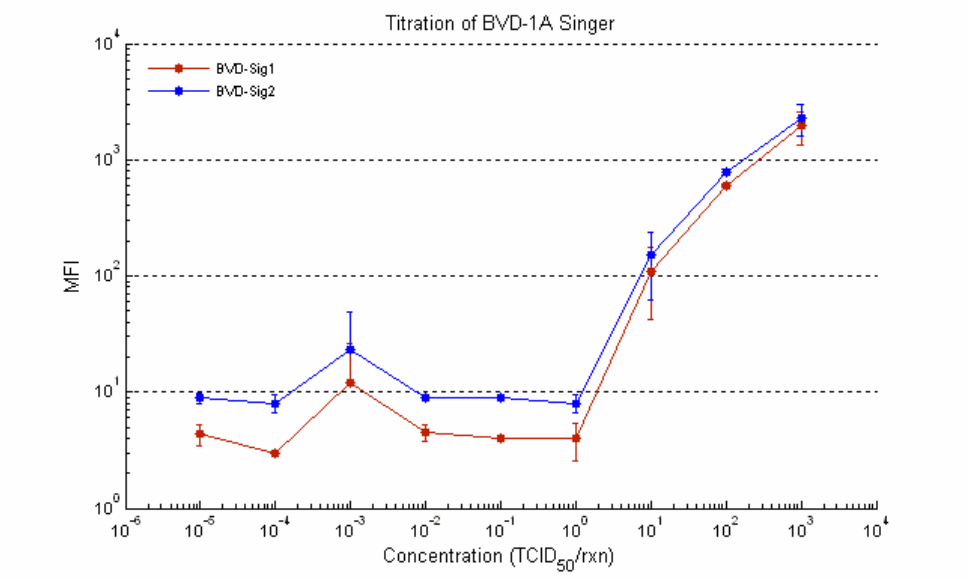


FIG. 19. Multiplex screening data for the two BVDV signatures against extracted nucleic acids from isolate BVD-1A (Singer). Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1 of the mean.

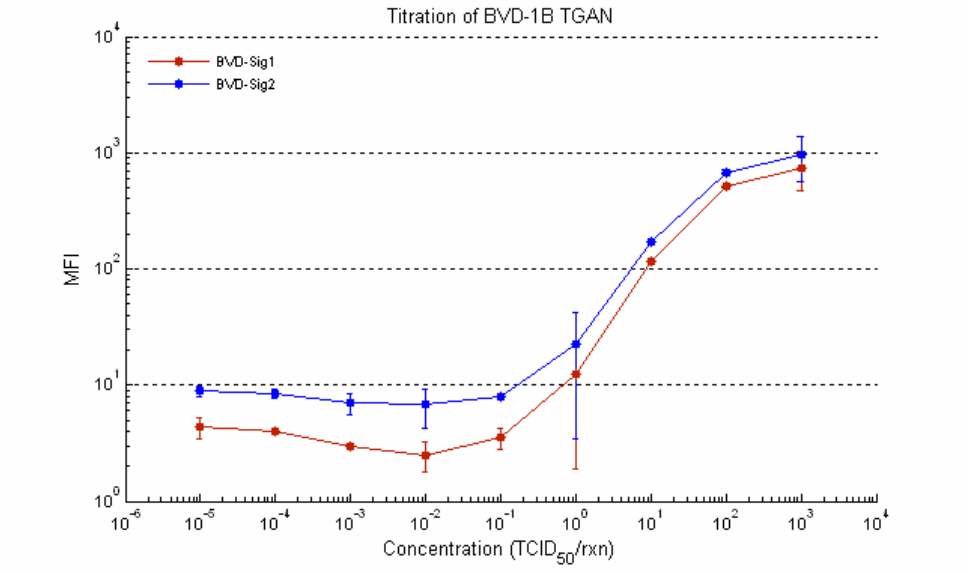


FIG. 20. Multiplex screening data for the two BVDV signatures against extracted nucleic acids from isolate BVD-1B (TGAN). Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1 of the mean.

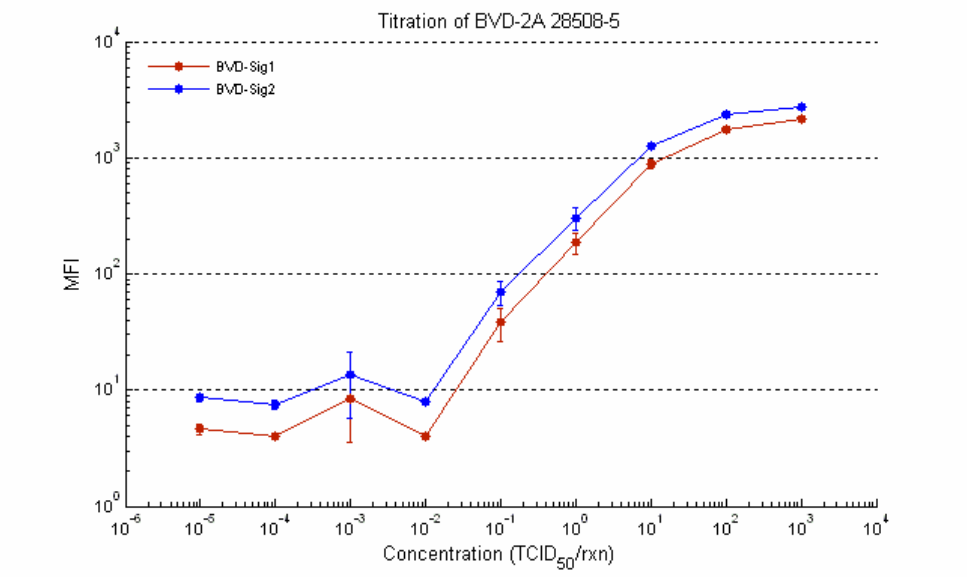


FIG. 21. Multiplex screening data for the two BVDV signatures against extracted nucleic acids from isolate BVD-2A (28508-5). Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1 of the mean.

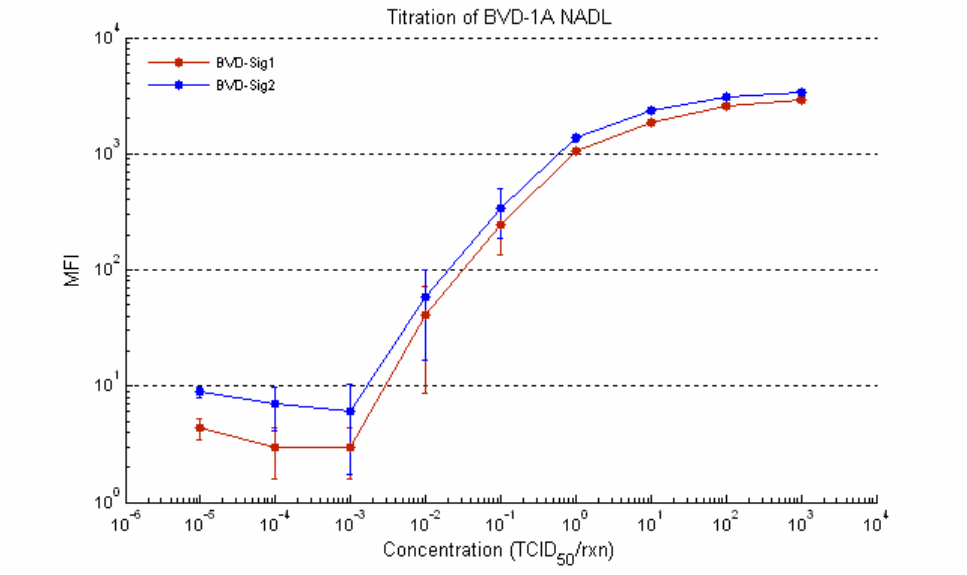


FIG. 22. Multiplex screening data for the two BVDV signatures against extracted nucleic acids from isolate BVD-1A (NADL). Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1 of the mean.

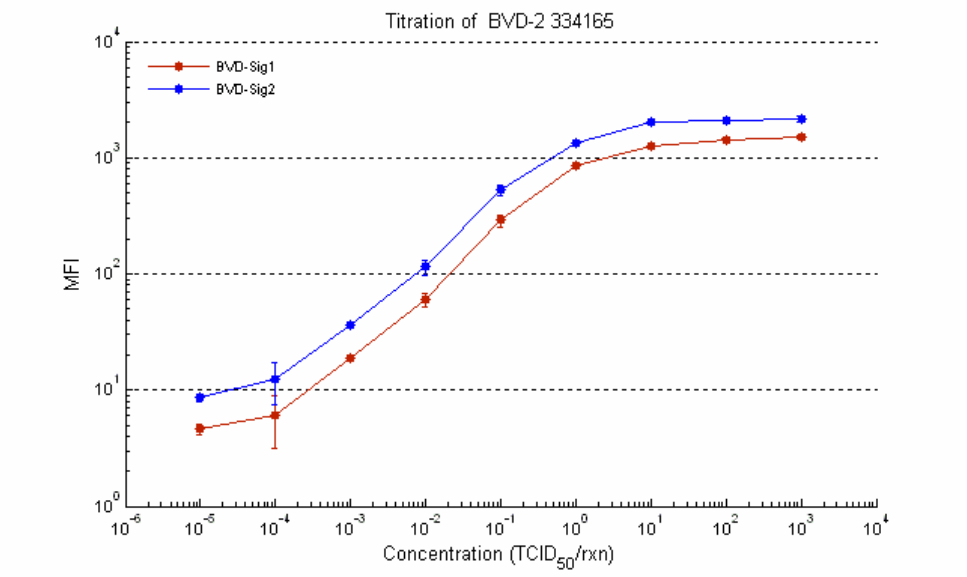


FIG. 23. Multiplex screening data for the two BVDV signatures against extracted nucleic acids from isolate BVD-2 (334165). Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1 of the mean.

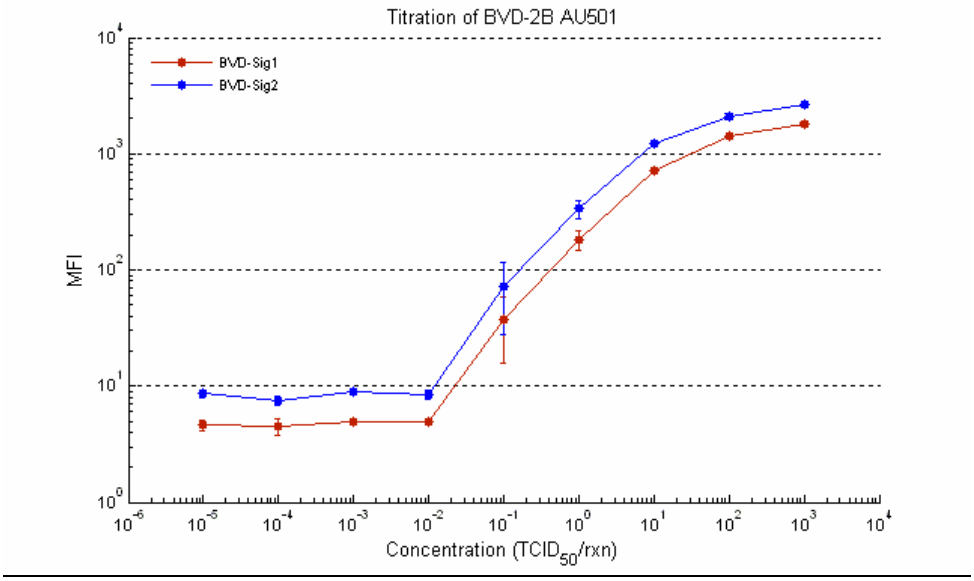


FIG. 24. Multiplex screening data for the two BVDV signatures against extracted nucleic acids from isolate BVD-2B (AU501). Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1 of the mean.

RESULTS: In 2005 we generated a BVDV signature that was screened in real-time and multiplexed PCR 1 that was determined suitable for the Version 1.0 panel. In 2006 tested this original signature against more BVDV genetic diversity than we had in 2005 and designed additional signatures to detect this genetic diversity of BVD. In combination with the BVD signature from the Version 1.0 panel, these two signatures have been currently added the Bovine panel and tested against various genotypes of BVDV. Each signature performed equivalently to one another when tested against the various BVDV isolates. Based on this data each signature could perform independently of one another for the detection of BVD-1 and BVD-2 isolates.

5. MALIGNANT CATARRHAL FEVER VIRUS (BOVINE PANEL)

OBJECTIVE: We were tasked by the Department of Homeland Security to develop specific and reliable Real-time RT- PCR signatures for of Malignant Catarrhal Fever Virus [MCF], among other major agriculturally-impacting viruses that are clinically indistinguishable from foot and mouth disease virus [FMDV]. The purpose of these assays (collectively with other virus-specific signatures developed in parallel) is to develop a species specific (i.e. bovine) multiplexed detection assay for Foot-and-Mouth disease rule-out for use in veterinary diagnostic laboratories. The signatures were designed to discriminate among multiple viral agents whose disease symptoms mimic those of Foot and Mouth Disease Virus [FMDV] and would be capable of detecting the AlHV-1 cause of MCF. This document describes the development of five optimal Real-time RT-PCR and multiplexed [MUX] PCR signatures to detect Malignant Catarrhal Fever [MCF] in a bovine-specific panel for FMDV-rule-out screening.

5.1. BACKGROUND AND ETIOLOGY OF MCF

Malignant catarrhal fever (MCF) is disease mainly effecting ruminants and rarely swine presenting as a variable complex of lesions making it an FMD look-alike disease. Incorporating MCF signatures in a Bovine panel could potentially allow for embedded FAD surveillance whilst testing for endemic domestic diseases of bovine. The disease is caused by either of two gammaherpesviruses; alcelaphine herpesvirus-1 (AIHV-1), of which the natural host is the wildebeest and herpesvirus-2 (OvHV-2), which is prevalent in all varieties of domestic sheep. These signatures should detect and differentiate MCF from FMDV in bovine samples including serum, oral/nasal swab in viral transport medium, and epithelial tissue.

MCF is principally a disease of domestic cattle, water buffalo, Bali cattle (banteng), American bison, and deer. In addition to these farmed animals, MCF has been described in a variety of captive ruminants in mixed zoologic collections. In some species, such as bison and some deer, MCF is acute and highly lethal, capable of affecting large numbers of animals. With occasional exceptions, the disease in cattle normally is seen sporadically and affects single animals. MCF is typically fatal; however, there are outbreaks in which several animals are affected, with evidence of recovery and mild or inapparent infections in some cases. It also occasionally presents as chronic alopecia and weight loss. Its distribution is essentially worldwide, mirroring that of the principal carriers, domestic sheep and wildebeest. MCF has long been a major problem in farmed

deer operations, and in recent years has emerged as a severe threat to the commercial bison industry.

MCF results from infection by one of several members of a group of closely related ruminant gammaherpesviruses of the Rhadinovirus genus. While the MCF group of ruminant rhadinoviruses currently comprises about 10 known members, only a few are known to be pathogenic under natural conditions. The principal carriers and their viruses are sheep (ovine herpesvirus-2), wildebeest (alcelaphine herpesvirus-1), and goats (caprine herpesvirus-2). Another strain of unidentified origin has caused MCF in white-tailed deer. Virtually all clinical cases are caused by the sheep or wildebeest viruses.

The viruses are maintained within the sheep and wildebeest populations in similar but not identical patterns. Lambs are infected usually at 1-2 mo of age by aerosol transmission from other individuals within the flock and begin to actively shed virus at ~6 mo of age. Shedding decreases at ~10 mo, with adults shedding at a much lower rate than adolescents. Wildebeest calves, in contrast, are infected in the perinatal period by horizontal and occasional intrauterine transmission, and actively shed virus until 3-4 mo of age. Transmission is by transfer of virus-laden nasal secretions by direct contact or poorly defined airborne routes. In Africa, most wildebeest-associated MCF is seen around the time of calving; however, sheep-associated MCF (SA-MCF) does not follow the same pattern. Ewes do not shed virus in placental tissues or secretions and do not experience more frequent shedding episodes around lambing time. The only rational and established factors contributing to seasonality of SA-MCF are climatic influences on virus survival and the age-related shedding patterns in lambs. The epidemiology of the caprine MCF virus appears similar to that of sheep[5].

5.2. MCF COMPREHENSIVE ASSAY SUMMARY

Bioinformatics

Virus name: *Malignant Catarrhal Fever* Virus

Project name: *emvfc alcelaphine*

Level of discrimination: *Species*

Total number of Genome Sequences available for alignment: 1
Number of Initial Signatures: 1108
Number of Signatures forwarded to bench screening: 200
Number of Signatures forwarded to PCR gel screening: 40
Number of Signatures forwarded to Real-time RT-PCR screening: 5

[5] *Source: The Merck Veterinary Manual*
http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/55700.htm&word=malignat%2ccatarrhal%2cfever%2cvirus

Real-time RT- PCR Screening Summary

TABLE 143. Final signatures down-selected in real-time RT-PCR screening (5).

#	LLNL Signature Designation	Sequence	#	LLNL Signature Designation	Sequence
1	emcf.94975.F	ATGCCAGTCACTGGCTCTCA	4	emcf_95416.F	TGGCCTACTTAAATGCTACTGTATCAA
	emcf.94976.R	GGGTGTTGTAGAATCCTGAAATGG		emcf_95417.R	AATACTAACACACAAAGTGCCCATAGC
	emcf.94977.P	CCAGGGTGCCACCGTGATCAAC		emcf_95418.P	CCTGTCATTAGTTTGCTTTCACTTTCCAAGAAGGT
2	emcf_95059.F	GTTCTGGAAACTGACCAAACAGTGT	5	emcf_95476.F	CAAAACTGGACAGATGTCTTTAGTTTG
	emcf_95060.R	AGTGGCACTTGAGTGTAACTTTTATTG		emcf_95477.R	TGGTTAAAAAGTGTGAGTTAAAATGCA
	emcf_95061.P	TGTATTTCCCTTATGCCTGCCAGAGTGC		emcf_95478.P	CACAGATTTTACAGACCTCAGTGGTTGACTTTGCTA
3	emcf_95155.F	CCCTGGAAGCTGTCATACAAAA			
	emcf_95156.R	AAACATTGGCATATCTTGCAAGGT			
	emcf_95157.P	TGAGACAACTGCAGCCCTGGACTCTACTG			

TABLE 144. Summary of wet-bench screening in signature down-selection.

	Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors	Target
Gel Screening	3	5	2	none	22	5
Real-time RT-PCR Screening	15	16	13	2 Aerosol Blocks	14	None to date

[1]Note**:** There are 752 pooled samples in each Aerosol Block.

TABLE 145. Limit of detection summary for the final set of *Malignant Catarrhal Fever* Virus signatures. Dose-response curves were generated for each signature using several reference targets for that organism. Dilutions were prepared in the 10-fold range over 5-logs in the detection limit range. Results were then averaged (n=3) and the limit of detection was determined to be the lowest measured concentration (shown here in ng units) that was detected. Nucleic acid is from total DNA extractions; thus for qualitative comparison only. "N" means no detectable PCR product.

Signature	Target Strains (ng total DNA units, relative LOD)		
	AIHV-1 (MN)	OvHV-2	AIHV-1 (WC11) Dilution factor[1]
94976	<0.03 ng	N	1:10E4
95060	<0.03 ng	N	1:10E4
95156	<0.03 ng	N	1:10E4

95417	<0.03 ng	N	1:10E3
94577	0.3 ng	N	1:10E2

Multiplexed PCR Screening Summary

TABLE 146. Backgrounds screening in multiplexed format for MCF at LLNL and PIADC.

	Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors[2]	Targets
Mux PCR Screening	54	135	16	0	43	3

[1]-There are 752 pooled samples in each Aerosol Block. [2]Near-neighbors indicated (43) apply to the near-neighbor panel that is not uniquely specific for MCF, but for the other panel constituents that were screened concurrently.

TABLE 147. Summary of the MCF signatures added to the bovine panel. Additional testing is in process at PIADC.

LLNL Signature Designation	MUX Group ID	Gene ID	Limit of detection[1]	Targets Screened	Near Neighbors Screened
94976	MCF-1	AlHV1gp10/911748	unavailable	3	43
95060	MCF-2	no specific gene associated with this signature	unavailable	3	43
95156	MCF-3	AlHV1gp66/911771	unavailable	3	43

[1] Targets used for MCF screening were untitered the multiplexed summary section reports this data as dilution series. Additional screening is underway at PIADC.

5.3. MCF SIGNATURE BIOINFORMATICS AND GENERATION INFORMATION

Bioinformatics process:
The LLNL Bioinformatics team have developed a computational process that uses available DNA sequence information for a given target organism to generate candidate DNA signature sequences that are conserved and unique. Signatures are primarily used to underlie reliable and specific pathogen detection assays. Evidence of the success of the process is that several of the Real-time RT- PCR signatures developed by LLNL are in use within the national BioWatch monitoring system and were generated by this process, and to date, has resulted in no false positives from a total of over 5 million assays performed.

The general approach is the following: All available complete genomes of different strains of the target species are compared using a multiple genome alignment programs. A *consensus gestalt* is formed from the alignments that contain the sequence conserved amongst all target inputs. (This step is bypassed if only one target sequence is available).

To determine that organism-conserved sequence does not occur in any other sequenced microbial organism, the consensus gestalt is compared against our immense, continuously updated database of external (Genbank) as well as private microbial sequence databases. A customized algorithm accomplishes this electronic subtraction, and the result is a *uniqueness gestalt* that is

mined for potential signature candidates. Final computational screening is done to verify that cross-reactions are not predicted.

Target Virus Information:
Virus name: *Malignant Catarrhal Fever Virus* (*Alcelaphine herpesvirus* 1).
Type: dsDNA virus.
Genome size: 130608 bp.

Primer/Probe Set Generation Information

Method: Alignment and All Microbe Database subtraction.

TABLE 148. K-path run id: S Segment: 13760. List of genomes used for alignment.

Genome Description		GI Number	Sequence Length (bp)
1	Alcelaphine herpesvirus 1, complete genome	10140926	130608

TABLE 149. List of parameters and settings used in generating candidate signatures using Primer 3 signature generation tool.

Primer3 Main Parameters	
Parameters	**Standard Settings**
PRIMER_OPT_SIZE	20
PRIMER_MIN_SIZE	18
PRIMER_MAX_SIZE	27
PRIMER_PRODUCT_OPT_SIZE	100
PRIMER_PRODUCT_SIZE_RANGE	71-600
PRIMER_OPT_TM	62
PRIMER_MIN_TM	61
PRIMER_MAX_TM	63
PRIMER_MIN_GC	20
PRIMER_MAX_GC	80
PRIMER_PICK_INTERNAL_OLIGO	1
PRIMER_INTERNAL_OLIGO_OPT_SIZE	31
PRIMER_INTERNAL_OLIGO_MIN_SIZE	18
PRIMER_INTERNAL_OLIGO_MAX_SIZE	36
PRIMER_INTERNAL_OLIGO_OPT_TM	72
PRIMER_INTERNAL_OLIGO_MIN_TM	71
PRIMER_INTERNAL_OLIGO_MAX_TM	73
Number of Primers/Probe Set generated:	**1108**

Signature Information

Source: LLNL
Project name: emcfv alcelaphine
Level of discrimination: Species.

Number of Initial Signatures: 1108

Number of Signatures forwarded to bench screening: 200
Number of Signatures forwarded to PCR gel screening: 40
Number of Signatures forwarded to Real-time RT-PCR screening: 5

Taqsim description

We used a computational Real-time RT- PCR simulator program, "Taqsim", to identify all potential targets for each signature from all sequences available in Genbank. Taqsim is a BLAST-based comparison of each signature as a triplet against all sequences in Genbank to identify the targets that are predicted to produce a Real-time RT- PCR reaction at 57 degrees primer annealing and 67 degrees for probe annealing (these temperatures are according to Primer 3 oligo Tm calculations). The first column of the excel spreadsheets list the signatures by the name, and the following columns report the predicted Genbank targets, coordinates of signature region, amplicon size, and accession #'s for each target.

Note: For a listing of computationally predicted product sequences and potential cross targets for each signature, please see Appendix II, Taqsim Run Data.

Signature bioinformatics

TABLE 150. Signature bioinformatics (a) emcf_94975 (b) emcf_95059 (c) emcf_95155 (d) emcf_95416 (e) emcf_95476.

	(a)	(b)	(c)
Forward Primer	emcf_95476.F	emcf_95059.F	emcf_95155.F
FWD Primer Length (bp)	20	25	22
FWD Primer TM (^{o}C)	62	62	61
FWD Primer GC Content (%)	55	44	45
Forward Location	21141	75080	106827
Reverse Primer	emcf_94976.R	emcf_95060.R	emcf_95156.R
Rev Primer Length (bp)	24	27	24
Rev Primer TM (^{o}C)	62	61	62
Probe Length (bp)	22	28	29
Probe TM (^{o}C)	71	71	72
Probe GC Content (%)	63	50	55
Probe location	21170	75123	106900
Probe strand	plus	plus	plus
Predicted Product Size	76	99	127

	(d)	(e)
Forward Primer	emcf_95416.F	emcf_95476.F
FWD Primer Length (bp)	27	27
FWD Primer TM (^{o}C)	61	61
FWD Primer GC Content (%)	37	37
Forward Location	3493	40851
Reverse Primer	emcf_95417.R	emcf_95477.R
Rev Primer Length (bp)	27	27
Rev Primer TM (^{o}C)	62	62
Rev Primer GC Content (%)	40	29
Reverse location	3630	40963
Probe Name	emcf_95418.P	emcf_95478.P

Probe Length (bp)	35	36
Probe TM (^{o}C)	71	71
Probe GC Content (%)	40	41
Probe location	3522	40906
Probe strand	plus	plus
Predicted Product Size	164	139

Target Region Gene Information

TABLE 151a-b. (a) Reference Genome used for Gene Information. (b) Gene information for each signature.

(a)

Genome Description	GI Number	Sequence Length (bp)
Alcelaphine herpesvirus 1, complete genome	gi|10140926 |ref| NC_002531.1	130608

(b)

				Gene Location		Gene Region Location	
Kpath Signature ID	**Signature**	**Gene/ID**	**Description**	**Start**	**End**	**Start**	**End**
643675	emcf_94975	AlHV1gp10/911748	DNA Polymerase; ORF09; similar to EBV BALF5, CMV UL54, HSV UL30	19428	22508	21141	21216
643820	emcf_95059	no specific gene associated with this signature	*	*	*	75080	75178
643975	emcf_95155	AlHV1gp66/911771	putative major envelope glycoprotein; ORF68; similar to EBV BFLF1, CMV UL52, HSV UL32	106760	108166	106827	106953
644440	emcf_95416	AlHV1gp03/911790	semaphorin homolog; A3; AHV-sema, similar to Vaccinia A39	3492	5453	3493	3656
644552	emcf_95476	AlHV1gp19/911757	glycoprotein H; ORF22; similar to EBV BXLF2, CMV UL75, HSV UL22	40059	42260	40851	40989

5.4. MCF GEL AND REAL-TIME PCR SCREENING REPORT

Wet-lab screening process. For assay development efficiency and to ensure a high level of selectivity and sensitivity, we have established a screening protocol that rigorously screens candidate DNA signatures to select the optimal sets for eventual field use. Candidate signatures that survive computational screening proceed to wet chemistry screening utilizing standard PCR with agarose gel electrophoresis and real-time PCR. This step ensures that the primers will react across strains representative of the diversity of the pathogen (avoid false negative), but will not

react with the nucleic acids of other organisms that could be present in a sample (avoid false positives). The collections of purified DNA templates used in this screening process are listed below:

1) Strain panels representative of the diversity of the target organism in nature.
2) Near neighbors-organisms that are closely related at the genetic level and therefore have the greatest likelihood of cross-reaction.
3) Eukaryotic DNA that may carry over from sample collection procedures.
4) Prokaryotic DNA that would be expected to be present in environmental samples and may also be present in Bovine and Porcine oral cavities.
5) Soil samples from around the country representing diverse climates and contains a complex mixture of organisms from diverse environmental collections.
6) Aerosol samples collected from aerosol collectors and extracted to test for potential cross-reaction with ubiquitous microbes.

Malignant Catarrhal Fever -Gel Screening Report

TABLE 152. List of targets screened. All MCF strains were extracted nucleic acids with unknown titers.

Virus	Strain[1]	Unique ID	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	Use stock titer	Titer Method
Malignant Catarrhal Fever	ET4499	N/A	PIADC	Unknown	Unknown	Unknown	Unknown	N/A (pg)	N/A
Malignant Catarrhal Fever	V1912 X648	N/A	PIADC	Unknown	Unknown	Unknown	Unknown	N/A (pg)	N/A
Malignant Catarrhal Fever	V1918 San Diego ZooSteer117	N/A	PIADC	Unknown	Unknown	Unknown	Unknown	N/A (pg)	N/A
Malignant Catarrhal Fever	V2201 Oaklahoma BCET91B006 Serial9109	N/A	PIADC	Unknown	Unknown	Unknown	Unknown	N/A (pg)	N/A
Malignant Catarrhal Fever	WC11 BTH50 6D	N/A	PIADC	Unknown	Unknown	Unknown	Unknown	N/A (pg)	N/A
Malignant Catarrhal Fever	AIHV-1 (MN)	N/A	PIADC: Hong Li in Washington State	Unknown	Sheep	Unknown	Ambion MagMax 96	N/A (pg)	N/A
Malignant Catarrhal Fever	OvHV-2	N/A	PIADC: Hong Li in Washington State	Unknown	IMP 7/1/73, 50BTH, 6 EBL	Unknown	Ambion MagMax 96	N/A (pg)	N/A
Malignant Catarrhal Fever	AvHV-1 (WC11 BTH50 6D)	N/A	PIADC	Unknown	MDBK	Unknown	Ambion MagMax 96	N/A (pg)	N/A

TABLE 153. List of near neighbors screened. All near-neighbors samples were extracted nucleic acids with unknown titers.

Virus[1]	Strain[1]	Unique ID	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	NA conc. used or stock titer	Titer Method
Border Disease virus	Coos Bay	4-6-92	NADC, Julia Ridpath	1.5 x 10^8 TCID50/mL	unknown	12/14/06	Trizol	1.18 x 10^6 TCID50/mL	Reed & Muench
Bovine Herpes Virus-1	California	NVSL 20032 111903	CAHFS	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Cooper	NVSL 86741 111903	CAHFS	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Los Angeles	Los Angeles ATCC VR188	ATCC (CAHFS)	1 x 10^5 TCID50/mL	(CAHFS) MDBK2(LLNL)MDBK1	5/17/07	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	RLB-106	ATCC VR793	ATCC (CAHFS)	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	A033640072	CAHFS, R. Mock, #5 Swab, 1BDFK1, 1/28/2004, A030020072	unknown	1BDFK1,	4/23/04	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	A040130066	CAHFS, R. Mock, #4 Lung, 1BFK1, 1/28/2004, A040130066	unknown	1BFK1	4/23/04	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	Texas A030020072	CAHFS, R. Mock, #80 Swab, 1BDFK1, 1/28/2004, A030020072	unknown	1BDFK1	4/23/2004	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	California	NVSL 200032	NVSL, CAHFS	unknown	MDBK CAHFS	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Virginia	NVSL 231221	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	California	NVSL 51619	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Minnesota	NVSL 86741	NVSL, CAHFS	unknown	MDBK CAHFS	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Minnesota	NVSL 97-10720	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Cooper	NVSL-Cooper RA 309	NVSL, CAHFS	unknown	MDBK CAHFS	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes-5	DN-599	A032540006	R. Mock, from lung tissue, 2BFK2, 2/3/2004	Unknown	CAHFS Lab 2BFK2 4/30/2004	6/3/2004	Phenol/Chloroform	40 pg/uL	N/A
Bovine Herpes-5	DN-599	A040150085	R. Mock, #2 Lung, 5BFK5, 2/3/04	Unknown	CAHFS Lab 5BFK5 4/23/2004	7/7/2004	Phenol/Chloroform	40 pg/uL	N/A
Bovine Herpes-5	D9402133	D9402133	Sonoma Co. 3/94 Severe necrotizing encephalitis, 1 week old calf	Unknown	CAHFS Lab (MDBK), (1 flasks) 11/19/2003	11/19/2003	Phenol/Chloroform	40 pg/uL	N/A

Bovine Herpes Virus	BHV 5	D9403153	CAHFS	unknown	unknown	unknown	unknown	40 pg/uL	unknown
Caprine Herpes Virus		VR462	ATCC	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Caprine Herpes Virus	D0201157	D0201157	CAHFS, Isolate D0201157 History: San Joaquin Co. 2/02 Aborted fetus-third trimester	unknown	CAHFS Lab (MDBK)	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Caprine Herpes Virus	S0201998	S0201998	CAHFS, Isolate S0201998 History: San Diego Co. 2/02 Aborted fetus-third trimester	unknown	CAHFS Lab (MDBK)	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-1	Santa Barbara	N/A	Bridget McLaughlin CAHFS	unknown	unknown	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-1	Georgia	N/A	Bridget McLaughlin CAHFS	unknown	unknown	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-2	Alberta	041 ODV 0401	NVSL	unknown	BHK	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-1 (SV123)	New Jersey (3TD) Deer, NJ 1955	041 ODV 0401	NVSL	1 x $10^{5.125}$ $TCID_{50}$/0.1 ml	+BHK-3 (12/7/83); (12/11/86); (12/18/86)	Unknown	Trizol	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A011120004	CAHFS, R. Mock, from brain tissue, 2BFK2, 2/3/2004, A011120004	unknown	CAHFS, 2BFK2	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A99043047	CAHFS, R. Mock, Spleen, 1BFK1, 2/3/2004, A99043047	unknown	CAHFS, 1BFK1	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A9904309	CAHFS	unknown	CAHFS, unknown	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Bovine 1247	Bovine 1247 ATCC VR2003	ATCC	unknown	CAHFS, unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	NVSL 002	NVSL 002 1/28/04	NVSL	unknown	CAHFS, unknown	1/29/03	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	LK	LK ATCC VR701	ATCC	unknown	CAHFS, unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	D990	D990	CAHFS	unknown	CAHFS, unknown	1/29/03	Phenol/ Chloroform	40 pg/uL	N/A
Feline Herpes	C-27	ATCC VR636	ATCC	unknown	CAHFS, unknown	unknown	unknown	40 pg/uL	N/A
Fowl Pox	Vaccine strain	Poxine™	Jeffers Livestock	unknown	ECE	5/17/07	Phenol/ Chloroform	40 pg/uL	N/A
Parainfluenza Virus type 3	C-243	TC-81335	LLNL	1 x 10^{5} TCID50/0.1 mL	(VL)MK14(LLNL)H292-1	5/17/07	Trizol	40 pg/uL	Reed and Muench
Porcine Coronavirus	AR310	ATCC VR-2384	LLNL	1 x $10^{4.25}$ $TCID_{50}$/0.1 ml	ST2				Reed and Muench

Porcine Herpes Pseudorabies	Shope	RA 180	CAHFS	1 x 10^5 TCID50/mL		5/17/07	Trizol	40 pg/uL	N/A
Pseudorabies		92-12013	NVSL 92-12013 bovine isolate Iowa December, 1991	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies		93-11745	NVSL 93-11745 Illinois December, 1992	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies	Shope	RA180	NVSL Shope (PRV) RA 180	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies Titered	Shope	N/A	NVSL	unknown		unknown	Phenol/ Chloroform		Reed & Muench
Respiratory Syncytial virus	Long	N/A		unknown	(VL)KB6 L2KB2HF DL2A549-1(LLNL)H eLa3	5/17/07	Trizol	40 pg/uL	N/A
Transmissible Gastroenteritis virus of hogs	Perdue	NVSL 9801	LLNL	1 x $10^{4.75}$ $TCID_{50}$/0.1 ml	ST 1		Trizol	40 pg/ul	Reed and Muench

TABLE 154. Backgrounds screening summary for MCF at LLNL and PIADC. No cross reactions were seen in gel screening against backgrounds. Signatures were considered successful if they produced expected product sizes for all MCF target strains without cross reacting with near-neighbor strains. All signatures were successful.

	Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors	Targets
Gel Screening	3	2	5	0	22	5
Real-time_PCR Screening	15	13	16	1504	13	3

[1-]There are 752 pooled samples in each Aerosol Block.

Gel Screening

Background gel tests were carried out in duplicate as 25ul reactions in 96 well PCR plates on MJ thermal cyclers. Each reaction mix consisted of 1X PCR Buffer [200mM Tris-HCL (pH 8.4), 500mM KCl], 1.5mM $MgCl_2$, 0.8mM each dNTP, 80ng BSA, 0.4uM each forward and reverse primers, 0.75U Platinum *Taq* DNA polymerase, PCR water and a variable amount of extracted DNA template. A total of 5ng of soil DNA or 1ng of Eukaryotic DNA or 200pg of Prokaryotic DNA was added to each 25ul reaction mix.

Reaction products were visualized by gel electrophoresis on 4% agarose gels. PCR product sizes are listed as visual estimates based on a 20bp ladder that was run on each gel for reference. If a signature screened against a background produced a PCR product size that fell below 100 base pairs greater in size than the predicted product size for a signature screened against its target, the signature was dropped from further screening. The theory behind this selection process is that a much larger than target PCR product would not cause inhibitory PCR competition. However, a PCR product of correct size or smaller would inhibit PCR through competition.

TABLE 155. Nucleic acid extracts used to challenge the initial set of candidate signatures. These select backgrounds are used to determine which signatures will be down-selected against in the screening process.

Nucleic Acid Extract Type	Description/ID
Soil Extract	D000505
Soil Extract	D000526
Soil Extract	D000558
Prokaryotic DNA Extract	*Bacillus cereus*
Prokaryotic DNA Extract	*Bacillus subtilis*
Eukaryotic DNA Extract	Bovine
Eukaryotic DNA Extract	Porcine
Eukaryotic DNA Extract	*Flea*
Eukaryotic DNA Extract	*Mosquito*
Eukaryotic DNA Extract	*Tick*

TABLE 156. Summary of gel screening against **target** and near neighbor extracted nucleic acid. For the five remaining MCF signatures. Results with predicted product size for targets: The product generated with the MCF V1918 San Diego ZooSteer117 did not form a PCR product with any of the signatures (negative or erroneous results indicated in red). The quality of this template may be of question. "N" indicates no detectable PCR product.

	emcf_94975.F	emcf_95059.F	emcf_95155.F	emcf_95416.F	emcf_95476.F
	emcf_94976.R	emcf_95060.R	emcf_95156.R	emcf_95417.R	emcf_95477.R
Predicted Product Size	76bp	99bp	127bp	164bp	192bp
MCF 500 Bovine ET4499	**3 x 80bp**	**3 x 99bp**	**3 x 120bp**	**3 x 160bp**	**3 x 135bp**
MCF V1912 X648	**3 x 75bp**	**3 x 90bp**	**3 x 120bp**	**N**	**3 x 140bp**
MCF V1918 San Diego ZooSteer117	**N**	**N**	**N**	**N**	**N**
MCF V2201 Oklahoma BCET91B006 Serial9109	**3 x 80bp**	**N**	**3 x 125bp**	**3 x 165bp**	**3 x 125bp**
MCF WC11 BTH50 6D	**3 x 80bp**	**3 x 100bp**	**3 x 120bp**	**3 x 160bp**	**3 x 140bp**
Rhadinovirus Caprine Herpes 2 S0201998	N	N	N	N	N
Alphaherpesvirinae Bovine Herpes 5 D9402133	N	N	N	N	N
V. Bovine Herpes 1 A030020072	N	N	N	N	N
V. Bovine Herpes 1 A033640072	N	N	N	N	N
V. Bovine Herpes 1 A040130066	N	N	N	N	N
V. Bovine Herpes 1 California	N	N	N	N	N

V. Bovine Herpes 1 Cooper	N	N	N	N	N
V. Bovine Herpes 1 Minnesota	N	N	N	N	N
V. Bovine Herpes 1 Virginia	N	N	N	N	N
V. Caprine Herpes 2 D0201157	N	N	N	N	N
V. Equine Herpes 1 A011120004	N	N	N	N	N
V. Equine Herpes 1 A99043047	N	N	N	N	N
V. Equine Herpes 2 D990	N	N	N	N	N
V. Equine Herpes 2 NVSL 0002	N	N	N	N	N
V. Pseudorabies (96-10866)	N	N	N	N	N
V. Pseudorabies (92_12013)	N	N	N	N	N
V. Pseudorabies (93-11745)	N	N	N	N	N
V. Pseudorabies (93-21246)	N	N	N	N	N
V. Pseudorabies (93-27020)	N	N	N	N	N
V. Pseudorabies Shope	N	N	N	N	N
Rhadinovirus Caprine herpes D0201157	N	N	N	N	N
Unclassified Gamma Herpes Virinae Ovine Herpes Virus2	N	N	N	N	N

Malignant Catarrhal Fever – Real-Time PCR Screening Report

Background real-time tests were carried out in triplicate as 25ul reactions in 96 well PCR plates on BioRad's iCYCLERs. Each reaction mix consisted of 1X PCR Buffer [200mM Tris-HCL (pH 8.4), 500mM KCl], 6mM $MgCl_2$, 0.2mM each dNTP, 80ng BSA, 0.2uM each forward and reverse primers, 0.4uM Probe with a 5' Fam and a 3' BHQ modification, 1.25U Platinum *Taq* DNA polymerase, PCR water and a variable amount of extracted DNA template. A total of 5ng of soil DNA or 1ng of Eukaryotic DNA or 200pg of Prokaryotic DNA was added to each 25ul reaction mix.

A real-time PCR reaction was deemed positive if a Ct (cycle threshold) value of below 36 cycles was observed at least 2 of the 3 times the reaction was performed.

TABLE 157. List of signatures that were considered successful through gel screening and were brought forward to be screened in real-time PCR format.

emcf_94975.F	ATGCCAGTCACTGGCTCTCA
emcf_94976.R	GGGTGTTGTAGAATCCTGAAATGG
emcf_94977.P	CCAGGGTGCCACCGTGATCAAC
emcf_95059.F	GTTCTGGAAACTGACCAAACAGTGT
emcf_95060.R	AGTGGCACTTGAGTGTAACTTTTATTG
emcf_95061.P	TGTATTTCCCTTATGCCTGCCAGAGTGC
emcf_95155.F	CCCTGGAAGCTGTCATACAAAA

emcf_95156.R	AAACATTGGCATATCTTGCAAGGT
emcf_95157.P	TGAGACAACTGCAGCCCTGGACTCTACTG
emcf_95416.F	TGGCCTACTTAAATGCTACTGTATCAA
emcf_95417.R	AATACTAACACACAAAGTGCCCATAGC
emcf_95418.P	CCTGTCATTAGTTTGCTTTCACTTTCCAAGAAGGT
emcf_95476.F	CAAAACTGGACAGATGTCTTTAGTTTG
emcf_95477.R	TGGTTAAAAAGTGTGAGTTAAAATGCA
emcf_95478.P	CACAGATTTTACAGACCTCAGTGGTTGACTTTGCTA

TABLE 158a-c. Real-time PCR background screening consisted of an extensive list of soil, prokaryotic and eukaryotic extracted DNAs in addition to 3 aerosol blocks, each consisting of 752 pooled samples (2 blocks screened #080403 and # 08190). All signatures passed real-time PCR background screening. None of the MCF signatures produced an amplicon when screened against the listed backgrounds. All signatures moved forward to be screened against the available *MCF* targets and near-neighbors.

(a) Total of 15 soils screened

D000426	D000109
D000407	D000107
D000541	D000019
D000557	D000028
D000558	D000086
D000526	D000542
D000528	D000054
D000532	

(b) Total of 16 Eukaryotes screened

Monkey	Sheep	Monkey	Sheep
Human	Dog	Human	Dog
Tick	Chicken	Tick	Chicken
Mouse	Cat	Mouse	Cat

(c) Total of 13 Prokaryotes screened

B. burgdorferi	E. coli	S. aureus
B. cereus	E. herbicola	S. pneumonia
B. globigii	H. influenza	S. typhimurium
B. subtilis	L. monocytogenes	
C. burnetti	P. aeriginosa	
B. burgdorferi	E. coli	
B. cereus	E. herbicola	
B. globigii	H. influenza	

TABLE 159. Each *MCF* signature was tested with six available near-neighbors. Each screening set was performed in triplicate indicated below as an average Ct value. "N" indicates no detectable PCR product in at least 2 of the 3 replicates after 35 cycles pf PCR. The PCR positive control, Bacillus thuringiensis, indicated that PCR results were reliable.

emcf_94975.F	emcf_95059.F	emcf_95155.F	emcf_95416.F	emcf_95476.F

	emcf_94976.R	emcf_95060.R	emcf_95156.R	emcf_95417.R	emcf_95477.R
Bovine Herpes Virus DN-599 0006	N	N	N	N	N
Bovine Herpes Virus-1 DN-599A040150085	N	N	N	N	N
Bovine Herpes Virus-1 RLB-106 ATCC #VR--793	N	N	N	N	N
Bovine HerpesVirus-1 A040130066	N	N	N	N	N
Bovine HerpesVirus-1 LA ATCC #VR-188	N	N	N	N	N
Bovine HerpesVirus--5 A032540006	N	N	N	N	N
Caprine Herpes #2 ATCC #VR-462	N	N	N	N	N
CHV D0201157	N	N	N	N	N
Equine Herpes Virus #1 Bov 1247 ATCC #VR-2003	N	N	N	N	N
Equine Herpes Virus #2 LK ATCC #VR-701	N	N	N	N	N
Equine Herpes Virus -1 A99043047	N	N	N	N	N
Equine Herpes Virus NVSL 0002	N	N	N	N	N
Felid Herpes Virus C-27 ATCC #VR-636	N	N	N	N	N
Pseudorabies Virus 92-12013	N	N	N	N	N
Pseudorabies Virus 93-27020	N	N	N	N	N

Lowest level of detection data:

TABLE 160. MCF titration data from PIADC. The target DNAs screened were total nucleic acid extracts, non-titered. The concentration in ng/ rxn (reaction) represents the total amount of target DNA added to each 25ul PCR reaction. Results are indicated as the average Ct value for each assay, n=3. "N" indicates no detectable PCR product after 35 cycles of PCR. The isolate AIHV-1 (WC11) was not quantified, so results are described in dilution factor units for qualitative comparison.

		AIHV-1(MN)		**OvHV-2**		**AIHV-1(WC11)**	
emcf_94976	Dilution	ng/Rxn	Ave. Ct	ng/Rxn	Ave. Ct	ng/Rxn	Ave. Ct
	1:10	291	17	64	N	unknown	25.5
	1:10E2	29.1	20.1	6.4	N	unknown	30.5
	1:10E3	2.91	24.3	0.64	N	unknown	34.9
	1:10E4	0.29	27.9	0.064	N	unknown	38.6
	1:10E5	0.03	31.3	0.0064	N	unknown	N
emcf_95060	Dilution	ng/Rxn	Ave. Ct	ng/Rxn	Ave. Ct	ng/Rxn	Ave. Ct
	1:10	291	15.5	64	N	unknown	24.9
	1:10E2	29.1	18.1	6.4	N	unknown	30.2

	1:10E3	2.91	23.3	0.64	N	unknown	33.3
	1:10E4	0.29	27.3	0.064	N	unknown	37.1
	1:10E5	0.03	30	0.0064	N	unknown	n
emcf_95156	Dilution	ng/Rxn	Ave. Ct	ng/Rxn	Ave. Ct	ng/Rxn	Ave. Ct
	1:10	291	18.4	64	N	unknown	18.3
	1:10E2	29.1	21.3	6.4	N	unknown	21.3
	1:10E3	2.91	26.3	0.64	N	unknown	26.3
	1:10E4	0.29	30.3	0.064	N	unknown	39
	1:10E5	0.03	33.1	0.0064	N	unknown	N
emcf_95417	Dilution	ng/Rxn	Ave. Ct	ng/Rxn	Ave. Ct	ng/Rxn	Ave. Ct
	1:10	291	20.2	64	N	unknown	28.4
	1:10E2	29.1	23.8	6.4	N	unknown	34.3
	1:10E3	2.91	28.6	0.64	N	unknown	37.2
	1:10E4	0.29	32.5	0.064	N	unknown	N
	1:10E5	0.03	35.8	0.0064	N	unknown	N
emcf_94577	Dilution	ng/Rxn	Ave. Ct	ng/Rxn	Ave. Ct	ng/Rxn	Ave. Ct
	1:10	291	17.9	64	N	unknown	29.1
	1:10E2	29.1	23.6	6.4	N	unknown	36
	1:10E3	2.91	28.1	0.64	N	unknown	N
	1:10E4	0.29	33.3	0.064	N	unknown	N
	1:10E5	0.03	n	0.0064	N	unknown	N

TABLE 161. Limit of detection summary for the final set of *Malignant Catarrhal Fever* Virus signatures. Dose-response curves were generated for each signature using several reference targets for that organism. Dilutions were prepared in the 10-fold range over 5-logs in the detection limit range. Results were then averaged (n=3) and the limit of detection was determined to be the lowest measured concentration (shown here in ng units) that was detected. Nucleic acid is from total DNA extractions; thus for qualitative comparison only. "N" means no detectable PCR product.

	Target Strains (ng total DNA units, relative LOD)		
Signature	**AIHV-1 (MN)**	**OvHV-2**	**AIHV-1 (WC11) Dilution factor[1]**
emcf_94976	<0.03 ng	N	1:10E4
emcf_95060	<0.03 ng	N	1:10E4
emcf_95156	<0.03 ng	N	1:10E4
emcf_95417	<0.03 ng	N	1:10E3
emcf_94577	0.3 ng	N	1:10E2

[1]MCF virus strain AIHV-1 (WC11) was not titered and thus is indicated as a dilution of stock with an unknown concentration.

Gel and Real-time Screening Summary: The LLNL Bioinformatics team generated 200 unique signatures, of which 40 continued on to gel screening. Of the 40 signatures screened in gel, only 18 did not cross react with background confounders. Based on data from primer pair screening, a set of 5 specific and reliable signatures were then further tested for suitability for real-time Taqman® fluorogenic PCR detection protocols.

5.5. MCF MULTIPLEXED PCR SCREENING REPORT

Screening and Down-selection Technical Approach:

Signatures that pass gel and real-time PCR screening are candidates for multiplexed assays. If the number of candidate signatures is large than a subset of the available signatures are selected based on measured sensitivity and specificity observed in real-time screening. In preliminary screening the background of individual signatures is measured by running "blank" (no sample added) controls and observing signature response. In some cases a primer-to-primer non-specific interference will eliminate candidate signatures. Signatures are then added iteratively to each multiplexed panel, titrated, and with each signature addition, collectively, all signatures are re-titrated to determine if the presence of one signature will interfere with the performance of another. During this process signatures are added or subtracted from the multiplexed panel depending on individual outcomes until all desirable signatures have been added.

Criterion for down-selection of multiplexed signatures

Signature specificity. In initial real-time PCR screening tests undergo target and near neighbor screening and screening against environmental confounders which assess individual signature specificity. In multiplex format this is repeated to verify that a more complex reaction system gives the same level of specificity.

Signature sensitivity. In initial real-time PCR screening tests undergo limit of detection testing where a dilution curve is generated for each signature. This determines individual signature sensitivity. In multiplex format this is repeated to verify that a more complex reaction system gives acceptable signature sensitivity.

Optimal signal to noise ratio. In the multiplex assay system the potential for cross reactivity is larger than in single reaction testing, this may cause unwanted PCR reaction products or non-specific cross-reactivity. Signatures are preferred to have a high signal to noise ratio, where desired signal is significantly greater than the background noise of the signature when there is no target nucleic acid in the reaction.

Compatibility with other signatures in multiplex. In some cases a primer set may interfere with other primers or probes in the multiplex and there by cause limit of detection shifting for one or more assay in the multiplex. For each scenario it must be evaluated whether the shift in detection level is significant enough to remove (what could be a very desirable signature) from the panel.

TABLE 162. Order details for signatures ordered for multiplexed assay screening and development.

ID	Mux ID	Modification details	Vendor
emcf_94975.BF	MCF_1	5'-/5Bio/ATGCCAG/iBiodT/CACTGGC/iBiodT/CTCA-3'	IDT DNA

emcf_94975.R	MCF_1	5'-GGGTGTTGTAGAATCCTGAAATGG-3'	IDT DNA
emcf_94975.FCP	MCF_1	5'- /5AmMC6//iSp18/GTTGATCACGGTGGCACCCTGG-3'	IDT DNA
emcf_94959.BF	MCF_2	5'-/5Bio/GTTCTGGAAAC/iBiodT/GACCAAACAG/iBiodT/GT-3'	IDT DNA
emcf_94960.R	MCF_2	5'-AGTGGCACTTGAGTGTAACTTTTATTG-3'	IDT DNA
emcf_94961.FCP	MCF_2	5'- /5AmMC6//iSp18/GCACTCTGGCAGGCATAAGGGAAATACA-3'	IDT DNA
emcf_95155.BF	MCF_3	5'-/5Bio/CCCTGGAAGC/iBiodT/GTCA/iBiodT/ACAAAA-3'	IDT DNA
emcf_95155.R	MCF_3	5'-AAACATTGGCATATCTTGCAAGGT-3'	IDT DNA
emcf_95155.FCP	MCF_3	5'- /5AmMC6//iSp18/CAGTAGAGTCCAGGGCTGCAGTTGTCTCA-3'	IDT DNA
emcf_95416.BF	MCF_4	5'-/5Bio/TGGCCTAC/iBiodT/TAAATGCTACTG/iBiodT/ATCAA-3'	IDT DNA
emcf_95416.R	MCF_4	5'-AATACTAACACACAAAGTGCCCATAGC-3'	IDT DNA
emcf_95416.FCP	MCF_4	5'- /5AmMC6//iSp18/ACCTTCTTGGAAAGTGAAAGCAAACTAATGACAGG-3'	IDT DNA
emcf_95476.BF	MCF_5	5'-/5Bio/CAAAAC/iBiodT/GGACAGATGTCT/iBiodT/TAGTTTG-3'	IDT DNA
emcf_95476.R	MCF_5	5'-TGGTTAAAAAGTGTGAGTTAAAATGCA-3'	IDT DNA
emcf_95476.FCP	MCF_5	5'- /5AmMC6//iSp18/TAGCAAAGTCAACCACTGAGGTCTGTAAAATCTGTG-3'	IDT DNA

Multiplex Data Analysis -Technical approach

Disease signature thresholds

Thresholds were initially determined using all available data from known negative samples (blanks) by plotting a histogram which showed the probability of a given sample generating a particular MFI. In the absence of cross reactivity, each signature in the multiplex assay could be treated as an individual signature result. For example, for a sample spiked with MCF virus, data for other disease signatures could contribute towards negative data, providing that cross reaction between disease signature sets was not observed. This is a way to increase the number of samples that can be used to establish thresholds.

TABLE 163. Individual signature thresholds for MCF. For FY06, threshold determinations have not yet been made as they require a significant number of tests (>500) to generate this information. This information will be updated when available.

Signature Name	Mux Assay name	Signature background in Tris NaCl Buffer (MFI +/-)	Signature background in Clinical matrices (MFI +/-)	Threshold
emcf_94959.BF emcf_94960.R emcf_94961.FCP	MCF-1	TBD	TBD	TBD
emcf_94975.BF emcf_94976.R emcf_94977.FCP	MCF-2	TBD	TBD	TBD
emcf_95155.BF emcf_95156.R emcf_95157.FCP	MCF-3	TBD	TBD	TBD

TABLE 164. List of targets screened in preliminary bovine multiplexed panel format and to be screened in the final bovine multiplexed panel at Plum island. All MCF strains were extracted DNAs with unknown titers.

Virus	Strain[1]	Unique ID	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	Use stock titer	Titer Method
Malignant Catarrhal Fever	AIHV-1 (MN)	N/A	PIADC: Hong Li in Washington State	Unknown	Sheep	Unknown	Ambion MagMax 96	N/A (pg)	N/A
Malignant Catarrhal Fever	OvHV-2	N/A	PIADC: Hong Li in Washington State	Unknown	IMP 7/1/73, 50BTH, 6 EBL	Unknown	Ambion MagMax 96	N/A (pg)	N/A
Malignant Catarrhal Fever	AvHV-1 (WC11 BTH50 6D)	N/A	PIADC	Unknown	MDBK	Unknown	Ambion MagMax 96	N/A (pg)	N/A

TABLE 165. List of near-neighbors screened against the Bovine Panel. All near-neighbors samples were extracted nucleic acids with unknown titers.

Virus	Strain[1]	Unique ID	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	NA conc. used or stock titer	Titer Method
Border Disease virus	Coos Bay	4-6-92	NADC, Julia Ridpath	1.5×10^8 TCID50/mL	unknown	12/14/06	Trizol	1.18×10^6 TCID50/mL	Reed & Muench
Bovine Herpes Virus-1	California	NVSL 20032 111903	CAHFS	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Cooper	NVSL 86741 111903	CAHFS	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Los Angeles	Los Angeles ATCC VR188	ATCC (CAHFS)	1×10^5 TCID50/mL	(CAHFS) MDBK2(LLNL)MDBK1	5/17/07	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	RLB-106	ATCC VR793	ATCC (CAHFS)	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	A033640072	CAHFS, R. Mock, #5 Swab, 1BDFK1, 1/28/2004, A030020072	unknown	1BDFK1,	4/23/04	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	A040130066	CAHFS, R. Mock, #4 Lung, 1BFK1, 1/28/2004, A040130066	unknown	1BFK1	4/23/04	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	Texas A030020072	CAHFS, R. Mock, #80 Swab, 1BDFK1, 1/28/2004, A030020072	unknown	1BDFK1	4/23/2004	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	California	NVSL 200032	NVSL, CAHFS	unknown	MDBK CAHFS	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Virginia	NVSL 231221	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	California	NVSL 51619	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Minnesota	NVSL 86741	NVSL, CAHFS	unknown	MDBK CAHFS	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Minnesota	NVSL 97-10720	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A

Bovine Herpes Virus-1	Cooper	NVSL-Cooper RA 309	NVSL, CAHFS	unknown	MDBK CAHFS	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes-5	DN-599	A032540006	R. Mock, from lung tissue, 2BFK2, 2/3/2004	Unknown	CAHFS Lab 2BFK2 4/30/2004	6/3/2004	Phenol/Chloroform	40 pg/uL	N/A
Bovine Herpes-5	DN-599	A040150085	R. Mock, #2 Lung, 5BFK5, 2/3/04	Unknown	CAHFS Lab 5BFK5 4/23/2004	7/7/2004	Phenol/Chloroform	40 pg/uL	N/A
Bovine Herpes-5	D9402133	D9402133	Sonoma Co. 3/94 Severe necrotizing encephalitis, 1 week old calf	Unknown	CAHFS Lab (MDBK), (1 flasks) 11/19/2003	11/19/2003	Phenol/Chloroform	40 pg/uL	N/A
Bovine Herpes Virus	BHV 5	D9403153	CAHFS	unknown	unknown	unknown	unknown	40 pg/uL	unknown
Caprine Herpes Virus		VR462	ATCC	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Caprine Herpes Virus	D0201157	D0201157	CAHFS, Isolate D0201157 History: San Joaquin Co. 2/02 Aborted fetus-third trimester	unknown	CAHFS Lab (MDBK)	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Caprine Herpes Virus	S0201998	S0201998	CAHFS, Isolate S0201998 History: San Diego Co. 2/02 Aborted fetus-third trimester	unknown	CAHFS Lab (MDBK)	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-1	Santa Barbara	N/A	Bridget McLaughlin CAHFS	unknown	unknown	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-1	Georgia	N/A	Bridget McLaughlin CAHFS	unknown	unknown	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-2	Alberta	041 ODV 0401	NVSL	unknown	BHK	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-1 (SV123)	New Jersey (3TD) Deer, NJ 1955	041 ODV 0401	NVSL	1 x $10^{5.125}$ $TCID_{50}$/0.1 ml	+BHK-3 (12/7/83); (12/11/86); (12/18/86)	Unknown	Trizol	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A011120004	CAHFS, R. Mock, from brain tissue, 2BFK2, 2/3/2004, A011120004	unknown	CAHFS, 2BFK2	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A990430 47	CAHFS, R. Mock, Spleen, 1BFK1, 2/3/2004, A99043047	unknown	CAHFS, 1BFK1	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A990430 9	CAHFS	unknown	CAHFS, unknown	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Bovine 1247	Bovine 1247 ATCC VR2003	ATCC	unknown	CAHFS, unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A

Equine Herpes 2	NVSL 002	NVSL 002 1/28/04	NVSL	unknown	CAHFS, unknown	1/29/03	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	LK	LK ATCC VR701	ATCC	unknown	CAHFS, unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	D990	D990	CAHFS	unknown	CAHFS, unknown	1/29/03	Phenol/ Chloroform	40 pg/uL	N/A
Feline Herpes	C-27	ATCC VR636	ATCC	unknown	CAHFS, unknown	unknown	unknown	40 pg/uL	N/A
Fowl Pox	Vaccine strain	Poxine™	Jeffers Livestock	unknown	ECE	5/17/07	Phenol/ Chloroform	40 pg/uL	N/A
Parainfluenza Virus type 3	C-243	TC-81335	LLNL	1×10^5 TCID50/0.1 mL	(VL)MK1 4(LLNL)H 292-1	5/17/07	Trizol	40 pg/uL	Reed and Muench
Porcine Coronavirus	AR310	ATCC VR-2384	LLNL	$1 \times 10^{4.25}$ $TCID_{50}$/0.1 ml	ST2				Reed and Muench
Porcine Herpes Pseudorabies	Shope	RA 180	CAHFS	1×10^5 TCID50/mL		5/17/07	Trizol	40 pg/uL	N/A
Pseudorabies		92-12013	NVSL 92-12013 bovine isolate Iowa December, 1991	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies		93-11745	NVSL 93-11745 Illinois December, 1992	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies	Shope	RA180	NVSL Shope (PRV) RA 180	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies Titered	Shope	N/A	NVSL	unknown		unknown	Phenol/ Chloroform		Reed & Muench
Respiratory Syncytial virus	Long	N/A		unknown	(VL)KB6 L2KB2HF DL2A549-1(LLNL)H eLa3	5/17/07	Trizol	40 pg/uL	N/A
Transmissible Gastroenteritis virus of hogs	Perdue	NVSL 9801	LLNL	$1 \times 10^{4.75}$ $TCID_{50}$/0.1 ml	ST 1		Trizol	40 pg/ul	Reed and Muench

TABLE 166. Panel membership for assay. The 3 down-selected MCF signatures were included in the **Bovine-specific multiplexed assay panel** for FMDV rule-out diagnostics. The below table describes the constituents of that multiplexed assay panel in which these signatures were tested.

Bead ID	Signature/ Target Name	Mux Group ID	Virus	Source	Forward Primer	Reverse Primer	Luminex Probe (-strand, FCP)
113	emcf_94975.F, emcf_94976.R, emcf_94977.P	MCF_1	Malignant Catarrhal Fever	LLNL	ATGCCAGTCACTGGCT CTCA	GGGTGTTGTAGAATCCTG AAATGG	GTTGATCACGGTGGCACC CTGG
114	emcf_95059.F, emcf_95060.R, emcf_95061.P	MCF_2	Malignant Catarrhal Fever	LLNL	GTTCTGGAAACTGACC AAACAGTGT	AGTGGCACTTGAGTGTAA CTTTTATTG	GCACTCTGGCAGGCATAA GGGAAATACA
115	emcf_95155.F, emcf_95156R, emcf_95157.P	MCF_3	Malignant Catarrhal Fever	LLNL	CCCTGGAAGCTGTCAT ACAAAA	AAACATTGGCATATCTTGC AAGGT	CAGTAGAGTCCAGGGCTG CAGTTGTCTCA
117	BVH__94666.F, BVH_94667.R, BVH_94668.P	BHV-94668	Bovine Herpes Virus	LLNL	GTGCCAGCCGCGTAAA AG	GACGACTCCGGGCTCTTT T	TCCTGGTTCCAGAGCGCTA ACATGGAG
118	BVH__94738.F, BVH_94739.R, BVH94740.P	BHV-94740	Bovine Herpes Virus	LLNL	TGAGGCCTATGTATGG GCAGTT	GCGCGCCAAACATAAGTA AA	AAATAACACGGTGTGCACT TAAATAAGATTCGCG
119	BPSV_95719.F, BPSV_95720.R, BPSV_95721.P	PPOX_1	Bovine Papular Stomatitis Virus	LLNL	GCAGATGCGCTCCTGG TT	GCACCTCTGCTGCTGCAA	CCGACTCCGACGTGGAGA ACGTG

120	BPSV_95722.F, BPSV_95723.R, BPSV_95724.P	PPOX_2	Bovine Papular Stomatitis Virus	LLNL	GATGGCCGTGCAGCTCTT	CGTACAAGATCACGGCCAACT	TGTACGGGCTCATGGGCTTCCG
122	BPSV_95731.F, BPSV_95732.R, BPSV_95733.P	PPOX_4	Bovine Papular Stomatitis Virus	LLNL	GCAGCAGTGCACCACGTAGT	CGCTGAACCCGTACATCCT	GACTTCGAGGCGGACAACAAGCG
132	FMDV.TC	FMDV.TC	Foot and Mouth Virus	Tetracore	ACTGGGTTTTACAAACCTGTGA	GCGAGTCCTGCCACGGA	GTCCCACGGCGTGCAAAGGA
133	FMDV.Pir	FMDV.Pir	Foot and Mouth Virus	Pirbright	CACYTYAAGRTGACAYTGRTACTGGTAC	CAGATYCCRAGTGWCICITGTTA	CCTCGGGGTACCTGAAGGGCATCC
134	BVD_1a	BVD_SIG1	Bovine Viral Diarrhea	UCD, CAHFS lab	GGTAGTCGTCAGTGGTTCGAC	CATGTGCCATGTACAGCAGAGAT	CCTCGTCCACGTGGCATCTCGAG
138	BVD_1821165.F, BVD_1821164.R, BVD_1821166.P	BVD_SIG2	Bovine Viral Diarrhea	LLNL	GGGAGTCGTCAATGGTTCGAC	TCCATGTGCCATGTACAGCAGAG	CCTCGTCCACITGGCATCTCGAG
135	BTV_1759932	BTV_1759932	Bluetongue Virus	LLNL	GCACCCTATATGTTTCCAGACCA	CAGCTAACTCTTCAGCCACACG	CTAACTCGTGGGCCAATCATCATCTTCTGT
136	BTV10_1810207	BTV10_1810207	Bluetongue Virus	LLNL	CAAACACAAAAGGCGGAGAAG	GGCGTTTAATCTGTCTTAGTCTTACGT	GAAACGCTTCTGCGTACGATGCGA
137	BTV10_1810199	BTV10_1810199	Bluetongue Virus	LLNL	CACATGTCGCTTAATTTGTCTTAACC	GCGGAGAAGGCTGCATT	ACGAAACGCTTCCGCGTACGATG
139	BTV10_1810205	BTV10_1810205	Bluetongue Virus	LLNL	TCAATTTTGGTAGAATTTGTTCATTCA	GCGGAGAAGGCTGCATTC	ACGAAACGCTTCCGCGTACGATG
163	VSV_1798943	VSV_1798943	Vesicular Stomatitis Virus	LLNL	CGCCACAAGGCAGAGATGT	TGTCAAATTCTGACTTAGCATACTTGC	GCATACTGCATCATATCAGGAGTCGGTTTTCTG
166	VSV_1798947	VSV_1798947	Vesicular Stomatitis Virus	LLNL	CCCAATCAATGCCATGATACA	CTCCAATGGAAGGGTCCAAA	TTTGAAAGTAGAACTGTGCAAGCCCGGTATC
167	VSV_1798949	VSV_1798949	Vesicular Stomatitis Virus	LLNL	GGCGCTCATTATAAAATTCGGA	ACATTTTCTCGTAGTAATGCAGCAG	GAAGTCCCTGTAATGGATTCCCATTCCATGT
169	VSV_1811408	VSV_1811408	Vesicular Stomatitis Virus	PIADC	CTCACAACATGGGTCCTGAA	TTCTTGACCTGGATACATCAT	GGCATAGYTCGTCTGCRACTTCCCT
160	RPV_1814853	RPV_1814853	Rinderpest Virus	LLNL	GGATCGCTGAAATGATCTGTGA	GGAGCCAGTTCACCCATTTG	CTGGCCAACCCTGCCTCCACTATGTA
161	RPV_1814855	RPV_1814855	Rinderpest Virus	LLNL	TGCATCTTATGTGACTTTGGTTCA	GGCTATCCGCACAGCTGAC	CAGTCCTCTCATCTGTTGTCGATCCGATGTA
162	RPV_1814856	RPV_1814856	Rinderpest Virus	LLNL	AACTCCTGACCTCATTCCTTGC	GGCTCTATAATCCCACTATGCCA	TGGCTCAGTGCATTCACAAAGACCTTGAATA
170	N/A	NC (MT-7)	Maritima	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
171	N/A	b-MT7 (FC)	N/A	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
172	N/A	MT7/Cy3 (IC)	N/A	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
173	Alien RNA	arRNA Control	None	Ambion	GACATCAAGGCTCAAACTAATTTTACC	CAAAGGCTGCCAACATAAAATG	CAAGCGTAAATGCAGCGTCCA

[1]FCP indicates that the probe is on the negative strand (Forward Compliment to the Probe).

5.5.1. BOVINE PANEL MULIPLEXED PCR DATA

Preliminary screening: Preliminary screening can be broken down into several steps. The signatures are preliminarily screened by first assessing how the signatures will perform under multiplex PCR conditions and whether or not the addition of the new signatures will adversely effect other signatures, these preliminary screening methods are described as “signature baseline screening” and “multiplex addition screening” respectively. First signature baseline screening tests the performance of the signature in a no-target reaction (blank), a “passing” score is indicated by a low (typically less than 100) MFI. All MCF signatures passed the signature baseline screening. Next in multiplex addition screening the new signature primers are added one-by –one to the other panel signatures to determine if there would be any cross-reactions between primer pairs in the panel. Further assessment of the signatures is done through target and near-neighbor screening and the determination of relative sensitivitiesThe signature down-selection for the MCF signature is further described in Table 26 below.

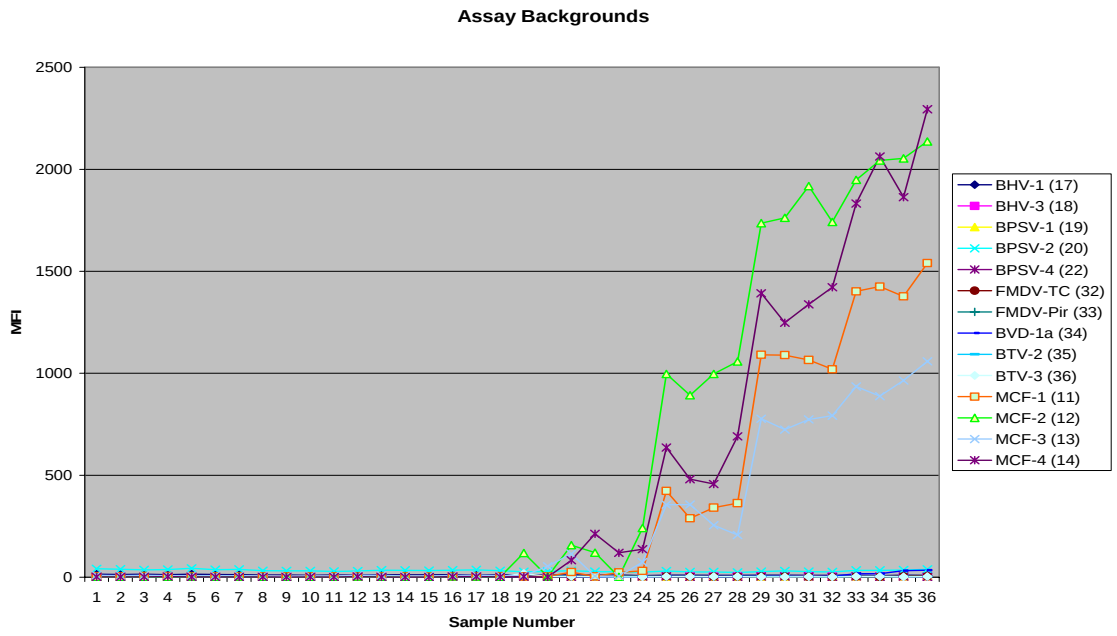


FIG. 25. Preliminary screening of the 4 MCF signatures when challenged with AlHV-1 WC11 target nucleic acid (samples #17-36) preceded by several "blank" samples (samples #1-16). Preliminary results show a low baseline MFI and sensitivity against target. Further screening will be conducted (multiplex addition) to determine if the signatures will be compatible with the other panel constituents.

TABLE 167. Additional preliminary screening of MCF signatures to determine signature backgrounds in the bovine panel. MCF signature emcf_95416.(MCF-4) and emcf_954176 (MCF-5) were observed to have higher than desired background MFIs (above 100) as shown below.

	MCF-1 (11)	MCF-2 (12)	MCF-3 (13)	MCF-4 (14)	MCF-5 (15)
Blank	19	10	17	124	229
Blank	21	9	16	132	237
Blank	25	11	18	144	286
Blank	24	10	17	132	267
Blank	24	9	16	126	263.5
Blank	19	8	14	141	240.5
Blank	20	10	15.5	152	250
Blank	18	8	14	126	225.5
Blank	19.5	8.5	18	108.5	237
Blank	24	9.5	16	125	261
Blank	22	10	17	107	247
Blank	23	10	19	134	277
Blank	22	9.5	17	117	244

Blank	22	9.5	16.5	134.5	268

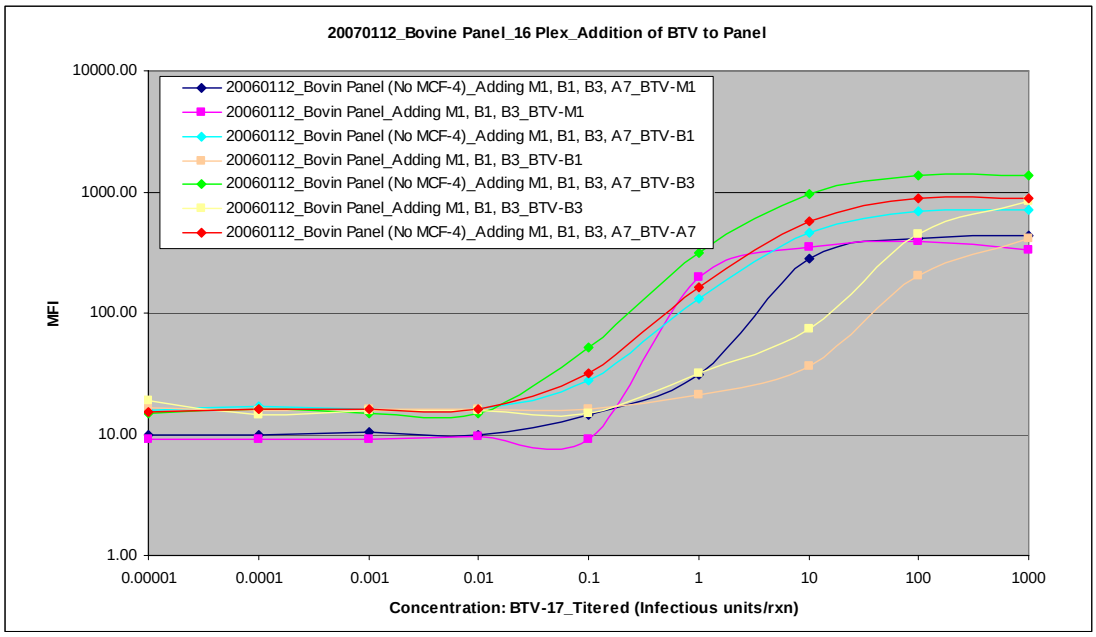


FIG. 26. The MCF-4 probe was noted for cross-reacting with BTV10_1810199 primers. In the absence of the MCF-4 signature, the BTV signatures markedly improve as seen in this plot. When BTV-A7 is present in the mix the relative sensitivity of the signature seems to improve for BTV-B1 and B3 and does not have much affect on BTV-M1. Based on this it was decided to be more optimal to have an additional BTV signature in the mix rather than the MCF-4 signature especially if it improves the other BTV signatures.

TABLE 168. Multiplexed assay down-selection summary. Signature baseline screening indicates the performance of the signature in a no-sample reaction (blank), a "passing" score is indicated by a low MFI. All signatures passed the signature baseline screening. In the multiplex addition screening the primers are added one-by –one to the other panel constituents to determine if there would be any cross-reactions between primer pairs in the panel. Signatures emcf_95416, (or MCF-4) and emf_95476 (or MCF-5) failed screening due to an observed cross-reaction with those signatures when added to the multiplexed panel. As a result no further screening was conducted with those signatures.

	Mux Screening: Signature Down Selection					
Signature	**Signature baseline screening**	**Multiplex addition screening**	**Target screening**	**Near-neighbor screening**	**Limit of detection screening**	**Noted cross-reactions**

MCF-1 (emcf_94975.F)	Pass	Pass	Pass	Pass	Pass	None
MCF-2 (emcf_95059.F)	Pass	Pass	Pass	Pass	Pass	None
MCF-3 (emcf_95155.F)	Pass	Pass	Pass	Pass	Pass	None
MCF-4 (emcf_95416.F)	Pass	Fail (12-29-06): Crossreacts with BTV10_1810199 (A7) signature	No further testing	No further testing	No further testing	MCF-4 probe crossreacts with BTV10_1810199 primers
MCF-5 (emcf_95476.F)	Pass	Fail (8-29-06): Crossreacts with MCF-4 (95416) signature	No further testing	No further testing	No further testing	MCF-5 primers crossreact with MCF-4 probe

Near-neighbor and Target screening: All three signatures were added to the Bovine panel. All three signatures exhibited a very low background response (<10 MFI) in the Bovine panel. The isolates used during the TaqMan screening phase were used to conduct multiplex screening at PIADC. The multiplex titration results in Fig 3 and 4 show that all three signatures responded similarly to both isolates. Similarly to TaqMan screening, when screened in multiplexed format against the OvHV-2 isolate, no reaction was observed as shown in Fig 5; TaqMan and multiplex target screening results where in agreement.

TABLE 169. Backgrounds screening in multiplexed format for MCF at LLNL and PIADC.

	Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors	Targets
Mux PCR Screening	54	135	16	0	43	3

[1]-There are 752 pooled samples in each Aerosol Block.

TABLE 170. Backgrounds screening in **multiplexed** format for all MCF signatures against the below listed **eukaryotes**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. None of the signatures cross-reacted with any of the samples listed below.

Description	MCF-1 (13)	MCF-2 (14)	MCF-3 (15)
BOVINE	9	12	14
CAT	7	11	13
CHICKEN	8	11	13
DOG	7	10	11
DROSOPHILA MELANOGASTER	7	8	11

EQUINE	7	11	11
FLEA	6	8	9
HUMAN	4	8	7
MONKEY	9	15	13
MOSQUITO	7	9	12
MOUSE	7	11	12
PIG / PORCINE	8	9	12
RABBIT	8	9	12
RAT	5	9	9
SHEEP	4	7	8
TICK	5	8	8

TABLE 171. Backgrounds screening in **multiplexed** format for all MCF signatures against the below listed **prokaryotes**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. None of the signatures cross-reacted with any of the samples listed below.

Description	MCF-1 (13)	MCF-2 (14)	MCF-3 (15)
Actinobacillus suis	11	11	16
Aneurinbacillus migulanus	10	12	16
Bacillus cereus	10	12	18
Bacillus globigii	12	14	22
Bacillus subtilis	8	7	13
Bacillus thuringiensis	13	12	21
Bifidobacterium denticum	10	11	16
Borrellia burgdorferi	13	12	20
Burkholderia capacia	11	11	20
Caulobacter vibriodes	8	9	13
Clavibacter michganensis	9	9	15
Clostridium butyricum	12	10	19
Corynebacterium pseudodipthericum	11	12	19
Cytophaga marinoflava	10	8	17
Erwina amylovora	11	10	18
Erwina herbicola	11	13	19
Escherichia coli	13	14	22
Geobacillus caldoxylosilyticus	11	10	16
Halomonas halmophila	9	15	14
Heamophilus influenza	10	11	18
Herbaspirillium seropedicae	13	13	20
Lactobacillus garvieae	8	11	13
Lactobacillus gasseri	10	11	16
Listeria monocytogenes	9	10	16
Listeria seeligeri	11	10	17
Micrococcus luteus	11	11	16
Moraxella lacunatica	11	10	18
Oceanospirillium ssp. Maris	13	11	21
Paenibacillus napthalaenovorans	11	12	17
Paracoccus dentrificans	11	10	17
Porphyrobacter sanguineus	11	10	18
Proteus mirabillis	10	10	16
Pseudomonas aeruginosae	10	11	17

Pseudomonas oleovorans	8	9	15
Rhizobium leguminosarum	13	14	21
Rhodococcus rhodochrous	8	10	12
Salmonella typhimurium	11	10	18
Simonsiella muelleri	10	10	15
Sphingomonas sp. (Alcaligenes sp)	10	10	16
Staphylococcus aureus	10	12	17
Streptococcus pneumoniae	11	12	17
Streptomyces scabiei	9	9	15
Tatlockia maceachernii	14	14	22
Vibrio paraheamolyticus	11	9	16
Xanthomonas translucens	10	10	16

TABLE 172. Backgrounds screening in **multiplexed** format for all MCF signatures against the below listed **soils**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. None of the signatures cross-reacted with any of the samples listed below.

Description	MCF-1 (13)	MCF-2 (14)	MCF-3 (15)
D 000107-49	15	13	23
D 000109 # 50	13	14	20
D 000402 # 53	15	18	20
D 000500 - 26 - 1	16	12	23
D 000501-14-1	16	11	19
D 000505 - 11 - 4	16	14	23
D 000521 - 23	16	12	23
D 000527 - 3	15	14	22
D 000531 - 21	15	16	19
D 000533 - 17 -1	16	16	21
D 000542 - 6	15	13	20
D 000550 - 20	14	12	22
D 000551 - 5	17	13	22
D 000561 - 8 - 6	16	13	22
D 000562 - 30 - 5	16	13	22
S 251	15	13	21
S 252	13	10	19
S 253	11	12	15
S 254	15	11	21
S 255	12	12	17
S 256	14	11	19
S 257	15	13	20
S 259	14	8	19
S 260	14	10	18
S 271	12	11	17
S 272	14	12	18
S 273	13	13	18
S 274	14	12	18
S 275	15	11	19

S 276	15	12	21
S 277	13	12	19
S 279	10	9	14
S 280	13	10	17
S 282	9	9	12
S 283	13	12	18
S 284	14	13	21
S 286	13	11	19
S 287	5	5	8
S 288	12	11	18
S 289	12	12	18
S 290	14	10	16
S 291	11	12	14
S 292	14	11	17
S 295	13	11	17
S 296	13	13	18
S 297	10	9	14
S 298	13	10	17
S 299	11	11	16
S 300	12	11	21
S 301	11	11	19
S 303	11	10	19
S 304	10	14	21
S 305	11	11	22
S 307	10	10	21

TABLE 173. Bovine Panel **Near-Neighbor** Screening (Data from 20070601) against MCF signatures. Values shown are Median Flourescence Intensity (MFI) units. “Blanks” are buffer-only samples (no nucleic acid added to reaction), shown below in red. Extracted nucleic acid was added to each PCR reaction in triplicate totaling 200pg/rxn. The data shows that none of the MCF signatures cross- reacted with any of the below listed near-neighbors from the Bovine panel constituents.

Description	**MCF-1 (13)**			**MCF-2 (14)**			**MCF-3 (15)**		
replicates	rep1	rep2	rep3	rep1	rep2	rep3	rep1	rep2	rep3
Blank	11	10	13	25	22	29	24	21	28
Blank	11	9	14	26	19	22	22	18	21
Blank	10	11	10	26	21	25	23	20	19
BHV A040150085	15	7	17	36	16	35	30	14	31
BHV (BFK)	13	12	12	22	24	22	28	22	22
BHV-1 A040130066	15	9	13	48	21	26	30	17	23
BHV-1 A033640072	14	9	12	33	21	38	27	17	23
BHV-1 ATCC VR 793	15	10	11	34	23	25	27	20	22
IBR CA 111903	13	2	16	26	5	28	28	4	26
IBR MN 111903	9	9	15	45	24	30	21	19	24
BHV-1 NVSL 231221	9	13	12	29	27	24	21	23	22
BHV-1 RA309	12	10	10	26	24	23	23	20	18

BHV-1 NVSL 97-10720	12	9	18	30	28	23	24	17	31
BHV-1 NVSL 51619	12	10	15	31	20	24	25	20	24
BHV-1 NVSL 86741	12	11	14	29	32	25	24	22	24
BHV-1 NVSL 200032	11	10	14	26	22	27	24	20	24
BHV-1 LA ATCC VR188	13	11	12	30	38	27	27	20	20
BHV-1 (IBR) Texas CAHFS A030020072	11	13	12	26	28	25	27	24	20
EHV-1 ATCC VR2003	11	10	13	25	26	22	24	21	21
EHV-1 A9904309	11	10	14	36	17	27	23	19	25
EHV-1 A011120004 CAHFS	12	9	11	26	19	18	26	18	19
EHV-1 NVSL 00002	12	9	11	26	24	35	23	20	19
EHV-2 ATCC VR701	11	11	11	27	18	19	25	20	20
EHV-1 A99043047 CAHFS	11	10	9	25	23	24	24	19	17
EHV-2 D990 CAHFS	13	10	10	36	22	21	24	21	19
Pseudorabies Titered	13	10	11	28	22	20	24	19	19
Pseudorabies NVSL 93-11745	12	10	12	28	27	19	25	19	19
Pseudorabies NVSL 92-12013	12	10	11	28	19	19	23	18	19
Pseudorabies RA180 CAHFS	11	9	11	22	23	19	21	18	19
Porcine Herpes Pseudorabies Shope	9	11	10	22	19	19	22	19	19
Feline Herpes ATCC VR636	10	10	11	25	23	22	22	19	20
Caprine Herpes ATCC VR462	11	9	11	29	23	16	24	21	20
Caprine Herpes S0201998 CAHFS	10	10	11	27	26	18	23	20	19
Caprine Herpes D0201157 CAHFS	12	11	12	25	22	19	23	19	19
BHV-5 A040150085 CAHFS	12	11	11	30	35	25	23	22	19
BHV-5 A032540006 CAHFS	10	10	12	30	25	16	22	18	19
BHV-5 D9403153 CAHFS	11	11	11	22	22	20	23	20	19
BHV-5 D9402133 CAHFS	8	10	10	24	19	18	16	18	19
BDV Coos Bay	11	10	10	24	20	19	21	18	17
EHD-1 Georgia	13	10	13	24	18	16	24	15	21
EHD-1 New Jersey	13	9	9	22	21	21	24	18	18
EHD-1 Santa Barbara	12	10	10	21	28	16	24	21	17
EHD-2 Alberta	10	14	10	30	32	16	21	27	18
Fowl Pox	12	14	10	26	32	18	23	28	17
Parainfluenza Type 3	10	14	9	25	24	16	20	26	17
Respiratory Syncytial	12	10	10	22	28	16	24	21	18

Multiplexed PCR Assay Titrations

Upon finalization of the assay panel constituents each agent assay is characterized in multiplexed PCR format by running dose-response (titration) curves against titered virus extracted from virus infected cell culture for each available agent strain. Each sample is tested in duplicate and reported as a mean value of the median fluorescence intensity units (MFI) against agent concentration (per reaction volume, 5uL) on a double log plot. Concentration units are reported in TCID50/rxn (assumes 5uL sample volume per reaction) or pfu/rxn depending on virus type and source. In some cases where titered virus was not available data is reported as picogram units (pg). Each assay is evaluated by its ability to detect agent strains and differentiate from near neighbors (specificity) and by its range of sensitivity to each strain relative to each signature tested for that agent. Because this method is not aimed at determination of analytical

sensitivities (requiring a more extensive sample set tested in clinical sample matrices which is not practical for this stage of development) it does however, provide tentative limits of detection that are used to measure the performance of each assay. For MCF, additional testing is pending for target screening at PIADC, the plots below represent the MCF assays **before the panel development was complete.**

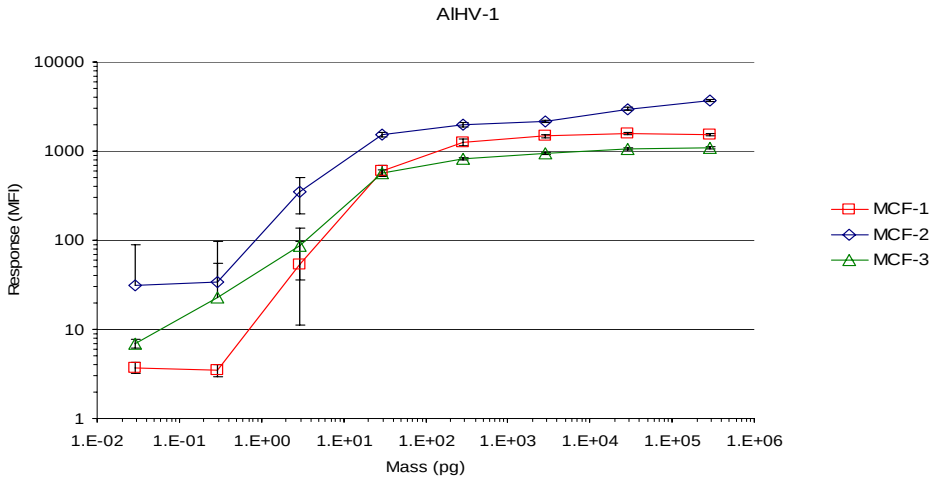


FIG. 27. **Preliminary** multiplex screening in a prototype 19 plex bovine panel data for the three MCF signatures against extracted nucleic acids from isolate AIHV-1 (MN). Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2). Error bars indicate ±1 of the mean.

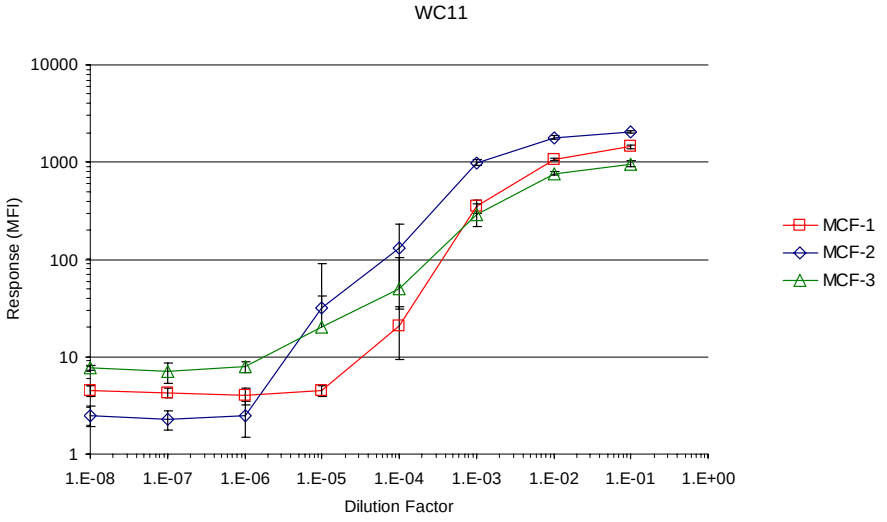


FIG 28. **Preliminary** multiplex screening in a prototype 19 plex bovine panel data for the three MCF signatures against extracted nucleic acids from isolate AIHV-1 (WC11). Serial dilution of

nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2). Error bars indicate ±1 of the mean.

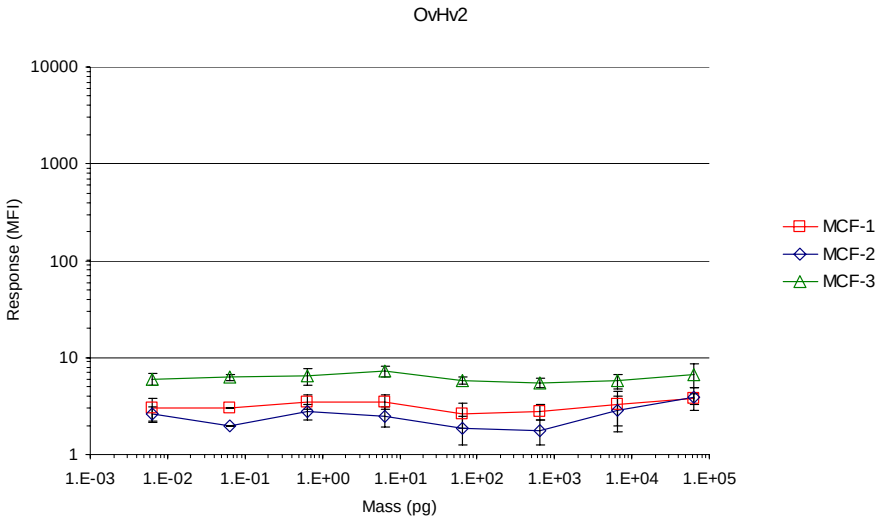


FIG. 29. **Preliminary** multiplex screening in a prototype 19 plex bovine panel data for the three MCF signatures against extracted nucleic acids from isolate OvHV-2. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2). Error bars indicate ±1 of the mean.

RESULTS: We used our computational DNA signature generation system to identify the regions of *Malignant Catarrhal Fever* that are not present in any other sequenced microbial organism in Genbank nor our internal sequence database. For *Malignant Catarrhal Fever* there was only one available AlHV1 genome to develop candidate signatures, so an alignment was not necessary. The bioinformatics group generated 1108 candidate signatures; 200 of these went to bench screening, however this was down-selected to 40 that were screened in gel-PCR format. Of this 40, 5 were moved forward into Real-time PCR testing and all of these 5 were forwarded to multiplexed assay development. These signatures were further down-selected in multiplexed and the remaining 3 were tested in multiplexed PCR format. These three signatures have been currently added the Bovine panel and with a limited number of target strains tested, all 3 signatures have been determined to perform equivalently. Additional target testing is being completed at Plum at the time of writing this report. Based on this preliminary data the three signatures would be effective in screening for detection of wildebeest–associated MCF. However these signatures are not able to detect OVHV as no sequenced genomes currently exist for this domestic virus which is the other cause of MCF. **This is a gap that needs to be addressed in future work.**

6. RINDERPEST VIRUS (BOVINE PANEL)

OBJECTIVE: We were tasked by the Department of Homeland Security to develop specific and reliable Real-time RT- PCR signatures for *Rinderpest Virus* [RPV], among other major agriculturally-impacting viruses that are clinically indistinguishable from foot and mouth disease virus [FMDV]. The purpose of these assays (collectively with other virus-specific signatures developed in parallel) is to develop a species specific (i.e. bovine) multiplexed detection assay for Foot-and-Mouth disease rule-out for use in veterinary diagnostic laboratories. The signatures were designed to meet two major criteria. First, they would be able to discriminate among multiple viral agents whose disease symptoms mimic those of Foot and Mouth Disease Virus [FMDV]. Secondly, these signatures would be uniquely capable of detecting all strains of RPV. This document describes the development of five optimal Real-time RT-PCR and multiplexed [MUX] PCR signatures to detect strains of *Rinderpest Virus* [RPV].

6.1. BACKGROUND AND ETIOLOGY OF RPV

Rinderpest is a disease of cloven-hoofed animals characterized by fever, necrotic stomatitis, gastroenteritis, lymphoid necrosis, and high mortality. In epidemic form, it is the most lethal plague known in cattle. All species of the order artiodactyls are variably susceptible to rinderpest. Susceptibility is high in African buffalo, giraffes, wild Suidae, Tragelaphinae, and breeds of cattle such as Ankole, Channel Islands, and Japanese Black; moderate in wildebeest and East African zebus; and mild in gazelles and small domestic ruminants. Rinderpest is subclinical in European pigs and hippopotami. It is endemic in many countries of Asia and Africa. Historically, rinderpest virus has been widely distributed throughout Europe and Africa but has never established itself in North America, Central America, the Caribbean Islands, South America, Australia, or New Zealand. Rinderpest is included in OIE List A.

The infectious agent is a morbillivirus, closely related to the viruses causing peste des petits ruminants (Peste Des Petits Ruminants: Introduction), canine distemper (Canine Distemper: Introduction), and measles. Strains of rinderpest virus may vary markedly in host range and virulence. Sera from recovered or vaccinated cattle cross-react with all strains in neutralization tests, but minor antigenic differences have been demonstrated. The virus is fragile and becomes rapidly inactivated by heat and light, but remains viable for long periods in chilled or frozen tissues.

Rinderpest virus is present in small amounts of nasal secretions 1-2 days before fever; levels are high in secretions and excretions during the first week of clinical disease and decrease rapidly as animals develop specific antibodies and begin to recover. Transmission requires direct or close indirect contact; infection is via the nasopharynx. There is no carrier state; the virus maintains itself by continual transmission among susceptible animals. In endemic areas, young cattle become infected after maternal immunity disappears and before vaccine immunity begins, with possible auxiliary cycles in sheep, goats, and wild ungulates. In epidemic areas, the virus infects

most susceptible animals and tends to limit itself unless the population is large enough to support endemicity[6].

6.2. RPV COMPREHENSIVE ASSAY SUMMARY

Bioinformatics

Virus name: *Rinderpest* Virus

Project name: *Rinderpest*

Level of discrimination: *species*

Total number of Genome Sequences available for alignment: 16
Number of Initial Signatures: 12
Number of Signatures forwarded to bench-screening: 9

Real-time PCR Screening Summary

TABLE 174. Final signatures down-selected in real-time RT-PCR screening (5).

#	LLNL Signature Designation	Sequence	#	LLNL Signature Designation	Sequence
1	Rinderpest_1811628_F	CGGTGAAAAGGTTGAGGGAGT	4	Rinderpest_1814856_F	AACTCCTGACCTCATTCCTTGC
	Rinderpest_1811628_R	TTCCTCATCTCCTCCCCAGA		Rinderpest_1814856_R	GGCTCTATAATCCCACTATGCCA
	Rinderpest_1811628_P	AGATGCTGACTCTATCCTGGTTCAATCAGGC		Rinderpest_1814856_P	TGGCTCAGTGCATTCACAAAGACCGAATA
2	Rinderpest_1814853_F	GGATCGCTGAAATGATCTGTGA	5	Rinderpest_1814893_F	AATAAACCGAGGATCGCTGAAATG
	Rinderpest_1814853_R	GGAGCCAGTTCACCCATTTG		Rinderpest_1814893_R	CTGAATTTGTTCTGGATTGAGTTC
	Rinderpest_1814853_P	TACATAGTGGAGGCAGGGTTGGCCAG		Rinderpest_1814893_P	TGTGACATTGATACCTACATAGTGGGCAGG
3	Rinderpest_1814855_F	TGCATCTTATGTGACTTTGGTTCA			
	Rinderpest_1814855_R	GGCTATCCGCACAGCTGAC			
	Rinderpest_1814855_P	CAGTCCTCTCATCTGTTGTCGATCCGATGTA			

TABLE 175. Summarizes wet-bench screening panels for signature down-selection.

Screening Method	Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors	Target
Gel Screening	5	5	5	none	0	0
Real-time PCR Screening	45	54	16	3 Aerosol Blocks	5	9

[6] Source: TheMerck Veterinary Manual
http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/56300.htm&word=rinderpest

Note: There are 752 pooled samples in each Aerosol Block.

TABLE 176. Limit of detection summary for the final set of Rinderpest signatures. Dose-response curves were generated for each signature using several reference targets for that organism. Dilutions were prepared in the 10-fold range over 4-logs in the detection limit range. Results were then averaged (n=3) and the limit of detection was determined to be the lowest measured concentration (in pg units/rxn) that was detected under these conditions. Units of measurement reflect total nucleic acid for qualitative comparison. Yellow highlighted cells indicate the signatures that had the lowest relative sensitivity against each isolate.

		Target Strains (pg total DNA units, relative LOD)								
Signature	Gene ID	India	Pakistan	Egypt	Nigerian Buffalo	Plowright	Yemen	RBOK	Pendik	Kuwait
1811628	Not available	0.1	0.1	0.1	0.1	0.1	0.01	0.01	0.01	0.1
1814853	Not available	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
1814855	Not available	0.1	1.0	0.01	0.1	0.1	1.0	0.01	0.1	1.0
1814856	Not available	0.1	1.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1
1814893	Not available	0.1	0.1	1.0	0.1	1.0	1.0	0.1	0.1	0.1

Multiplexed PCR Screening Summary

TABLE 177. Backgrounds screening in multiplexed format for RPV at LLNL and PIADC.

	Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors[2]	Targets
Mux PCR Screening	54	135	16	0	48 (3)	7

[1]-There are 752 pooled samples in each Aerosol Block. [2]Near-neighbors indicated (43) apply to the near-neighbor panel that is not uniquely specific for PRRS, but for the other panel constituents that were screened concurrently.

TABLE 178. Signature summary for RPV multiplexed signatures in the bovine panel.

LLNL Signature Designation	MUX Group ID	Gene ID	Limit of detection[1]	Targets Screened	Near Neighbors Screened
RPV_1814853	RPV_1814853	RPVgp1/3021777	pending	7	48
RPV_1814855	RPV_1814855	RPVgp7/3021780	pending	7	48
RPV_1814856	RPV_1814856	RPVgp7/3021780	pending	7	48

[1] The relative "Limit of detection" is described as a range of lowest and highest sensitivity determined by multiple target strains screened and is described further in the Multiplex results summary report section.

6.3. RPV SIGNATURE BIOINFORMATICS AND GENERATION INFORMATION

Bioinformatics process:

The LLNL Bioinformatics team have developed a computational process that uses available DNA sequence information for a given target organism to generate candidate DNA signature sequences that are conserved and unique. Signatures are primarily used to underlie reliable and specific pathogen detection assays. Evidence of the success of the process is that several of the Real-time RT-PCR signatures that are used in the national BioWatch monitoring system were

generated by this process, and to date, has resulted in no false positives from a total of over 5 million assays performed.

The general approach is the following: All available complete genomes of different strains of the target species are compared using a multiple genome alignment programs. A *consensus gestalt* is formed from the alignments that contain the sequence conserved amongst all target inputs. (This step is bypassed if only one target sequence is available).

To determine that organism-conserved sequence which does not occur in any other sequenced microbial organism, the consensus gestalt is compared against our immense, continuously updated database of microbial organism. A customized algorithm accomplishes this electronic subtraction, and the result is a *uniqueness gestalt* that is mined for potential signature candidates. A final computational screening is done to verify that cross-reactions are not detected.

Target Virus Information:
Virus name: *Rinderpest* Virus.
Type: ssRNA negative-strand virus
Genome size: 15882 bp

Primer/Probe Set Generation Information

Method: Alignment and All Microbe Database subtraction.

TABLE 179. K-path run id: 103110. List of genomes used for alignment. Genome sequences list and information.

	Genome Description	GI Number	Sequence Length (bp)
1	Rinderpest virus, complete genome	56410431	15882
2	Rinderpest virus Rinderpest egypt from USDA on Aug 30 2006 10:12AM	No information	15882
3	Rinderpest virus Rinderpest kuwait from USDA on Aug 30 2006 10:13AM	No information	15882
4	Rinderpest virus Rinderpest nigbuf from USDA on Aug 30 2006 10:13AM	No information	15882
5	Rinderpest virus Rinderpest pakchong from USDA on Aug 30 2006 10:14AM	No information	15882

TABLE 180. K-path run id: 103137. List of genomes used for alignment.

	Genome Description	GI Number	Sequence Length (bp)
1	Rinderpest virus, complete genome	56410431	15882
2	Rinderpest virus Rinderpest egypt from USDA on Aug 30 2006 10:12AM	No information	15882
3	Rinderpest virus Rinderpest nigbuf from USDA on Aug 30 2006 10:13AM	No information	15882
4	Rinderpest virus Rinderpest nigsokoto from USDA on Aug 30 2006 10:13AM	No information	15882

5	Rinderpest virus Rinderpest sokoto from USDA on Aug 30 2006 10:14AM	No information	15882

TABLE 181. K-path run id: 103146. List of genomes used for alignment.

	Genome Description	GI Number	Sequence Length (bp)
1	Rinderpest virus, complete genome	56410431	15882
2	Rinderpest virus Rinderpest egypt from USDA on Aug 30 2006 10:12AM	No information	15882
3	Rinderpest virus Rinderpest nigbuf from USDA on Aug 30 2006 10:13AM	No information	15882
4	Rinderpest virus Rinderpest nigsokoto from USDA on Aug 30 2006 10:13AM	No information	15882
5	Rinderpest virus Rinderpest pakchong from USDA on Aug 30 2006 10:14AM	No information	15882
6	Rinderpest virus Rinderpest sokoto from USDA on Aug 30 2006 10:14AM	No information	15882

TABLE 182. List of parameters and settings used in generating candidate signatures using Primer 3.

Primer3 Main Parameters	
Parameters	**Standard Settings**
PRIMER_OPT_SIZE	20
PRIMER_MIN_SIZE	18
PRIMER_MAX_SIZE	27
PRIMER_PRODUCT_OPT_SIZE	100
PRIMER_PRODUCT_SIZE_RANGE	71-600
PRIMER_OPT_TM	62
PRIMER_MIN_TM	61
PRIMER_MAX_TM	63
PRIMER_MIN_GC	20
PRIMER_MAX_GC	80
PRIMER_PICK_INTERNAL_OLIGO	1
PRIMER_INTERNAL_OLIGO_OPT_SIZE	31
PRIMER_INTERNAL_OLIGO_MIN_SIZE	18
PRIMER_INTERNAL_OLIGO_MAX_SIZE	36
PRIMER_INTERNAL_OLIGO_OPT_TM	72
PRIMER_INTERNAL_OLIGO_MIN_TM	71
PRIMER_INTERNAL_OLIGO_MAX_TM	73
Number of Primers/Probe Set generated:	**12**

Signature Information
Source: LLNL
Project name: Rinderpest
Level of discrimination: species

Number of Initial Signatures: 12

Number of Signatures forwarded to gel screening: 9
Number of Signatures forwarded to real-time screening: 5
Number of Final Signatures: 5

Taqsim description
We used a computational Real-time PCR simulator program, "Taqsim", to identify all potential targets for each signature from all sequences available in genbank. Taqsim is a BLAST-based comparison of each signature as a triplet against all sequences in genbank to identify the targets that are predicted to produce a Real-time PCR reaction at 57 degrees primer annealing and 67 degrees for probe annealing (these temperatures are according to Primer 3 oligo Tm calculations). The first column of the excel spreadsheets list the signatures by the name, and the following columns report the predicted genbank targets, coordinates of signature region, amplicon size, and accession #'s for each target.

Note: For a listing of computationally predicted product sequences and potential targets for each signature, please see Appendix II, Taqsim Run Data.
Signature bioinformatics

TABLE 183 Signature bioinformatics for each final signature.

Forward Primer	1811628_F	1814853_F	1814855_F	1814856_F	1814893_F
FWD Primer Length (bp)	21	22	24	22	26
FWD Primer TM (^{o}C)	55	53	54	55	56
FWD Primer GC Content (%)	52	46	38	50	39
Forward Location	2154	853	12750	13756	843
Reverse Primer	1811628_R	1814853_R	1814855_R	1814856_R	1814893_R
Rev Primer Length (bp)	20	20	19	23	25
Rev Primer TM (^{o}C)	54	54	55	54	53
Rev Primer GC Content (%)	55	55	63	48	36
Reverse location	2228	1020	12929	13848	1057
Probe Name	1811628_P	1814853_P	1814855_P	1814856_P	1814893_P
Probe Length (bp)	31	26	31	31	32
Probe TM	63	63	62	62	62

(°C)					
Probe GC Content (%)	48	58	48	42	47
Probe location	2178	885	12855	13795	870
Probe strand	plus	plus	minus	minus	plus
Predicted Product Size	94	187	198	115	239

Target Region Gene Information

TABLE 184a-b (a) Reference Genome used for Gene Information. (b) Gene information for each signature.

(a)

Genome Description	GI Number	Sequence Length (bp)
Rinderpest virus (strain Kabete O), complete genome	56410431	15882

(b)

			Gene Location		Target Region Location	
Kpath Signature ID	Gene/ID	Description	Start	End	Start	End
1811628	RPVgp2/3021775; RPVgp3/3021779	P(phosphoprotein)protein; C protein	1807; 1829	3330; 2362	2154	2247
1814853	RPVgp1/3021777	N(nucleocapsid) protein	108	1685	853	1039
1814855	RPVgp7/3021780	L(polymerase) protein	9222	15773	12750	12947
1814856	RPVgp7/3021780	L(polymerase) protein	9222	15773	13756	13870
1814893	RPVgp1/3021777	N(nucleocapsid) protein	108	1685	843	1081

6.4. RPV GEL AND REAL-TIME PCR SCREENING REPORT

Wet-lab screening process. To ensure extremely high selectivity and sensitivity, we have established a screening protocol that rigorously screens candidate DNA signatures to select the optimal sets for eventual field use. Candidate signature primer pairs that survive computational screening proceed to wet chemistry screening utilizing standard PCR with agarose gel electrophoresis. This step ensures that the primers will react across strains representative of the diversity of the pathogen (avoid false negative), but will not react with the nucleic acids of other organisms that could be present in a sample (avoid false positives). The collection of purified DNA templates used in this screening process is listed below:

1) Strain panels representative of the diversity of the target organism in nature.
2) Near neighbors-organisms that are closely related at the genetic level and therefore have the greatest likelihood of cross-reaction.

3) Eukaryotic DNA that may carry over from sample collection procedures.
4) Prokaryotic DNA that would be expected to be present in environmental samples and may also be present in Bovine and Porcine oral cavities.
5) Forty five soil samples from around the country representing diverse climates and contain a complex mixture of organisms from diverse environmental collections
6) Aerosol samples: 752 samples collected from aerosol collectors and pooled for background testing purposes.

Rinderpest TaqMan Screening Report

11-21-2006

LLNL did not have access to target or near-neighbor RNAs for this screening so we did all background screening at LLNL and sent the down selected signatures to Max Rasmussen to perform the target and near-neighbor screening at the Plum Island animal disease center.

Rinderpest Gel Screening Report

TABLE 185. Screening summary for Rinderpest at LLNL and Plum Island Animal Disease Center (PIADC).

	Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors	Targets
Gel Screening	5	5	5	none	0	0
Real-time_PCR Screening	45	54	16	3 Aerosol Blocks[1]	5	9

[1]-There are 752 pooled samples in each Aerosol Block.

Rinderpest - Gel Screening Report

Background gel screening was carried out in duplicate as 25ul reactions in 96 well PCR plates on MJ thermal cyclers. Each reaction mix consisted of 1X PCR Buffer [200mM Tris-HCL (pH 8.4), 500mM KCl], 1.5mM $MgCl_2$, 0.8mM each dNTP, 80ng BSA, 0.4uM each forward and reverse primers, 0.75U Platinum *Taq* DNA polymerase, PCR water and a variable amount of extracted DNA template. A total of 5ng of soil DNA or 1ng of Eukaryotic DNA or 200pg of Prokaryotic DNA was added to each 25ul reaction mix. Background template data and extraction protocols are available upon request.

The target near-neighbors are RNA, therefore when target and near-neighbor screening is done it will be performed using the Clontech Powerscript RT-PCR kit. Each reaction will be performed in triplicate as per the manufacturer's suggested protocol, replacing probe with PCR water, in a 25ul volume with a total of 200pg TRIZOL extracted RNA.

Reaction products were visualized by gel electrophoresis on 4% agarose gels. PCR product sizes are listed as visual estimates based on a 20bp ladder that was run on each gel for reference. I f a

signature screened against a background produced a PCR product size that fell below 100 base pairs greater in size than the predicted product size for a signature screened against its target, the signature was dropped from further screening. The theory behind this selection process is that a much larger than target PCR product would not cause inhibitory PCR competition. However, a PCR product of correct size or smaller would inhibit PCR through competition.

TABLE 186. Lists 9 signatures developed by the LLNL Bioinformatics group for Rinderpest detection forwarded to gel screening. (Signatures that did not pass the initial gel screening did not have probes ordered and are colored red.)

Rinderpest_1811628_F	CGGTGAAAAGGTTGAGGGAGT
Rinderpest_1811628_R	TTCCTCATCTCCTCCCCAGA
Rinderpest_1811628_P	AGATGCTGACTCTATCCTGGTTCAATCAGGC
Rinderpest_1814853_F	GGATCGCTGAAATGATCTGTGA
Rinderpest_1814853_R	GGAGCCAGTTCACCCATTTG
Rinderpest_1814853_P	TACATAGTGGAGGCAGGGTTGGCCAG
Rinderpest_1814855_F	TGCATCTTATGTGACTTTGGTTCA
Rinderpest_1814855_R	GGCTATCCGCACAGCTGAC
Rinderpest_1814855_P	CAGTCCTCTCATCTGTTGTCGATCCGATGTA
Rinderpest_1814856_F	AACTCCTGACCTCATTCCTTGC
Rinderpest_1814856_R	GGCTCTATAATCCCACTATGCCA
Rinderpest_1814856_P	TGGCTCAGTGCATTCACAAAGACCTTGAATA
Rinderpest_1814893_F	AATAAACCGAGGATCGCTGAAATGAT
Rinderpest_1814893_R	CTGAATTTGTTCTGGATTGAGTTCT
Rinderpest_1814893_P	TGTGACATTGATACCTACATAGTGGAGGCAGG
Rinderpest_1811276_F	TCTCGCAGTCACGATCAGG
Rinderpest_1811276_R	GAGTCAGCATCTTCGACTCCC
Rinderpest_1811276_P	CAACCTTTTCACCGCTGTGATCATAAACATG
Rinderpest_1814857_F	ATGGCAGAGGAGCAAGCCTA
Rinderpest_1814857_R	CCATGCTGCAAGGGCTTC
Rinderpest_1814857_P	CATGTCAACAAAGGTCTGGAGTGCATCAA
Rinderpest_1814859_F	GGGTCAAAAGCTAGGGCTGA
Rinderpest_1814859_R	TTATGTCCCCACCCAAGGC
Rinderpest_1814859_P	TATCATTATTTGGACCCAGCCTCAGAGACCC
Rinderpest_1814861_F	CAGGCTTGAGGCAATCACAG
Rinderpest_1814861_R	GCCAGCTCCCTCCATGTCTA
Rinderpest_1814861_P	CGATACCCGTTGATGATAATCCCGCAAAATA

TABLE 187. Lists the nucleic acid extracts used to challenge the initial set of candidate signatures. These select backgrounds are used to determine which signatures will be down-selected against in the screening process.

Nucleic Acid Extract Type	Description/ID
Soil Extract	S251
Soil Extract	S255
Soil Extract	S256
Soil Extract	S260
Soil Extract	S271

Prokaryotic DNA Extract	*P. aeruginosae*
Prokaryotic DNA Extract	*L. monocytogenes*
Prokaryotic DNA Extract	*S. aureus*
Prokaryotic DNA Extract	*S. typhimurium*
Prokaryotic DNA Extract	*S. pneumonia*
Eukaryotic DNA Extract	Chicken
Eukaryotic DNA Extract	Sheep
Eukaryotic DNA Extract	Flea
Eukaryotic DNA Extract	Pig
Eukaryotic DNA Extract	Bovine

TABLE 188a-b. Results summary of background gel screening against the aforementioned nucleic acid extracts. Signatures in red were dropped from further screening because they made PCR product with background DNAs of a similar size that would compete with a target. Of the 9 signatures screened, 4 were eliminated from further screening due to non-specific cross-reactions. "N" indicates no detectable PCR product. Smallest band size is listed. "+" indicates multiple larger bands as well.

(a)

Signature	Expected Product	S251	S255	S256	S260	S271	Flea	Chicken	Sheep	Porcine	Bovine
1814893	94 bp	N	N	N	N	N	N	N	N	N	N
1811276	187 bp	N	N	N	N	N	N	N	N	N	N
1811628	198 bp	N	N	N	N	N	N	N	N	N	N
1814853	115 bp	600+	N	250+	250+	N	N	N	N	N	N
1814855	239 bp	300+	300+	300+	300+	N	N	300+	N	300	N
1814856	158 bp	200+	200+	200+	N	N	N	600	N	100+	200+
1814857	108 bp	100+	100+	100+	100+	100+	N	100+	100+	100+	100+
1814859	134 bp	100+	100+	100+	N	N	N	300	N	N	100+
1814861	149 bp	100+	100+	100+	N	N	N	N	N	N	N

(b)

Signature	Expected Product	*L. monocytogenes*	*P. aergosea*	*S. pneumonea*	*S. typhimurium*	*S. aureus*
1814893	94 bp	N	N	N	N	N
1811276	187 bp	N	N	N	N	N
1811628	198 bp	N	N	N	N	N
1814853	115 bp	N	N	N	N	N
1814855	239 bp	N	N	N	N	N
1814856	158 bp	N	N	N	N	N
1814857	108 bp	100+	N	N	N	600
1814859	134 bp	N	N	N	N	N
1814861	149 bp	N	N	N	N	N

SUMMARY OF GEL SCREENING RESULTS: 9 Rinderpest signatures were initially selected for bench screening. The signatures were down selected to 5 signatures which successfully passed all criteria and moved forward to real-time screening.

Rinderpest – Real-Time PCR Screening Report

Background real-time tests were carried out in triplicate as 25ul reactions in 96 well PCR plates on BioRad's iCYCLERs. Each reaction mix consisted of 1X PCR Buffer [200mM Tris-HCL (pH 8.4), 500mM KCl], 6mM $MgCl_2$, 0.2mM each dNTP, 80ng BSA, 0.2uM each forward and reverse primers, 0.4uM Probe with a 5' Fam and a 3' BHQ modification, 1.25U Platinum *Taq* DNA polymerase, PCR water and a variable amount of extracted DNA template. A total of 5ng of soil DNA or 1ng of Eukaryotic DNA or 200pg of Prokaryotic DNA was added to each 25ul reaction mix.

Target and near-neighbor screening has not been performed as of yet. However, because the target is RNA, we will use the Clontech Powerscript RT-PCR kit. This work will be performed by Max Rassmussen at Plum Island. Each reaction will be performed in triplicate as per the manufacturer's suggested protocol, in a 25ul volume with a total of 200pg TRIZOL extracted RNA.

A real-time PCR reaction is deemed positive if a Ct (cycle threshold) value of below 36 cycles is observed at least 2 of the 3 times the reaction was performed.

TABLE 189. List of signatures that were considered successful through gel screening and were brought forward to be screened in real-time PCR format.

Rinderpest_1811628_F	CGGTGAAAAGGTTGAGGGAGT
Rinderpest_1811628_R	TTCCTCATCTCCTCCCCAGA
Rinderpest_1811628_P	AGATGCTGACTCTATCCTGGTTCAATCAGGC
Rinderpest_1814853_F	GGATCGCTGAAATGATCTGTGA
Rinderpest_1814853_R	GGAGCCAGTTCACCCATTTG
Rinderpest_1814853_P	TACATAGTGGAGGCAGGGTTGGCCAG
Rinderpest_1814855_F	TGCATCTTATGTGACTTTGGTTCA
Rinderpest_1814855_R	GGCTATCCGCACAGCTGAC
Rinderpest_1814855_P	CAGTCCTCTCATCTGTTGTCGATCCGATGTA
Rinderpest_1814856_F	AACTCCTGACCTCATTCCTTGC
Rinderpest_1814856_R	GGCTCTATAATCCCACTATGCCA
Rinderpest_1814856_P	TGGCTCAGTGCATTCACAAAGACCTTGAATA
Rinderpest_1814893_F	AATAAACCGAGGATCGCTGAAATGAT
Rinderpest_1814893_R	CTGAATTTGTTCTGGATTGAGTTCT
Rinderpest_1814893_P	TGTGACATTGATACCTACATAGTGGAGGCAGG

TABLE 190a-c. Real-time PCR background screening consisted of an extensive list of soil, prokaryotic and eukaryotic extracted DNAs in addition to 3 aerosol blocks, each consisting of 752 pooled samples. All signatures passed real-time PCR background screening. None of the

Rinderpest signatures produced an amplicon when screened against the listed backgrounds. All signatures moved forward to be screened against the available Rinderpest targets and near-neighbors at the Center for Animal Disease at Plum Island.

(a) Total of 45 soils screened

D000402	D000531	S252	S271	S280	S290	S300
D000109	D000542	S253	S272	S282	S291	S301
D000107	D000533	S254	S273	S283	S292	S303
D000500	D000561	S255	S274	S284	S295	S304
D000505	D000562	S256	S275	S286	S296	S305
D000521	D000501	S257	S276	S287	S297	S307
D000551	D000550	S259	S277	S288	S298	
D000527	S251	S260	S279	S289	S299	

(b) Total of 16 Eukaryotes screened

Bovine	Drosophila	Monkey	Rabbit
Cat	Equine	Mosquito	Rat
Chicken	Flea	Mouse	Sheep
Dog	Human	Porcine	Tick

(c) Total of 54 Prokaryotes screened

A. suis	*C. butyricum*	*L. gasseri*	*P. oleovorans*
A migulanus	*C. pseudodipthericum*	*L. monocytogenes*	*R. leguminosarum*
B. cereus	*C. marinoflava*	*L. seeligeri*	*R. rhodochrous*
B. globigii	*E. amylovora*	*M. luteus*	*S. typhimurium*
B. subtilis	*E. herbicola*	*M. lacunatica*	*S. muelleri*
B thuringiensis	*E. coli*	*O. ssp. Maris*	*Alcaligenes sp.*
B. denticum	*G.caldoxylosilyticus*	*P. napthalaenovorans*	*S. aureus*
B. burgdorferi	*H. halmophila*	*P. dentrificans*	*S. pneumoniae*
B. capacia	*H. influenza*	*P sanguineus*	*S. scabiei*
C. vibriodes	*H. seropedicae*	*P. mirabillis*	*T. maceachernii*
C. michganensis	*L. garviease*	*P aeruginosae*	*V. paraheamolyticus*
			X. translucens

TABLE 191. Final signatures sent to Plum Island for target and near-neighbor screening.

Rinderpest_1811628_F	CGGTGAAAAGGTTGAGGGAGT
Rinderpest_1811628_R	TTCCTCATCTCCTCCCCAGA
Rinderpest_1811628_P	AGATGCTGACTCTATCCTGGTTCAATCAGGC
Rinderpest_1814853_F	GGATCGCTGAAATGATCTGTGA
Rinderpest_1814853_R	GGAGCCAGTTCACCCATTTG
Rinderpest_1814853_P	TACATAGTGGAGGCAGGGTTGGCCAG
Rinderpest_1814855_F	TGCATCTTATGTGACTTTGGTTCA
Rinderpest_1814855_R	GGCTATCCGCACAGCTGAC
Rinderpest_1814855_P	CAGTCCTCTCATCTGTTGTCGATCCGATGTA
Rinderpest_1814856_F	AACTCCTGACCTCATTCCTTGC

Rinderpest_1814856_R	GGCTCTATAATCCCACTATGCCA
Rinderpest_1814856_P	TGGCTCAGTGCATTCACAAAGACCTTGAATA
Rinderpest_1814893_F	AATAAACCGAGGATCGCTGAAATGAT
Rinderpest_1814893_R	CTGAATTTGTTCTGGATTGAGTTCT
Rinderpest_1814893_P	TGTGACATTGATACCTACATAGTGGAGGCAGG

TABLE 192. Lists the target nucleic acid extracts used to screen signatures for sensitivity. The Rinderpest virus isolates were propagated in Vero cells. Qiagen RNeasy mini spin columns were used to extract the viral RNA from cell culture media.

Virus	Serotype	Isolate	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	Use stock titer ($TCID_{50}$/mL)	Titer Method
Rinderpest Virus	India	V02280	PIADC	Unknown	2 VERO	Unknown	Qiagen Rneasy Mini kit	3.16 x 10^6	Spearman-karber
Rinderpest Virus	Pakistan	V02284	PIADC	Unknown	1 VERO	Unknown	Qiagen Rneasy Mini kit	5.01 x 10^5	Spearman-karber
Rinderpest Virus	Egypt	V02285	PIADC	Unknown	1 VERO	Unknown	Qiagen Rneasy Mini kit	3.98 x 10^5	Spearman-karber
Rinderpest Virus	Kuwait	V02278	PIADC	Unknown	2 VERO	Unknown	Qiagen Rneasy Mini kit	3.98 x 10^6	Spearman-karber
Rinderpest Virus	Nigeria buffalo	V02279	PIADC	Unknown	2 VERO	Unknown	Qiagen Rneasy Mini kit	3.16 x 10^6	Spearman-karber
Rinderpest Virus	Plowright Vaccine Master seed, Kabete O-strain	V02057	PIADC	Unknown	92BK, 2VERO, Master seed	Unknown	Qiagen Rneasy Mini kit	3.98 x 10^6	Spearman-karber
Rinderpest Virus	RBOK vaccine	V02325	PIADC	Unknown	unknown	Unknown	Qiagen Rneasy Mini kit	1 x 10^7	Spearman-karber
Rinderpest Virus	Yemen	V#02286	PIADC	Unknown	PI Vero 1	Unknown	Qiagen Rneasy Mini kit	1 x 10^6.5 TCID50 / ml	Spearman-karber
Rinderpest Virus	Pendik	V#02917	PIADC	Unknown	Vero P-18	Unknown	Qiagen Rneasy Mini kit	1x 10^5 TCID50 / ml	Spearman-karber

TABLE 193. Near-neighbor nucleic acid extract used to screen signatures for target specificity. The viruses were propagated in Vero cells. Qiagen RNeasy mini spin columns were used to extract the viral RNA from cell culture media. All signatures in table 9 were screened in

triplicate with 200 pg of extracted RNA per reaction against all 5 isolates of PPRV. None of the signatures produced a PCR product in any of the near-neighbor screenings.

Virus	Serotype	Isolate	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	Use stock titer ($TCID_{50}$/mL)	Titer Method
Peste de Petits Ruminants virus (PPRV)	RCA	V02297	PIADC	Unknown	2 LK, 6 VERO	Unknown	Qiagen Rneasy Mini kit	6.31 x 10^4	Spearman-Karber
Peste de Petits Ruminants virus (PPRV)	Burkina Faso	V02299	PIADC	Unknown	1 LS, 10 VERO	Unknown	Qiagen Rneasy Mini kit	7.94 x 10^3	Spearman-Karber
Peste de Petits Ruminants virus (PPRV)	Egypt	V02306	PIADC	Unknown	10 VERO	Unknown	Qiagen Rneasy Mini kit	2.51 x 10^6	Spearman-Karber
Peste de Petits Ruminants virus (PPRV)	Dorcas	V02322	PIADC	Unknown	8 VERO	Unknown	Qiagen Rneasy Mini kit	5.01 x 10^5	Spearman-Karber
Peste de Petits Ruminants virus (PPRV)	Ghana	V02329	PIADC	Unknown	7 LK, 6 VERO	Unknown	Qiagen Rneasy Mini kit	5.01 x 10^4	Spearman-Karber

TABLE 194. Each Rinderpest signature was tested with the available target strains, shown here as column headings. Each screening set was performed in triplicate indicated below as an average Ct value.

	India	Pakistan	Egypt	Nigerian Buffalo	Plowright	Yemen	RBOK	Pendik	Kuwait
1811628	21.4	20.8	20.1	20.8	22.8	21.5	23.8	19.9	20.1
1814853	21.8	23.8	20.8	21.6	22.7	22.8	21.2	20	21.8
1814855	21	24.9	19.9	20.5	22.1	25.7	20.2	22.9	24.3
1814856	25.3	22.8	21.1	21.3	22.6	22.5	21.5	23.6	21.5
1814893	23.3	22.8	23.9	24.2	24.3	23.2	22.4	23.9	22.2

Rinderpest Real-Time Screening Conclusions:

The 5 signatures that advanced to real-time screening against target and near-neighbors all produced valid Ct values against targets. All signatures were also screened against Peste de Petits Ruminants virus, (PPRV) a near-neighbor. This virus is a member of the family Paramyxoviridae, genus *Morbillivirus* and is antigenically close to rinderpest virus. None of the down selected signatures produced a PCR product with PPRV. Of the 9 initial signatures screened, 5 signatures have met all the criteria for a successful signature.

Lowest level of detection data:

TABLE 195. Rinderpest titration data. The target RNAs screened are listed in the top row of the table. The concentration in the second from the right column represents the total amount of target RNA added to each 25ul PCR reaction. Results are indicated as the average Ct value for each assay, n=3. "N" indicates no detectable PCR product after 35 cycles of PCR.

Signature	Concentration	India	Pakistan	Egypt	Nigerian Buffalo	Plowright	Yemen	RBOK	Pendik	Kuwait
1811628	100pg	23.3	22.7	22.5	22.9	23.3	20.9	20.7	20	22.1
	10pg	26.7	26.2	26	26.4	26.8	24.1	24.8	26.1	25.3
	1pg	30.6	30	29.5	29.6	30.5	28.8	27.7	28	28.8
	100fg	33.7	33.2	32.9	32.9	33.5	31.4	31	31.6	32.2
	10fg	N	N	N	N	N	34.4	34.6	34.1	N
1814853	100pg	23.5	23	22.8	22.8	24.6	24.4	22.8	23.7	24.5
	10pg	26.4	27.4	25.8	26.3	27.7	27.8	26.2	26.8	26.3
	1pg	30.9	30.2	29.9	29.8	31.8	31.9	29.6	30.4	29.4
	100fg	34.3	33.3	33	33.1	35.5	34.8	33.3	34.3	33.3
	10fg	N	N	N	N	N	N	N	N	N
1814855	100pg	23.9	26.8	22.3	23	23.6	28.5	22.9	24.4	26.4
	10pg	27.4	31.1	25.8	26.8	27.4	32	26.8	27.6	29.8
	1pg	31.6	34.2	30.1	30.1	30.5	N	28.9	29.8	33.7
	100fg	35	N	N	32.9	32.9	N	32.3	33.4	N
	10fg	N	N	33.4	N	N	N	34.8	N	N
1814856	100pg	23.4	26.1	22.7	23.5	24.6	24.2	23.2	20	23
	10pg	27.2	29.7	26.3	26.3	27.9	28.3	26.8	26.1	26.3
	1pg	30.4	32.7	29.9	29.6	32.1	32.1	30.2	28	29.7
	100fg	34.8	N	33.4	34.1	35.1	34.9	33.4	31.6	33.2
	10 fg	N	N	N	N	N	N	N	34.1	N
1814893	100pg	23.7	22.7	23.9	24.1	24.8	24.3	23.7	24.9	22.3
	10pg	27.1	26.5	27.6	27.2	28.2	28.3	26.7	28	25.2
	1pg	31.4	29.8	31.5	30.5	32.4	32.6	29.9	31.6	27.9
	100fg	35	33.9	N	34.4	N	N	33.5	35	32.4
	10fg	N	N	N	N	N	N	N	N	N

TABLE 196. Limit of detection summary for the final set of Rinderpest signatures. Dose-response curves were generated for each signature using several reference targets for that organism. Dilutions were prepared in the 10-fold range over 4-logs in the detection limit range. Results were then averaged (n=3) and the limit of detection was determined to be the lowest measured concentration (in pg units) that was detected under these conditions. Units of measurement reflect total RNA for qualitative comparison.

Signature	Gene ID	Target Strains (pg total DNA units, relative LOD)								
		India	Pakistan	Egypt	Nigerian Buffalo	Plowright	Yemen	RBOK	Pendik	Kuwait
1811628	Not available	0.1	0.1	0.1	0.1	0.1	0.01	0.01	0.01	0.1
1814853	Not available	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
1814855	Not available	0.1	1.0	0.01	0.1	0.1	1.0	0.01	0.1	1.0
1814856	Not available	0.1	1.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1
1814893	Not available	0.1	0.1	1.0	0.1	1.0	1.0	0.1	0.1	0.1

6.5. RPV MULTIPLEXED PCR SCREENING REPORT

SCREENING AND DOWN-SELECTION TECHNICAL APPROACH:

Signatures that pass gel and real-time PCR screening are candidates for multiplexed assays. If the number of candidate signatures is large than a subset of the available signatures are selected based on measured sensitivity and specificity observed in real-time screening. In preliminary screening the background of individual signatures is measured by running "blank" (no sample added) controls and observing signature response. In some cases a primer-to-primer non-specific interference will eliminate candidate signatures. Signatures are then added iteratively to each multiplexed panel, titrated, and with each assay addition, collectively, all signatures are re-titrated to determine if the presence of one signature will interfere with the performance of another. During this process signatures are added or subtracted from the multiplexed panel depending on individual outcomes until all desirable signatures have been added.

Criterion for down-selection of multiplexed signatures

Signature specificity. In initial real-time PCR screening tests undergo target and near neighbor screening and screening against environmental confounders which assess individual signature specificity. In multiplex format this is repeated to verify that a more complex reaction system gives the same level of specificity.

Signature sensitivity. In initial real-time PCR screening tests undergo limit of detection testing where a dilution curve is generated for each signature. This determines individual signature sensitivity. In multiplex format this is repeated to verify that a more complex reaction system gives acceptable signature sensitivity.

Optimal signal to noise ratio. In the multiplex assay system the potential for cross reactivity is larger than in single reaction testing, this may cause unwanted PCR reaction products or non-specific cross-reactivity. Signatures are preferred to have a high signal to noise ratio, where desired signal is significantly greater than the background noise of the signatures when there is no target nucleic acid in the reaction.

Compatibility with other signatures in multiplex. In some cases a primer set may interfere with other primers or probes in the multiplex and there by cause limit of detection shifting for one or more signature in the multiplex. For each scenario it must be evaluated whether the shift in detection level is significant enough to remove (what could be a very desirable signature) from the panel.

TABLE 197. Order details for RPV signatures ordered for multiplexed assay screening and development.

ID	Modification detail	Vendor
Rinderpest_1628.BF	5'-/5Bio/CGGTGAAAAGGTTGAGGGAGT-3'	Biosearch
Rinderpest_1628.FCP	5'-/5AmMC6/iSp18GCCTGATTGAACCAGGATAGAGTCAGCATCT-3'	Biosearch
Rinderpest_1628.R	5'-TCCTCATCTCCTCCCCAGA-3'	Biosearch

Rinderpest_4853.BF	5'-/5Bio/GGATCGCTGAAATGATCTGTGA-3'	Biosearch
Rinderpest_4853.FCP	5'-/5AmMC6/iSp18CTGGCCAACCCTGCCTCCACTATGTA-3'	Biosearch
Rinderpest_4853.R	5'-GGAGCCAGTTCACCCATTTG-3'	Biosearch
Rinderpest_4855.BF	5'-/5Bio/TGCATCTTATGTGACTTTGGTTCA-3'	Biosearch
Rinderpest_4855.FCP	5'-/5AmMC6/iSp18CAGTCCTCTCATCTGTTGTCGATCCGATGTA-3'	Biosearch
Rinderpest_4855.R	5'-GGCTATCCGCACAGCTGAC-3'	Biosearch
Rinderpest_4856.BF	5'-/5Bio/AACTCCTGACCTCATTCCTTGC-3'	Biosearch
Rinderpest_4856.FCP	5'-/5AmMC6/iSp18TGGCTCAGTGCATTCACAAAGACCTTGAATA-3'	Biosearch
Rinderpest_4856.R	5'-GGCTCTATAATCCCACTATGCCA-3'	Biosearch
Rinderpest_4893.BF	5'-/5Bio/AATAAACCGAGGATCGCTGAAATGAT-3'	Biosearch
Rinderpest_4893.FCP	5'-/5AmMC6/iSp18CCTGCCTCCACTATGTAGGTATCAATGTCACA-3'	Biosearch
Rinderpest_4893.R	5'-CTGAATTTGTTCTGGATTGAGTTCT-3'	Biosearch

Multiplex Data Analysis -Technical approach

Disease signature thresholds

Thresholds were initially determined using all available data from known negative samples (blanks) by plotting a histogram which showed the probability of a given sample generating a particular MFI. In the absence of cross reactivity, each signature in the multiplex assay could be treated as an individual signature result. For example, for a sample spiked with RPV, data for other disease signatures could contribute towards negative data, providing that cross reaction between disease signature sets was not observed. This is a way to increase the number of samples that can be used to establish thresholds.

TABLE 198. Individual signature thresholds for RPV signatures. For FY06, threshold determinations have not yet been made as they require a significant number of tests (>500) to generate this information. This information will be updated when available.

Signature Name	Mux Assay name	Signature background in Tris NaCl Buffer (MFI +/-)	Signature background in Clinical matrices (MFI +/-)	Threshold
RPV_1814853	RPV_1814853	TBD	TBD	TBD
RPV_1814855	RPV_1814855	TBD	TBD	TBD
RPV_1814856	RPV_1814856	TBD	TBD	TBD

TABLE 199. Lists the target nucleic acid extracts used to screen signatures for sensitivity. The Rinderpest virus isolates were propagated in Vero cells. Qiagen RNeasy mini spin columns were used to extract the viral RNA from cell culture media.

Virus	Isolate	Unique ID	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	Use stock titer ($TCID_{50}$/mL)	Titer Method
Rinderpest	India	V02280	PIADC	Unknown	2 VERO	Unknown	Qiagen	3.16 x	Spearman-

Virus							Rneasy Mini kit	10^6	karber
Rinderpest Virus	Pakistan	V02284	PIADC	Unknown	1 VERO	Unknown	Qiagen Rneasy Mini kit	5.01 x 10^5	Spearman-karber
Rinderpest Virus	Egypt	V02285	PIADC	Unknown	1 VERO	Unknown	Qiagen Rneasy Mini kit	3.98 x 10^5	Spearman-karber
Rinderpest Virus	Kuwait	V02278	PIADC	Unknown	2 VERO	Unknown	Qiagen Rneasy Mini kit	3.98 x 10^6	Spearman-karber
Rinderpest Virus	Nigeria buffalo	V02279	PIADC	Unknown	2 VERO	Unknown	Qiagen Rneasy Mini kit	3.16 x 10^6	Spearman-karber
Rinderpest Virus	Plowright Vaccine Master seed, Kabete O-strain	V02057	PIADC	Unknown	92BK, 2VERO, Master seed	Unknown	Qiagen Rneasy Mini kit	3.98 x 10^6	Spearman-karber
Rinderpest Virus	RBOK vaccine	V02325	PIADC	Unknown	unknown	Unknown	Qiagen Rneasy Mini kit	1 x 10^7	Spearman-karber

TABLE 200. Near-neighbor nucleic acid extract used to screen signatures for target specificity. The viruses were propagated in Vero cells. Qiagen RNeasy mini spin columns were used to extract the viral RNA from cell culture media. All signatures in table 9 were screened in triplicate with 200 pg of extracted RNA per reaction against all 5 isolates of PPRV. None of the signatures produced a PCR product in any of the near-neighbor screenings.

Virus	Isolate	Unique ID	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	Use stock titer ($TCID_{50}$/mL)	Titer Method
Peste de Petits Ruminants virus (PPRV)	RCA	V02297	PIADC	Unknown	2 LK, 6 VERO	Unknown	Qiagen Rneasy Mini kit	6.31 x 10^4	Spearman-Karber
Peste de Petits Ruminants virus (PPRV)	Burkina Faso	V02299	PIADC	Unknown	1 LS, 10 VERO	Unknown	Qiagen Rneasy Mini kit	7.94 x 10^3	Spearman-Karber
Peste de Petits Ruminants virus (PPRV)	Egypt	V02306	PIADC	Unknown	10 VERO	Unknown	Qiagen Rneasy Mini kit	2.51 x 10^6	Spearman-Karber
Peste de Petits Ruminants virus (PPRV)	Dorcas	V02322	PIADC	Unknown	8 VERO	Unknown	Qiagen Rneasy Mini kit	5.01 x 10^5	Spearman-Karber
Peste de Petits Ruminants	Ghana	V02329	PIADC	Unknown	7 LK, 6 VERO	Unknown	Qiagen Rneasy Mini kit	5.01 x 10^4	Spearman-Karber

virus (PPRV)									

TABLE 201. List of additional near-neighbors screened against the Bovine Panel. All near-neighbors samples were extracted nucleic acids with unknown titers. Further screening of available near-neighbors will be conducted at PIADC in July 2007.

Virus	Strain[1]	Unique ID	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	NA conc. used or stock titer	Titer Method
Border Disease virus	Coos Bay	4-6-92	NADC, Julia Ridpath	1.5 x 10^8 TCID50/mL	unknown	12/14/06	Trizol	1.18 x 10^6 TCID50/mL	Reed & Muench
Bovine Herpes Virus-1	California	NVSL 20032 111903	CAHFS	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Cooper	NVSL 86741 111903	CAHFS	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Los Angeles	Los Angeles ATCC VR188	ATCC (CAHFS)	1 x 10^5 TCID50/mL	(CAHFS) MDBK2(LLNL)MDBK1	5/17/07	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	RLB-106	ATCC VR793	ATCC (CAHFS)	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	A033640072	CAHFS, R. Mock, #5 Swab, 1BDFK1, 1/28/2004, A030020072	unknown	1BDFK1,	4/23/04	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	A040130066	CAHFS, R. Mock, #4 Lung, 1BFK1, 1/28/2004, A040130066	unknown	1BFK1	4/23/04	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	Texas A030020072	CAHFS, R. Mock, #80 Swab, 1BDFK1, 1/28/2004, A030020072	unknown	1BDFK1	4/23/2004	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	California	NVSL 200032	NVSL, CAHFS	unknown	MDBK CAHFS	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Virginia	NVSL 231221	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	California	NVSL 51619	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Minnesota	NVSL 86741	NVSL, CAHFS	unknown	MDBK CAHFS	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Minnesota	NVSL 97-10720	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Cooper	NVSL-Cooper RA 309	NVSL, CAHFS	unknown	MDBK CAHFS	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes-5	DN-599	A032540006	R. Mock, from lung tissue, 2BFK2, 2/3/2004	Unknown	CAHFS Lab 2BFK2 4/30/2004	6/3/2004	Phenol/Chloroform	40 pg/uL	N/A
Bovine Herpes-5	DN-599	A040150085	R. Mock, #2 Lung, 5BFK5, 2/3/04	Unknown	CAHFS Lab 5BFK5 4/23/2004	7/7/2004	Phenol/Chloroform	40 pg/uL	N/A
Bovine Herpes-5	D9402133	D9402133	Sonoma Co. 3/94 Severe	Unknown	CAHFS Lab	11/19/2003	Phenol/Chloroform	40 pg/uL	N/A

			necrotizing encephalitis, 1 week old calf		(MDBK), (1 flasks) 11/19/2003				
Bovine Herpes Virus	BHV 5	D9403153	CAHFS	unknown	unknown	unknown	unknown	40 pg/uL	unknown
Caprine Herpes Virus		VR462	ATCC	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Caprine Herpes Virus	D0201157	D0201157	CAHFS, Isolate D0201157 History: San Joaquin Co. 2/02 Aborted fetus-third trimester	unknown	CAHFS Lab (MDBK)	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Caprine Herpes Virus	S0201998	S0201998	CAHFS, Isolate S0201998 History: San Diego Co. 2/02 Aborted fetus-third trimester	unknown	CAHFS Lab (MDBK)	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-1	Santa Barbara	N/A	Bridget McLaughlin CAHFS	unknown	unknown	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-1	Georgia	N/A	Bridget McLaughlin CAHFS	unknown	unknown	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-2	Alberta	041 ODV 0401	NVSL	unknown	BHK	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-1 (SV123)	New Jersey (3TD) Deer, NJ 1955	041 ODV 0401	NVSL	1 x $10^{5.125}$ $TCID_{50}$/0.1 ml	+BHK-3 (12/7/83); (12/11/86); (12/18/86)	Unknown	Trizol	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A011120004	CAHFS, R. Mock, from brain tissue, 2BFK2, 2/3/2004, A011120004	unknown	CAHFS, 2BFK2	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A99043047	CAHFS, R. Mock, Spleen, 1BFK1, 2/3/2004, A99043047	unknown	CAHFS, 1BFK1	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A9904309	CAHFS	unknown	CAHFS, unknown	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Bovine 1247	Bovine 1247 ATCC VR2003	ATCC	unknown	CAHFS, unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	NVSL 002	NVSL 002 1/28/04	NVSL	unknown	CAHFS, unknown	1/29/03	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	LK	LK ATCC VR701	ATCC	unknown	CAHFS, unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	D990	D990	CAHFS	unknown	CAHFS, unknown	1/29/03	Phenol/ Chloroform	40 pg/uL	N/A
Feline Herpes	C-27	ATCC VR636	ATCC	unknown	CAHFS, unknown	unknown	unknown	40 pg/uL	N/A
Fowl Pox	Vaccine strain	Poxine™	Jeffers Livestock	unknown	ECE	5/17/07	Phenol/ Chloroform	40 pg/uL	N/A
Parainfluenza Virus type 3	C-243	TC-81335	LLNL	1 x 10^{5} TCID50/0.1	(VL)MK1 4(LLNL)H	5/17/07	Trizol	40 pg/uL	Reed and Muench

				mL	292-1				
Porcine Coronavirus	AR310	ATCC VR-2384	LLNL	1 x $10^{4.25}$ $TCID_{50}$/0.1 ml	ST2				Reed and Muench
Porcine Herpes Pseudorabies	Shope	RA 180	CAHFS	1 x 10^{5} TCID50/mL		5/17/07	Trizol	40 pg/uL	N/A
Pseudorabies		92-12013	NVSL 92-12013 bovine isolate Iowa December, 1991	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies		93-11745	NVSL 93-11745 Illinois December, 1992	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies	Shope	RA180	NVSL Shope (PRV) RA 180	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies Titered	Shope	N/A	NVSL	unknown		unknown	Phenol/ Chloroform		Reed & Muench
Respiratory Syncytial virus	Long	N/A		unknown	(VL)KB6 L2KB2HF DL2A549-1(LLNL)H eLa3	5/17/07	Trizol	40 pg/uL	N/A
Transmissible Gastroenteritis virus of hogs	Perdue	NVSL 9801	LLNL	1 x $10^{4.75}$ $TCID_{50}$/0.1 ml	ST 1		Trizol	40 pg/ul	Reed and Muench

TABLE 202. Panel membership for signature. The 3 RPV signatures were included in the **Bovine-specific multiplexed assay panel** for FMDV rule-out diagnostics. The below table describes the constituents of that multiplexed assay panel in which these signatures were tested.

Bead ID	Signature/ Target Name	Mux Group ID	Virus	Source	Forward Primer	Reverse Primer	Luminex Probe (-strand, FCP)
113	emcf_94975.F, emcf_94976.R, emcf_94977.P	MCF_1	Malignant Catarrhal Fever	LLNL	ATGCCAGTCACTGGCTC TCA	GGGTGTTGTAGAATCCTGA AATGG	GTTGATCACGGTGGCACCC TGG
114	emcf_95059.F, emcf_95060.R, emcf_95061.P	MCF_2	Malignant Catarrhal Fever	LLNL	GTTCTGGAAACTGACCA AACAGTGT	AGTGGCACTTGAGTGTAAC TTTTATTG	GCACTCTGGCAGGCATAAG GGAAATACA
115	emcf_95155.F, emcf_95156R, emcf_95157.P	MCF_3	Malignant Catarrhal Fever	LLNL	CCCTGGAAGCTGTCATA CAAAA	AAACATTGGCATATCTTGC AAGGT	CAGTAGAGTCCAGGGCTGC AGTTGTCTCA
117	BVH__94666.F, BVH_94667.R, BVH_94668.P	BHV-94668	Bovine Herpes Virus	LLNL	GTGCCAGCCGCGTAAA AG	GACGACTCCGGGCTCTTTT	TCCTGGTTCCAGAGCGCTA ACATGGAG
118	BVH__94738.F, BVH_94739.R, BVH94740.P	BHV-94740	Bovine Herpes Virus	LLNL	TGAGGCCTATGTATGG GCAGTT	GCGCGCCAAACATAAGTAA A	AAATAACACGGTGTGCACTT AAATAAGATTCGCG
119	BPSV_95719.F, BPSV_95720.R, BPSV_95721.P	PPOX_1	Bovine Papular Stomatitis Virus	LLNL	GCAGATGCGCTCCTGG TT	GCACCTCTGCTGCTGCAA	CCGACTCCGACGTGGAGAA CGTG
120	BPSV_95722.F, BPSV_95723.R, BPSV_95724.P	PPOX_2	Bovine Papular Stomatitis Virus	LLNL	GATGGCCGTGCAGCTC TT	CGTACAAGATCACGGCCAA CT	TGTACGGGCTCATGGGCTT CCG
122	BPSV_95731.F, BPSV_95732.R, BPSV_95733.P	PPOX_4	Bovine Papular Stomatitis Virus	LLNL	GCAGCAGTGCACCACG TAGT	CGCTGAACCCGTACATCCT	GACTTCGAGGCGGACAACA AGCG
132	FMDV.TC	FMDV.TC	Foot and Mouth Virus	Tetracore	ACTGGGTTTTACAAACC TGTGA	GCGAGTCCTGCCACGGA	GTCCCACGGCGTGCAAAGG A
133	FMDV.Pir	FMDV.Pir	Foot and Mouth Virus	Pirbright	CACYTYAAGRTGACAYT GRTACTGGTAC	CAGATYCCRAGTGWCICIT GTTA	CCTCGGGGTACCTGAAGGG CATCC
134	BVD_1a	BVD_SIG1	Bovine Viral Diarrhea	UCD, CAHFS lab	GGTAGTCGTCAGTGGTT CGAC	CATGTGCCATGTACAGCAG AGAT	CCTCGTCCACGTGGCATCT CGAG
138	BVD_1821165.F, BVD_1821164.R, BVD_1821166.P	BVD_SIG2	Bovine Viral Diarrhea	LLNL	GGGAGTCGTCAATGGT TCGAC	TCCATGTGCCATGTACAGC AGAG	CCTCGTCCACITGGCATCTC GAG
135	BTV_1759932	BTV_1759932	Bluetongue Virus	LLNL	GCACCCTATATGTTTCC AGACCA	CAGCTAACTCTTCAGCCAC ACG	CTAACTCGTGGGCCAATCAT CATCTTCTGT
136	BTV10_1810207	BTV10_181020 7	Bluetongue Virus	LLNL	CAAACACAAAAGGCGG AGAAG	GGCGTTTAATCTGTCTTAG TCTTACGT	GAAACGCTTCTGCGTACGA TGCGA
137	BTV10_1810199	BTV10_181019	Bluetongue	LLNL	CACATGTCGCTTAATTT	GCGGAGAAGGCTGCATT	ACGAAACGCTTCCGCGTAC

		9	Virus		GTCTTAACC		GATG
139	BTV10_1810205	BTV10_1810205	Bluetongue Virus	LLNL	TCAATTTTGGTAGAATTTGTTCATTCA	GCGGAGAAGGCTGCATTC	ACGAAACGCTTCCGCGTACGATG
163	VSV_1798943	VSV_1798943	Vesicular Stomatitis Virus	LLNL	CGCCACAAGGCAGAGATGT	TGTCAAATTCTGACTTAGCATACTTGC	GCATACTGCATCATATCAGGAGTCGGTTTTCTG
166	VSV_1798947	VSV_1798947	Vesicular Stomatitis Virus	LLNL	CCCAATCAATGCCATGATACA	CTCCAATGGAAGGGTCCAAA	TTTGAAAGTAGAACTGTGCAAGCCCGGTATC
167	VSV_1798949	VSV_1798949	Vesicular Stomatitis Virus	LLNL	GGCGCTCATTATAAAATTCGGA	ACATTTTCTCGTAGTAATGCAGCAG	GAAGTCCCTGTAATGGATTCCCATTCCATGT
169	VSV_1811408	VSV_1811408	Vesicular Stomatitis Virus	PIADC	CTCACAACATGGGTCCTGAA	TTCTTGACCTGGATACATCAT	GGCATAGYTCGTCTGCRACTTCCCT
160	RPV_1814853	RPV_1814853	Rinderpest Virus	LLNL	GGATCGCTGAAATGATCTGTGA	GGAGCCAGTTCACCCATTTG	CTGGCCAACCCTGCCTCCACTATGTA
161	RPV_1814855	RPV_1814855	Rinderpest Virus	LLNL	TGCATCTTATGTGACTTTGGTTCA	GGCTATCCGCACAGCTGAC	CAGTCCTCTCATCTGTTGTCGATCCGATGTA
162	RPV_1814856	RPV_1814856	Rinderpest Virus	LLNL	AACTCCTGACCTCATTCCTTGC	GGCTCTATAATCCCACTATGCCA	TGGCTCAGTGCATTCACAAAGACCTTGAATA
170	N/A	NC (MT-7)	Maritima	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
171	N/A	b-MT7 (FC)	N/A	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
172	N/A	MT7/Cy3 (IC)	N/A	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
173	Alien RNA	arRNA Control	None	Ambion	GACATCAAGGCTCAAACTAATTTTACC	CAAAGGCTGCCAACATAAAATG	CAAGCGTAAATGCAGCGTCCA

[1]FCP indicates that the probe is on the negative strand (Forward Compliment to the Probe).

6.5.1. BOVINE PANEL MULTIPLEXED PCR DATA

Preliminary screening: Preliminary screening can be broken down into several steps. The signatures are preliminarily screened by first assessing how the signatures will perform under multiplex PCR conditions and whether or not the addition of the new signatures will adversely effect other signatures, these preliminary screening methods are described as “signature baseline screening” and “multiplex addition screening” respectively. First signature baseline screening tests the performance of the signature in a no-target reaction (blank), a “passing” score is indicated by a low (typically less than 100) MFI. All RPV signatures passed the signature baseline screening. Next in multiplex addition screening the new signature primers are added one-by –one to the other panel signatures to determine if there would be any cross-reactions between primer pairs in the panel. Further assessment of the signatures is done through target and near-neighbor screening and the determination of relative sensitivities. The signature down-selection for the RPV signatures is further described in Table 26 below.

TABLE 203. Prelimnary screening of RPV signatures by adding step-wise into the bovine panel. Numerous cross-reactions observed with primer and probe combinations when added step-wise. The addition of primers for assay RPV-4893 and RPV-4856 noticibly cross-react with other panel constituents.

Description	RPV-1628 (43)	RPV-4853 (60)	RPV-4855 (61)	RPV-4856 (62)	RPV-4893 (63)
Blank RPV-1628	6	20	10	31	13
Blank RPV-1628	5	13	10	30	11
Blank RPV-1628	5	14	10	28	10
Blank RPV-1628	5	12	9	24	11

Blank RPV-4853	11	8	8	9	12
Blank RPV-4853	10	9	8	9	11
Blank RPV-4853	10	8	9	9	11
Blank RPV-4853	10	8	9	9.5	12
Blank RPV-4855	48	8	9	8	93
Blank RPV-4855	48	8	7	10	93
Blank RPV-4855	45	8	8	8.5	78
Blank RPV-4855	44	8	8	8	87
Blank RPV-4856	9	9	9	8.5	21
Blank RPV-4856	8	9	8.5	8	19
Blank RPV-4856	8	9	8	9	20
Blank RPV-4856	10	9	8	9	19
Blank RPV-4893	5	8	338	12	10
Blank RPV-4893	4	8	360	12	10
Blank RPV-4893	5	8	309	11	9
Blank RPV-4893	5	8	343	10.5	10
Blank RPV-1628,4853	8	14	9	16	11
Blank RPV-1628,4853	8	9	9	12.5	10
Blank RPV-1628,4853	8	19	8	13	11
Blank RPV-1628,4853	8	9	8	12	11
Blank RPV-1628,4853,4855	32	10	9	14	61
Blank RPV-1628,4853,4855	35	15	8	14	80
Blank RPV-1628,4853,4855	34	9	8	13	84
Blank RPV-1628,4853,4855	33	30	9	15	69
Blank RPV-1628,4853,4855,4856,	32	41	10	231	84
Blank RPV-1628,4853,4855,4856	30	18	8	243	87
Blank RPV-1628,4853,4855,4856	30	38	10	234	73
Blank RPV-1628,4853,4855,4856	35	16	10	218	80
Blank RPV-1628,4853,4855,4856,4893	38	19	359	690	73
Blank RPV-1628,4853,4855,4856,4893	42	21	375	767	107
Blank RPV-1628,4853,4855,4856,4893	38	14	324	661	69
Blank RPV-1628,4853,4855,4856,4893	40	19	411	745	99

TABLE 204. Multiplexed assay down-selection summary. Signature baseline screening indicates the performance of the signature in a no-sample reaction (blank), a “passing” score is indicated by a low MFI. All signatures passed the assay baseline screening. In the multiplex addition screening the primers are added one-by –one to the other panel constituents to determine if there would be any cross-reactions between primer pairs in the panel. RPV-1811628 and

RPV-1814893 cross-reacted with other panel constituents during the multiplex addition screening and thus were not carried forth in screening.

Signature	Mux Screening: Assay Down Selection					
	Signature baseline screening	Multiplex addition screening	Target screening	Near-neighbor screening	Limit of detection screening	Noted cross-reactions
RPV_1811628	Pass	Failed: Cross-reacted with VSV-8947	No further testing	No further testing	No further testing	Cross-reacted with VSV-8947 and mildly with RPV 4853 and 4856
RPV_1814853	Pass	Pass	Pass	Pass	Pass	Pass
RPV_1814855	Pass	Pass	Pass	Pass	Pass	Pass
RPV_1814856	Pass	Pass	Pass	Pass	Pass	Pass
RPV_1814893	Pass	Failed: cross-reacted with 3 other RPV signatures	No further testing	No further testing	No further testing	Cross-reacted with RPV-4855 and 4856

Near-neighbor and Target screening: Three RPV signatures were added to the Bovine panel. The signatures exhibited a reasonably low background response (<50 MFI) in the Bovine panel. Target screening for RPV was conducted at Plum Island Animal Disease Center. Preliminary (non-target) screening was conducted at LLNL.

TABLE 205. Backgrounds screening in multiplexed format for RPV at LLNL and PIADC.

	Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors	Targets
Mux PCR Screening	54	135	16	0	48	7

[1]-There are 752 pooled samples in each Aerosol Block.

TABLE 206. Backgrounds screening in **multiplexed** format for down-selected RPV signatures against the below listed **eukaryotes**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. RPV-1814853 cross-reacted with several of the eukaryotes at a low to moderate level (shown in red). This testing has not yet been repeated. The other 2 RPV signatures did not show any significant cross-reactions.

Description	RPV-1814853 (60)	RPV-1814855 (61)	RPV-1814856 (62)
BOVINE	92	37	5
CAT	174	19	4
CHICKEN	35	8	4

DOG	37	10	3
DROSOPHILA MELANOGASTER	9	6	3
EQUINE	168	27	3
FLEA	8	5	3
HUMAN	29	6	3
MONKEY	293	25	4
MOSQUITO	8	7	4
MOUSE	45	12	3
PIG / PORCINE	17	8	4
RABBIT	7	6	4
RAT	11	5	3
SHEEP	50	24	3
TICK	51	10	3

TABLE 207. Backgrounds screening in **multiplexed** format for the three RPV signatures against the listed **prokaryotes**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. None of the signatures cross-reacted with any of the samples listed below.

Description	RPV-1814853 (60)	RPV-1814855 (61)	RPV-1814856 (62)
Erwina amylovora	21	9	6
Actinobacillus suis	21	9	5
Aneurinbacillus migulanus	11	11	6
Bacillus cereus	18	10	5
Bacillus globigii	14	12	6
Bacillus subtilis	15	10	5
Bacillus thuringiensis	14	11	6
Bifidobacterium denticum	12	8	5
Borrellia burgdorferi	17	10	5
Burkholderia capacia	13	9	6
Caulobacter vibriodes	13	6	5
Clavibacter michganensis	10	8	5
Clostridium butyricum	14	9	6
Corynebacterium pseudodiphthericum	13	8	6
Cytophaga marinoflava	9	8	5
Erwina herbicola	14	10	6
Escherichia coli	15	11	6
Geobacillus caldoxylosilyticus	12	8	5
Halomonas halmophila	16	8	5
Haemophilus influenza	15	11	5
Herbaspirillium seropedicae	13	8	5
Lactobacillus garvieae	12	7	5
Lactobacillus gasseri	14	8	6
Listeria monocytogenes	14	8	5
Listeria seeligeri	15	9	6
Micrococcus luteus	17	10	6
Moraxella lacunatica	18	9	5
Oceanospirillium ssp. Maris	13	8	5
Paenibacillus napthalaenovorans	16	10	5
Paracoccus dentrificans	15	11	6

Porphyrobacter sanguineus	12	9	4
Proteus mirabillis	13	9	5
Pseudomonas aeruginosae	14	12	6
Pseudomonas oleovorans	11	8	5
Rhizobium leguminosarum	18	11	5
Rhodococcus rhodochrous	11	7	5
Salmonella typhimurium	22	11	6
Simonsiella muelleri	12	9	5
Sphingomonas sp. (Alcaligenes sp)	12	8	6
Staphylococcus aureus	17	9	6
Streptococcus pneumoniae	13	10	6
Streptomyces scabiei	13	8	5
Tatlockia maceachernii	16	9	5
Vibrio paraheamolyticus	11	8	5
Xanthomonas translucens	14	8	6

TABLE 208. Backgrounds screening in **multiplexed** format for the three RPV signatures against the below listed **soils**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. None of the signatures cross-reacted with any of the samples listed below.

Description	RPV-1814853 (60)	RPV-1814855 (61)	RPV-1814856 (62)
D 000107-49	17	13	9
D 000109 # 50	17	11	9
D 000402 # 53	28	12	10
D 000500 - 26 - 1	27	12	9
D 000501-14-1	28	11	10
D 000505 - 11 - 4	34	12	9
D 000521 - 23	18	12	8
D 000527 - 3	20	12	9
D 000531 - 21	26	12	9
D 000533 - 17 -1	16	11	10
D 000542 - 6	28	12	10
D 000550 - 20	26	12	9
D 000551 - 5	22	12	8
D 000561 - 8 - 6	21	12	9
D 000562 - 30 - 5	16	11	10
S 251	19	11	8
S 252	15	11	9
S 253	18	11	8
S 254	23	11	10
S 255	14	10	8
S 256	19	12	8
S 257	17	14	9
S 259	13	10	9
S 260	14	10	9
S 271	15	10	8
S 272	20	11	9

S 273	21	11	9
S 274	17	12	9
S 275	15	10	9
S 276	15	10	10
S 277	25	12	9
S 279	15	9	8
S 280	15	11	10
S 282	11	9	8
S 283	16	11	9
S 284	16	12	9
S 286	23	10	8
S 287	10	8	7
S 288	16	10	9
S 289	13	9	9
S 290	17	9	9
S 291	21	9	9
S 292	26	10	9
S 295	13	11	9
S 296	31	11	9
S 297	14	9	8
S 298	17	10	9
S 299	15	10	9
S 300	11	6	4
S 301	10	6	4
S 303	9	6	4
S 304	11	7	5
S 305	15	6	5
S 307	9	6	4

TABLE 209. Bovine Panel **Near-Neighbor** Screening (Data from 20070601) against RPV signatures. Values shown are Median Flourescence Intensity (MFI) units. “Blanks” are buffer-only samples (no nucleic acid added to reaction), shown below in red. Extracted nucleic acid was added to each PCR reaction in triplicate totaling 200pg/rxn. The data shows that the RPV signatures did not cross- reacted with any listed near-neighbors of the Bovine panel constituents.

Description	**RPV-4853 (60)**			**RPV-4855 (61)**			**RPV-4856 (62)**		
replicate	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Blank	15	23	19	9	10	10	5	5	5
Blank	27	22	17	10	9	8	5	5	5
BHV A040150085	29	9	30	20	6	15	6	4	6
BHV (BFK)	30	22	19	12	10	14	5	5	5
BHV-1 A040130066	60	23	24	15	11	16	6	4	6
BHV-1 A033640072	21	32	16	15	9	10	6	4	4
BHV-1 ATCC VR 793	28	16	30	17	8	13	5	5	5
IBR CA 111903	23	4	20	12	3	16	5	2	6
IBR MN 111903	13	17	20	9	7	12	5	5	5
BHV-1 NVSL 231221	27	25	36	13	12	10	4	5	5

BHV-1 RA309	34	31	31	16	10	11	5	5	5
BHV-1 NVSL 97-10720	21	76	22	12	8	18	5	5	6
BHV-1 NVSL 51619	29	28	32	16	8	12	6	5	5
BHV-1 NVSL 86741	24	20	24	10	10	15	5	5	5
BHV-1 NVSL 200032	21	14	17	9	10	11	5	6	6
BHV-1 LA ATCC VR188	16	23	28	16	10	15	5	5	5
BHV-1 (IBR) Texas CAHFS A030020072	20	26	23	15	10	10	6	6	4
EHV-1 ATCC VR2003	50	37	41	11	13	15	5	6	6
EHV-1 A9904309	24	19	41	12	15	19	5	6	5
EHV-1 A011120004 CAHFS	50	13	19	13	9	11	5	4	4
EHV-1 NVSL 00002	38	18	23	15	10	9	5	4	5
EHV-2 ATCC VR701	37	58	33	11	17	27	5	4	5
EHV-1 A99043047 CAHFS	17	17	27	12	9	12	5	5	5
EHV-2 D990 CAHFS	94	51	45	17	11	19	5	5	5
Pseudorabies Titered	22	17	23	14	9	9	6	5	4
Pseudorabies NVSL 93-11745	29	11	20	13	9	12	5	5	5
Pseudorabies NVSL 92-12013	13	20	22	11	6	8	5	5	4
Pseudorabies RA180 CAHFS	49	13	19	12	8	10	5	5	4
Porcine Herpes Pseudorabies Shope	17	20	21	7	11	11	4	5	5
Feline Herpes ATCC VR636	19	21	16	7	9	9	4	5	4
Caprine Herpes ATCC VR462	20	18	23	11	8	10	5	4	5
Caprine Herpes S0201998 CAHFS	28	28	24	11	13	11	5	5	5
Caprine Herpes D0201157 CAHFS	26	56	21	11	18	11	5	4	5
BHV-5 A040150085 CAHFS	69	94	39	28	29	15	5	5	5
BHV-5 A032540006 CAHFS	40	16	16	13	13	13	5	4	5
BHV-5 D9403153 CAHFS	25	25	13	9	9	11	5	5	5
BHV-5 D9402133 CAHFS	51	28	17	9	9	9	4	5	4
BDV Coos Bay	21	44	13	11	8	9	5	5	5
EHD-1 Georgia	20	13	19	11	9	17	5	4	5
EHD-1 New Jersey	15	24	19	14	10	12	6	5	4
EHD-1 Santa Barbara	27	11	13	15	8	9	5	4	5
EHD-2 Alberta	10	27	15	8	20	11	5	5	4
Fowl Pox	26	23	19	14	16	11	4	6	4
Parainfluenza Type 3	41	45	16	12	19	8	4	5	4
Respiratory Syncytial	18	23	20	10	16	7	4	5	5

RESULTS: We used our computational DNA signature generation system to identify the regions of Rinderpest that are not present in any other sequenced microbial organism in our database. We mined the resulting sequence for triplet oligonucleotides that meet the parameters of Real-time PCR. The LLNL bench screening includes real-time and multiplexed PCR format against extensive background confounders, near neighbors and target nucleic acid panels. The five signatures that emerged from Real-time PCR screening as being the most specific, reliable, and robust were selected for multiplexed assay development and are described in this document.

These signatures were further down-selected in multiplexed PCR format and reduced to three signatures. These three signatures have been currently added the Bovine panel and further testing against targets and near-neighbors is in process at PIADC.

7. VESICULAR STOMATITIS VIRUS (BOVINE AND PORCINE PANELS)

OBJECTIVE: We were tasked by the Department of Homeland Security to develop specific and reliable Real-time RT- PCR tests for Vesicular stomatitis Virus [VSV], among other major agriculturally-impacting viruses that are clinically indistinguishable from foot and mouth disease virus [FMDV]. The purpose of these assays (collectively with other virus-specific signatures developed in parallel) is to develop a species specific (i.e. bovine, porcine) multiplexed detection assay for Foot-and-Mouth disease rule-out for use in veterinary diagnostic laboratories. The signatures were designed to meet two major criteria. First, they would be able to discriminate among multiple viral agents whose disease symptoms mimic those of Foot and Mouth Disease Virus [FMDV]. Secondly, these signatures would be uniquely capable of detecting all species of VSV. This document describes the development of fourteen optimal Real-time RT-PCR and multiplexed [MUX] PCR signatures to detect both species of Vesicular stomatitis Virus [VSV]]. We were not able to develop under this project year signatures that detect serotypes 2 & 3 of the Indiana species of VSV. There are no complete genome sequences for Indiana serotypes 2 or 3. However, our multiplex signatures are able to detect New Jersey and Serotype 1 of Indiana.

7.1. BACKGROUND AND ETIOLOGY OF VSV

Vesicular stomatitis is a viral disease caused by 2 distinct species of vesicular stomatitis virus—New Jersey and Indiana. Vesiculation, ulceration, and erosion of the oral and nasal mucosa and epithelial surface of the tongue, coronary bands, and teats are typically observed in clinical cases, along with crusting lesions of the muzzle, ventral abdomen, and sheath. Clinical disease has been observed in cattle, horses, and pigs and very rarely in sheep, goats, and llamas. Serologic evidence of exposure has been found in many species including cervids, nonhuman primates, rodents, birds, dogs, antelope, and bats. The viruses are zoonotic and may cause influenza-like disease in people working in close contact with the virus (e.g., laboratory exposure, direct contact with lesions in infected animals).

Vesicular Stomatitis Virus (VSV) is a member of the family *Rhabdoviridae*, order *Mononegavirales*, genus *Vesiculovirus*. The genome of these viruses is a single molecule of negative-sense RNA that encodes five major proteins: glycoprotein, matrix protein, nucleoprotein, large protein and phosphoprotein. The genus also contains approximately 35 serologically distinct arboviruses. The New Jersey and Indiana serotypes of VSV, along with six additional VSV serotypes are known to cause vesicular disease in horses, cattle, swine and humans. Foot and mouth disease virus (FMDV), swine vesicular disease virus (SVDV), and vesicular exanthema and swine vesicular disease virus (VESV) along with VSV all produce similar symptoms of blisters or vesicles around the mouth and nostrils, the tongue, on the teats

and at the hoof skin junctions or coronary bands. In most countries they are notifiable and if suspected must be reported to the authorities.

The viruses are members of the family Rhabdoviridae and genus Vesiculovirus. Vesicular stomatitis viruses are the prototypes of the Vesiculovirus genus. They are bullet shaped and generally 180 nm long and 75 nm wide. The genomic structure is a single strand of negative sense RNA composed of 5 genes (N, P, M, G, and L, representing the nucleocapsid protein, phosphoprotein, matrix protein, glycoprotein, and the large protein, respectively). Although there are many members of the Vesiculovirus genus, the New Jersey and Indiana serotypes are of particular interest in the Western hemisphere. These 2 viruses are similar in size and morphology but generate distinct neutralizing antibodies in infected animals. They have both been isolated in recent outbreaks in the USA.

Vesicular stomatitis is seen sporadically in the USA. Outbreaks historically occurred in all regions of the country but since the 1980s have been limited to southwestern states. Vesicular stomatitis viruses are endemic in South America, Central America, and parts of Mexico but have not been seen naturally outside the Western hemisphere. The virus can be transmitted through direct contact with infected animals with clinical disease (those with lesions) or by blood-feeding insects. In the southwestern USA, black flies (Simulidae) are the most likely biologic insect vector. In endemic areas, sand flies (*Lutzomyia*) are proven biologic vectors. Other insects may act as mechanical vectors. The prevalence of clinical cases in a herd is generally low (10-20%), but seroprevalence within the herd may approach 100%. No reservoir or amplifying host of vesicular stomatitis viruses has been identified[7].

7.2. VSV COMPREHENSIVE ASSAY SUMMARY

Bioinformatics

Virus name: *Vesicular Stomatitis* Virus

Project name: *VSV update run; VSV external reworked signatures.*

Level of discrimination: *Strain*

Total number of Genome Sequences used for alignment: 4
Number of Initial Signatures: 14
Number of Signatures forwarded to PCR gel screening: 14
Number of Signatures forwarded to Real-time RT-PCR screening: 14

[7] *Source: The Merck Veterinary Manual*
http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/52500.htm&word=Vesicular%2cstomatitis%2cvirus

Real-time RT- PCR Screening Summary

TABLE 210. Final signatures down-selected in real-time RT-PCR screening (14).

#	LLNL Signature Designation	Sequence	#	LLNL Signature Designation	Sequence
1	VSV_1798941_F	AGAACCAGCGCAGATGACAA	8	VSV_1798948_F	TCATGCCGAGGACAGTTCTCTA
	VSV_1798941_R	CAGCCCATCCATGAGCTTTT		VSV_1798948_R	TCAAACCTCCGTACACTGCG
	VSV_1798941_P	TCAGGCATTTGTGTTCTGCCCACTCTGTATA		VSV_1798948_P	CGGCCTCTTCAAATGAGCCAGACTTCT
2	VSV_1798942_F	AAATGATGCTTCCAGGCCAA	9	VSV_1798949_F	GGCGCTCATTATAAAATTCGGA
	VSV_1798942_R	GTCGGGCATTCCTTGCTCT		VSV_1798949_R	ACATTTTCTCGTAGTAATGCAGCAG
	VSV_1798942_P	AAATTGACAAGGCCGATTCATACATGCCTTA		VSV_1798949_P	ACATGGAATGGGAATCCATTACAGGGACTTC
3	VSV_1798943_F	CGCCACAAGGCAGAGATGT	10	VSV_1811405_F	AAGAGATGGTCACGAGTGAC
	VSV_1798943_R	TGTCAAATTCTGACTTAGCATACTTGC		VSV_1811405_R	GAGCATTTGTGGAAACCGAGC
	VSV_1798943_P	CAGAAAACCGACTCCTGATATGATGCAGTATGC		VSV_1811405_P	TGTGTCACCAATGACCAAATACCCA
4	VSV_1798944_F	CCTCATGATCATCCCTTTAAAAGTC	11	VSV_1811406_F	GCTTGCACAGTTCTACTT
	VSV_1798944_R	TGTTTCAACACCTCTGACCTATTCA		VSV_1811406_R	GACAAAGACATGCCTGACAC
	VSV_1798944_P	TTCAAGATTTTGGAGATAAATGGCATGAACTTCC		VSV_1811406_P	TGTTGTATTTGGACCCTTCCATTGGAGG
5	VSV_1798945_F	ACCAGACTTGATGCGTGTTCA	12	VSV_1811407_F	CGGAGGATTGACGACTAATGC
	VSV_1798945_R	TGATTATCACCTTGTGCCAAGAC		VSV_1811407_R	TCAAACCATCCGAGCCATTC
	VSV_1798945_P	TCAATTCAACCTCCCAACGAGTTTGTTGG		VSV_1811407_P	CCGCCACAAGGCAGAGATGTGGT
6	VSV_1798946_F	CCGATTTTCCGTGGAGTGAT	13	VSV_1811408_F	CTCACAACATGGGTCCTGAA
	VSV_1798946_R	GGGTATTTGGTCATTGGTGACA		VSV_1811408_R	TTCTTGACCTGGATACATCAT
	VSV_1798946_P	ACTCGTGACCATCTCTTGGTCTCTAACCCTCT		VSV_1811408_P	AGGGAAGTYGCAGACGARCTATGCC
7	VSV_1798947_F	CCCAATCAATGCCATGATACA	14	VSV_1811409_F	CTCACAACATGGGTCCTGAA
	VSV_1798947_R	CTCCAATGGAAGGGTCCAAA		VSV_1811409_R	TTCTTGCCCCGGATACATCAT
	VSV_1798947_P	TTTGAAAGTAGAACTGTGCAAGCCCGGTATC		VSV_1811409_P	AGGGAAGTCGCAGATGAGCTGTGCC

TABLE 211. Summary of wet-bench screening in signature down-selection.

Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors	Targets

Gel Screening	5	5	5	none	none	16
Real-time RT-PCR Screening	45	54	16	3 Aerosol Blocks	none	16

Note: There are 752 pooled samples in each Aerosol Block.

TABLE 212. All 14 of the VSV signatures made the appropriate PCR product with at least one VSV isolate and none of the signatures were eliminated due to producing PCR product with backgrounds. Further screening is on going at Plum Island. From our preliminary data, 8 signatures were chosen to be tested in the multiplex assay, (MUX) format. The decision was based on the isolate the signature was designed to detect and the sensitivity it expressed in LOD screening. Below is the list of signatures moved onto MUX testing.

Signatures designed to detect VSV Indiana:	LOD against VSV Indiana
1798941	10fg
1798943	10fg
1798947	10fg
1798949	10fg
1811405	100fg
1811406	100fg
Signatures designed to detect VSV New Jersey:	**LOD against VSV New Jersey**
1811408	1fg
1811409	100fg

Multiplexed PCR Screening Summary

TABLE 213. Backgrounds screening in multiplexed format for VSV bovine and porcine panels at LLNL and PIADC. Pending near neighbor and target screening to be conducted at pIADC in Aug, 2007.

	Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors[2]	Targets
Mux PCR Screening	54	135	16	0	43 (+pending)	7(2 pending)

[1]-There are 752 pooled samples in each Aerosol Block. [2]Near-neighbors indicated (43) apply to the near-neighbor panel that is not uniquely specific for PRRS, but for the other panel constituents that were screened concurrently.

TABLE 214. Signature summary for VSV multiplexed signatures in the bovine panel.

LLNL Signature Designation	Panel Designation	MUX Group ID	Gene ID	Relative Limit of detection[1]	Targets Screened
VSV_1798943 (IN)	Bovine /Porcine	VSV_1798943	VSIVgp1/1489831	$1x10^{-2}$ $TCID_{50}$/rxn	IN serotype 1
VSV_1798947 (IN)	Bovine /Porcine	VSV_1798947	VSIVgp5/1489835	$1x10^{-2}$ $TCID_{50}$/rxn	IN serotype 1
VSV_1798949 (IN)	Bovine /Porcine	VSV_1798949	VSIVgp5/1489835	$1x10^{-2}$ $TCID_{50}$/rxn	IN serotype 1

VSV_1811408 (NJ)	Bovine /Porcine	VSV_1811408	nucleocapsid (N) protein	$1x10^{-3}$ $TCID_{50}$/rxn	NJ

[1] The relative "Limit of detection" for the VSV signatures reflects signature-specific targets (New Jersey and Indiana strains)

TABLE 215. Signature summary for VSV multiplexed signatures in the porcine panel.

LLNL Signature Designation	Panel Designation	MUX Group ID	Gene ID	Relative Limit of detection[1]	Targets Screened
VSV_1798943 (IN)	Bovine /Porcine	VSV_1798943	VSIVgp1/1489831	$1x10^{-2}$ $TCID_{50}$/rxn	IN serotype 1
VSV_1798947 (IN)	Bovine /Porcine	VSV_1798947	VSIVgp5/1489835	$1x10^{-2}$ $TCID_{50}$/rxn	IN serotype 1
VSV_1798949 (IN)	Bovine /Porcine	VSV_1798949	VSIVgp5/1489835	$1x10^{-2}$ $TCID_{50}$/rxn	IN serotype 1
VSV_1811405 (IN)	Porcine	VSV_1811405	VSIVgp5/1489835	$5x10^{0}$ $TCID_{50}$/rxn	IN serotype 1
VSV_1811408 (NJ)	Bovine /Porcine	VSV_1811408	nucleocapsid (N) protein	$1x10^{-3}$ $TCID_{50}$/rxn	1, New Jersey
VSV_1811409 (NJ)	Porcine	VSV_1811409	nucleocapsid (N) protein	$1x10^{-1}$ $TCID_{50}$/rxn	1, New Jersey

[1] The relative "Limit of detection" for the VSV signatures reflects signature-specific targets (New Jersey and Indiana strains)

7.3. VSV SIGNATURE BIOINFORMATICS AND GENERATION INFORMATION

Bioinformatics process:
The LLNL Bioinformatics team have developed a computational process that uses available DNA sequence information for a given target organism to generate candidate DNA signature sequences that are conserved and unique. Signatures are primarily used to underlie reliable and specific pathogen detection assays. Evidence of the success of the process is that several of the Real-time RT- PCR signatures that are used in the national BioWatch monitoring system were generated by this process, and to date, has resulted in no false positives from a total of over 5 million assays performed.

The general approach is the following: All available complete genomes of different strains of the target species are compared using a multiple genome alignment programs. A *consensus gestalt* is formed from the alignments that contain the sequence conserved amongst all target inputs. (This step is bypassed if only one target sequence is available).

To determine that organism-conserved sequence which does not occur in any other sequenced microbial organism, the consensus gestalt is compared against our immense, continuously updated database of microbial organism. A customized algorithm accomplishes this electronic subtraction, and the result is a *uniqueness gestalt* that is mined for potential signature candidates. A final computational screening is done to verify that cross-reactions are not detected.

Target Organism Information:
Organism name: *Vesicular Stomatitis* Virus
Type: ssRNA negative-strand virus
Genome size: 11161 bp .

Primer/Probe Set Generation Information

Method: Alignment and All Microbe Database subtraction.

Note: Five of the fourteen VSV signatures were reworked signatures from collaborator Luis Rodriguez and they are: VSV_1811405, VSV_1811406, VSV_1811407, VSV_1811408 and VSV_1811409. This set was assigned Kpath id number 102604 although it was not an actual signature generation run.

TABLE 216. K-path run id: 98780. List of genomes used for alignment.

	Genome Description	GI Number	Sequence Length (bp)
1	Vesicular stomatitis Indiana virus strain 94GUB, complete genome	23305074	11336
2	Vesicular stomatitis Indiana virus strain 85CLB, complete genome	23305068	11155
3	Vesicular stomatitis Indiana virus strain 98COE, complete genome	23305062	11162
4	Vesicular stomatitis Indiana virus, complete genome	9627229	11161

TABLE 217. List of main parameters and settings used in generating candidate signatures using Primer 3 signature generation tool.

Primer3 Main Parameters	
Parameters	**Standard Settings**
PRIMER_OPT_SIZE	20
PRIMER_MIN_SIZE	18
PRIMER_MAX_SIZE	27
PRIMER_PRODUCT_OPT_SIZE	100
PRIMER_PRODUCT_SIZE_RANGE	71-600
PRIMER_OPT_TM	62
PRIMER_MIN_TM	61
PRIMER_MAX_TM	63
PRIMER_MIN_GC	20
PRIMER_MAX_GC	80
PRIMER_PICK_INTERNAL_OLIGO	1
PRIMER_INTERNAL_OLIGO_OPT_SIZE	31
PRIMER_INTERNAL_OLIGO_MIN_SIZE	18
PRIMER_INTERNAL_OLIGO_MAX_SIZE	36
PRIMER_INTERNAL_OLIGO_OPT_TM	72
PRIMER_INTERNAL_OLIGO_MIN_TM	71
PRIMER_INTERNAL_OLIGO_MAX_TM	73
Number of Primers/Probe Set generated:	**9**

Signature Information

Source: LLNL
Project name: VSV update run; VSV external reworked signatures.
Level of discrimination: Strain

Number of Initial Signatures: 14
Number of Signatures forwarded to gel bench-screening: 14
Number of Signatures forwarded to real-time RT-PCR Taqman screening: 14
Number of Final real-time RT-PCR Signatures: 14

Taqsim description

We used a computational Real-time RT- PCR simulator program, "Taqsim", to identify all potential targets for each signature from all sequences available in Genbank. Taqsim is a BLAST-based comparison of each signature as a triplet against all sequences in Genbank to identify the targets that are predicted to produce a Real-time RT- PCR reaction at 57 degrees primer annealing and 67 degrees for probe annealing (these temperatures are according to Primer 3 oligo Tm calculations). The first column of the excel spreadsheets list the signatures by the name, and the following columns report the predicted Genbank targets, coordinates of signature region, amplicon size, and accession #'s for each target.

Note: For a listing of computationally predicted product sequences and potential cross targets for each signature, please see Appendix II, Taqsim Run Data.

Signature Bioinformatics

TABLE 218. Detailed signature bioinformatics.

Signature	Size		Sequence	Length	T_m	GC
1798941	105bp	F	AGAACCAGCGCAGATGACAA	20bp	61.43°	50.00%
		R	CAGCCCATCCATGAGCTTTT	20bp	60.06°	50.00%
		P(-)	TCAGGCATTTGTGTTCTGCCCACTCTGTATA	31bp	69.03°	45.16%
1798942	174bp	F	AAATGATGCTTCCAGGCCAA	20bp	59.73°	45.00%
		R	GTCGGGCATTCCTTGCTCT	19bp	61.59°	57.89%
		P(+)	AAATTGACAAGGCCGATTCATACATGCCTTA	31bp	66.77°	38.71%
1798943	160bp	F	CGCCACAAGGCAGAGATGT	19bp	61.81°	57.89%
		R	TGTCAAATTCTGACTTAGCATACTTGC	27bp	61.49°	37.04%
		P(+)	CAGAAAACCGACTCCTGATATGATGCAGTATGC	33bp	68.16°	45.45%
1798944	191bp	F	CCTCATGATCATCCCTTTAAAAGTC	25bp	58.96°	40.00%
		R	TGTTTCAACACCTCTGACCTATTCA	25bp	61.60°	40.00%
		P(+)	TTCAAGATTTTGGAGATAAATGGCATGAACTTCC	34bp	65.82°	35.29%
1798945	192bp	F	ACCAGACTTGATGCGTGTTCA	21bp	61.62°	47.62%
		R	TGATTATCACCTTGTGCCAAGAC	23bp	60.32°	43.48%
		P(+)	TCAATTCAACCTCCCAACGAGTTTGTTGG	29bp	67.24°	44.83%
1798946	81bp	F	CCGATTTTCCGTGGAGTGAT	20bp	59.32°	50.00%
		R	GGGTATTTGGTCATTGGTGACA	22bp	60.05°	45.45%
		P(-)	ACTCGTGACCATCTCTTGGTCTCTAACCCTCT	32bp	70.50°	50.00%
1798947	173bp	F	CCCAATCAATGCCATGATACA	21bp	57.78°	42.86%
		R	CTCCAATGGAAGGGTCCAAA	20bp	59.07°	50.00%
		P(-)	TTTGAAAGTAGAACTGTGCAAGCCCGGTATC	31bp	68.32°	45.16%
1798948	173bp	F	TCATGCCGAGGACAGTTCTCTA	22bp	62.15°	50.00%
		R	TCAAACCTCCGTACACTGCG	20bp	61.57°	55.00%
		P(-)	CGGCCTCTTCAAATGAGCCAGACTTCT	27bp	68.15°	51.85%
1798949	116bp	F	GGCGCTCATTATAAAATTCGGA	22bp	58.41°	40.91%
		R	ACATTTTCTCGTAGTAATGCAGCAG	25bp	61.04°	40.00%
		P(+)	ACATGGAATGGGAATCCATTACAGGGACTTC	31bp	67.81°	45.16%

1811405	86bp	F	AAGAGATGGTCACGAGTGAC	20bp	58.29°	50.00%
		R	GAGCATTTGTGGAAACCGAGC	21bp	61.54°	52.38%
		P(+)	TGTGTCACCAATGACCAAATACCCA	25bp	64.25°	44.00%
1811406	80bp	F	GCTTGCACAGTTCTACTT	18bp	53.77°	44.44%
		R	GACAAAGACATGCCTGACAC	20bp	58.18°	50.00%
		P(+)	TGTTGTATTTGGACCCTTCCATTGGAGG	28bp	66.52°	46.43%
1811407	67bp	F	CGGAGGATTGACGACTAATGC	21bp	59.97°	52.38%
		R	TCAAACCATCCGAGCCATTC	20bp	59.66°	50.00%
		P(+)	CCGCCACAAGGCAGAGATGTGGT	23bp	68.80°	60.87%
1811408	70bp	F	CTCACAACATGGGTCCTGAA	20bp	58.95°	50.00%
		R	TTCTTGACCTGGATACATCAT	21bp	55.59°	38.10%
		P(+)	AGGGAAGTYGCAGACGARCTATGCC	25bp	63.40°	52.00%
1811409	70bp	F	CTCACAACATGGGTCCTGAA	20bp	58.95°	50.00%
		R	TTCTTGCCCCGGATACATCAT	21bp	60.90°	47.62%
		P(+)	AGGGAAGTCGCAGATGAGCTGTGCC	25bp	70.19°	60.00%

Target Region Gene Information

TABLE 219a-b. (a) Reference Genomes used for Gene Information. (b) Gene information for each signature.

(a)

Genome Description	GI Number	Sequence Length (bp)
Vesicular stomatitis Indiana virus, complete genome	9627229|ref|NC_001560.1	7285
Vesicular stomatitis virus nucleocapsid protein (N) mRNA	37528739|gb|AY383716.1	1269

(b)

Gene Location			Target Region Location			
Kpath Signature ID	Gene/ID	Description	Start	End	Start	End
1798941	VSIVgp1/1489831	nucleocapsid (N) protein	51	1376	439	
1798942	VSIVgp1/1489831	nucleocapsid (N) protein	51	1376	842	1015
1798943	VSIVgp1/1489831	nucleocapsid (N) protein	51	1376	1169	1327
1798944	VSIVgp5/1489835	polymerase (L protein)	4723	11095	5981	6171
1798945	VSIVgp5/1489835	polymerase (L protein)	4723	11095	6688	6879
1798946	VSIVgp5/1489835	polymerase (L protein)	4723	11095	7070	7150
1798947	VSIVgp5/1489835	polymerase (L	4723	11095	7216	7388

		protein)				
1798948	VSIVgp5/1489835	polymerase (L protein)	4723	11095	8869	9041
1798949	VSIVgp5/1489835	polymerase (L protein)	4723	11095	9671	9786
1811405	VSIVgp5/1489835	polymerase (L protein)	4723	11095	7106	7190
1811406	VSIVgp5/1489835	polymerase (L protein)	4723	11095	7332	7410
1811407	VSIVgp1/1489831	nucleocapsid (N) protein	51	1376	1146	1211
1811408	N	nucleocapsid (N) protein	1	1269	733	801
1811409	N	nucleocapsid (N) protein	1	1269	733	801

7.4. VSV GEL AND REAL-TIME PCR SCREENING REPORT

Wet-lab screening process. To ensure extremely high selectivity and sensitivity, we have established a screening protocol that rigorously screens candidate DNA signatures to select the optimal sets for eventual field use. Candidate signature primer pairs that survive computational screening proceed to wet chemistry screening utilizing standard PCR with agarose gel electrophoresis. This step ensures that the primers will react across strains representative of the diversity of the pathogen (avoid false negative), but will not react with the nucleic acids of other organisms that could be present in a sample (avoid false positives). The collection of purified DNA templates used in this screening process is listed below:

1) Strain panels representative of the diversity of the target organism in nature.
2) Near neighbors-organisms that are closely related at the genetic level and therefore have the greatest likelihood of cross-reaction.
3) Eukaryotic DNA that may carry over from sample collection procedures.
4) Prokaryotic DNA that would be expected to be present in environmental samples and may also be present in Bovine and Porcine oral cavities.
5) Fifty four soil samples from around the country representing diverse climates and contain a complex mixture of organisms from diverse environmental collections
6) Aerosol samples: 752 samples collected from aerosol collectors and pooled for background testing purposes.

Vesicular Stomatitis Virus (VSV) TaqMan Screening Report

February 22, 2007

The LLNL Bioinformatics team generated 14 unique signatures for bench screening using the sequenced New Jersey and Indiana species of VSV; of these 14 signatures, signatures VSV_1811408, and VSV_1811409 are specific to the New Jersey strain and the others are specific to the Indiana strain. For this screening we were limited with the amount of available target templates and were unable to obtain near neighbor templates, so after initial screening at LLNL we sent our down selected signatures to Plum Island for more extensive target and near neighbor screening. We were able to obtain RNA from one New Jersey and one Indiana VSV strain for our target screening at LLNL.

TABLE 220. List of Targets screened at LLNL.

Virus	Strain	Unique ID	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	Use stock titer	Titer Method
VSV	New Jersey	N/A	NVSL	10^7 $TCID_{50}$/mL	Unknown	9/14/06	Trizol	10^7 $TCID_{50}$/mL	Reed & Muench
VSV	Indiana	N/A	NVSL	10^8 $TCID_{50}$/mL	Unknown	9/14/06	Trizol	10^6 $TCID_{50}$/mL	Reed & Muench

TABLE 221. List of targets screened at Plum Island.

Virus	Species-serotype	Isolate	Source	Original Titer (Log10 TCID50)	Passage History	Extraction date/ID	Extraction Method	Use stock titer	Titer Method
VSV	Indiana - 2	Salt	PIADC	Unknown	Unknown	Unknown	Qiagen Rneasy Mini Kit	Unknown	N/A
VSV	Indiana - 2	Maipu	PIADC	Unknown	Unknown	Unknown	Qiagen Rneasy Mini Kit	Unknown	N/A
VSV	Indiana - 2	Cocal	PIADC	Unknown	Unknown	Unknown	Qiagen Rneasy Mini Kit	Unknown	N/A
VSV	Indiana - 2	Parana (118)	PIADC	Unknown	Unknown	Unknown	Qiagen Rneasy Mini Kit	Unknown	N/A
VSV	Indiana - 3	Alagoas (146)	PIADC	Unknown	Unknown	Unknown	Qiagen Rneasy Mini Kit	Unknown	N/A
VSV	Indiana - 3	Alagoas (174)	PIADC	Unknown	Unknown	Unknown	Qiagen Rneasy Mini Kit	Unknown	N/A
VSV	New Jersey	NJ95COB	PIADC: Luis Rodriguez	1 x E9	Unknown	Unknown	Qiagen Rneasy Mini Kit	1 x E9	Spearman-Karber
VSV	New Jersey	NJ89GAS	PIADC: Luis Rodriguez	1 x E9.25	Unknown	Unknown	Qiagen Rneasy Mini Kit	1 x E9.25	Spearman-Karber
VSV	New Jersey	NJ0604NME	PIADC: Luis	1 x E7.75	Unknown	Unknown	Qiagen Rneasy Mini	1 x E7.75	Spearman-Karber

			Rodriguez				Kit		
VSV	New Jersey	NJ1184HDB	PIADC: Luis Rodriguez	1 x E7.5	Unknown	Unknown	Qiagen Rneasy Mini Kit	1 x E7.5	Spearman-Karber
VSV	Indiana-1	IN97COE	PIADC: Luis Rodriguez	Unknown	Unknown	Unknown	Qiagen Rneasy Mini Kit	Unknown	N/A
VSV	Indiana-1	IN98NME	PIADC: Luis Rodriguez	Unknown	Unknown	Unknown	Qiagen Rneasy Mini Kit	Unknown	N/A
VSV	Indiana-1	IN97CMB	PIADC: Luis Rodriguez	1 x E7.5	Unknown	Unknown	Qiagen Rneasy Mini Kit	1 x E7.5	Spearman-Karber
VSV	Indiana-1	IN94GUB	PIADC: Luis Rodriguez	1 x E9.59	Unknown	Unknown	Qiagen Rneasy Mini Kit	1 x E9.59	Spearman-Karber

TABLE 222. Backgrounds screening summary for VSV at LLNL. No cross reactions were seen in gel screening against backgrounds. Signatures were considered successful if they produced expected product sizes for VSV New Jersey or VSV Indiana or both.

	Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors	Targets
Gel Screening	5	5	5	none	none	16
Real-time RT-_PCR Screening	45	54	16	3_Aerosol Blocks[1]	none	16

[1]-There are 752 pooled samples in each Aerosol Block.

Vesicular Stomatitis Virus (VSV) - Gel Screening Report

Background gel screening was carried out in duplicate as 25ul reactions in 96 well PCR plates on MJ thermal cyclers. Each reaction mix consisted of 1X PCR Buffer [200mM Tris-HCL (pH 8.4), 500mM KCl], 1.5mM $MgCl_2$, 0.8mM each dNTP, 80ng BSA, 0.4uM each forward and reverse primers, 0.75U Platinum *Taq* DNA polymerase, PCR water and a variable amount of extracted DNA template. A total of 5ng of soil DNA or 1ng of Eukaryotic DNA or 200pg of Prokaryotic DNA was added to each 25ul reaction mix. Background template data and extraction protocols are available upon request.

Because the target is RNA, we used the Clontech Powerscript RT-PCR kit. Each reaction was performed in triplicate as per the manufacturer's suggested protocol, replacing probe with PCR water, in a 25ul volume with a total of 200pg TRIZOL extracted RNA.

Reaction products were visualized by gel electrophoresis on 4% agarose gels. PCR product sizes are listed as visual estimates based on a 20bp ladder that was run on each gel for reference. I f a signature screened against a background produced a PCR product size that fell below 100 base pairs greater in size than the predicted product size for a signature screened against its target, the

signature was dropped from further screening. The theory behind this selection process is that a much larger than target PCR product would not cause inhibitory PCR competition. However, a PCR product of correct size or smaller would inhibit PCR through competition.

TABLE 223. List of signatures screened in gel format: 14 signatures were gel screened.

VSV-1798941_F	AGAACCAGCGCAGATGACAA
VSV-1798941_R	CAGCCCATCCATGAGCTTTT
VSV-1798941_P	TCAGGCATTTGTGTTCTGCCCACTCTGTATA
VSV-1798942_F	AAATGATGCTTCCAGGCCAA
VSV-1798942_R	GTCGGGCATTCCTTGCTCT
VSV-1798942_P	AAATTGACAAGGCCGATTCATACATGCCTTA
VSV-1798943_F	CGCCACAAGGCAGAGATGT
VSV-1798943_R	TGTCAAATTCTGACTTAGCATACTTGC
VSV-1798943_P	CAGAAAACCGACTCCTGATATGATGCAGTATGC
VSV-1798944_F	CCTCATGATCATCCCTTTAAAAGTC
VSV-1798944_R	TGTTTCAACACCTCTGACCTATTCA
VSV-1798944_P	TTCAAGATTTTGGAGATAAATGGCATGAACTTCC
VSV-1798945_F	ACCAGACTTGATGCGTGTTCA
VSV-1798945_R	TGATTATCACCTTGTGCCAAGAC
VSV-1798945_P	TCAATTCAACCTCCCAACGAGTTTGTTGG
VSV-1798946_F	CCGATTTTCCGTGGAGTGAT
VSV-1798946_R	GGGTATTTGGTCATTGGTGACA
VSV-1798946_P	ACTCGTGACCATCTCTTGGTCTCTAACCCTCT
VSV-1798947_F	CCCAATCAATGCCATGATACA
VSV-1798947_R	CTCCAATGGAAGGGTCCAAA
VSV-1798947_P	TTTGAAAGTAGAACTGTGCAAGCCCGGTATC
VSV-1798948_F	TCATGCCGAGGACAGTTCTCTA
VSV-1798948_R	TCAAACCTCCGTACACTGCG
VSV-1798948_P	CGGCCTCTTCAAATGAGCCAGACTTCT
VSV-1798949_F	GGCGCTCATTATAAAATTCGGA
VSV-1798949_R	ACATTTTCTCGTAGTAATGCAGCAG
VSV-1798949_P	ACATGGAATGGGAATCCATTACAGGGACTTC
VSV_1811405_F	AAGAGATGGTCACGAGTGAC
VSV_1811405_R	GAGCATTTGTGGAAACCGAGC
VSV_1811405_P	TGTGTCACCAATGACCAAATACCCA
VSV_1811406_F	GCTTGCACAGTTCTACTT
VSV_1811406_R	GACAAAGACATGCCTGACAC
VSV_1811406_P	TGTTGTATTTGGACCCTTCCATTGGAGG
VSV_1811407_F	CGGAGGATTGACGACTAATGC
VSV_1811407_R	TCAAACCATCCGAGCCATTC
VSV_1811407_P	CCGCCACAAGGCAGAGATGTGGT
VSV_1811408_F	CTCACAACATGGGTCCTGAA
VSV_1811408_R	TTCTTGACCTGGATACATCAT
VSV_1811408_P	AGGGAAGTYGCAGACGARCTATGCC
VSV_1811409_F	CTCACAACATGGGTCCTGAA
VSV_1811409_R	TTCTTGCCCCGGATACATCAT
VSV_1811409_P	AGGGAAGTCGCAGATGAGCTGTGCC

TABLE 224. Nucleic acid extracts used to challenge the initial set of candidate signatures. These select backgrounds are used to determine which signatures will be down-selected in the screening process.

Nucleic Acid Extract Type	Description/ID
Soil Extract	s251
Soil Extract	s255
Soil Extract	s256
Soil Extract	s260
Soil Extract	s271
Prokaryotic DNA Extract	*Bacillus thuringiensis*
Prokaryotic DNA Extract	*Borrelia burgdorferi*
Prokaryotic DNA Extract	*Erwinia herbicola*
Prokaryotic DNA Extract	*Rhizobium leguminosarum*
Prokaryotic DNA Extract	*Streptococcus pneumoniae*
Eukaryotic DNA Extract	Bovine
Eukaryotic DNA Extract	Equine
Eukaryotic DNA Extract	Flea
Eukaryotic DNA Extract	Pig
Eukaryotic DNA Extract	Tick

TABLE 225. Gel screening results: Signature VSV_1798942 did produce product with some of the backgrounds. However, the product sizes were not close to the predicted target product size so we chose to keep this signature in the screening process. None of the other signatures made any PCR product when screened with the listed backgrounds so all 14 VSV signatures moved forward to be screened against the available targets at LLNL

	VSV_1798942
Expected Product Size	174bp
Bovine	2 x 500bp
Equine	2 x 500bp
Soil s256	2 x >500bp

TABLE 226a-b. Results with predicted product size for targets: The product generated with signature 1811405 and the VSV New Jersey template is in red because it did not produce the correct size product. 13 of the 14 signatures produced the expected product sizes with the 2 LLNL VSV isolates. Signature 1811406 produced the correct product size with the Indiana VSV isolate, but failed to produce the correct size amplicon with the New Jersey isolate. Because the signature did detect both isolates we decided to move it into real-time RT-PCR format.

(a)

	1798941	**1798942**	**1798943**	**1798944**	**1798945**	**1798946**	**1798947**
Predicted Product Size	105bp	174bp	159bp	191bp	192bp	81bp	173bp
VSV New Jersey	3 x 100bp	3 x 180bp	3 x 160bp	3 x 200bp	3 x 200bp	3 x 80bp	3 x 180bp
VSV Indiana	3 x 110bp	3 x 180bp	3 x 160bp	3 x 200bp	3 x 200bp	3 x 80bp	3 x 180bp

(b)

	1798948	**1797949**	**1811405**	**1811406**	**1811407**	**1811408**	**1811409**
Predicted Product Size	173	116	85	79	66	69	69
VSV New Jersey	3 x 180bp	3 x 120bp	3 x 50bp*	3 x 80bp	3 x 70bp	3 x 70bp	3 x 70bp

VSV Indiana	3 x 180bp	3 x 120bp	3 x 90bp	3 x 80bp	3 x 70bp	3 x 70bp	3 x N

*Upon further screening with probe, this signature did not produce a PCR product when screened against VSV New Jersey.

Vesicular Stomatitis Virus (VSV) – Real-time RT- PCR Screening Report

Background real-time RT-PCR tests were carried out in triplicate as 25ul reactions in 96 well PCR plates on BioRad's iCYCLERs. Each reaction mix consisted of 1X PCR Buffer [200mM Tris-HCL (pH 8.4), 500mM KCl], 6mM $MgCl_2$, 0.2mM each dNTP, 80ng BSA, 0.2uM each forward and reverse primers, 0.4uM Probe with a 5' Fam and a 3' BHQ modification, 1.25U Platinum *Taq* DNA polymerase, PCR water and a variable amount of extracted DNA template. A total of 5ng of soil DNA or 1ng of Eukaryotic DNA or 200pg of Prokaryotic DNA was added to each 25ul reaction mix.

Because the target is RNA, we used the Clontech Powerscript RT-PCR kit. Each reaction was performed in triplicate as per the manufacturer's suggested protocol; in a 25ul volume with a total of 200pg TRIZOL extracted RNA.

TABLE 227(a-c). Real-time RT- PCR background screening consisted of an extensive list of soil, Prokaryotic and Eukaryotic extracted DNAs along with 3 aerosol blocks which are listed below. All 14 signatures passed real-time RT- PCR background screening. None of the signatures produced an amplicon when screened against the listed backgrounds. All signatures moved forward to be screened against the 2 LLNL VSV isolates.

(a) Total of 45 soils screened

D000402	D000531	S252	S271	S280	S290	S300
D000109	D000542	S253	S272	S282	S291	S301
D000107	D000533	S254	S273	S283	S292	S303
D000500	D000561	S255	S274	S284	S295	S304
D000505	D000562	S256	S275	S286	S296	S305
D000521	D000501	S257	S276	S287	S297	S307
D000551	D000550	S259	S277	S288	S298	
D000527	S251	S260	S279	S289	S299	

(b) Total of 16 Eukaryotes screened

Bovine	Drosophila	Monkey	Rabbit
Cat	Equine	Mosquito	Rat
Chicken	Flea	Mouse	Sheep
Dog	Human	Porcine	Tick

(c) Total of 54 Prokaryotes screened

A. suis	*C. butyricum*	*L. gasseri*	*P. oleovorans*
A migulanus	*C. pseudodipthericum*	*L. monocytogenes*	*R. leguminosarum*
B. cereus	*C. marinoflava*	*L. seeligeri*	*R. rhodochrous*
B. globigii	*E. amylovora*	*M. luteus*	*S. typhimurium*
B. subtilis	*E. herbicola*	*M. lacunatica*	*S. muelleri*

B thuringiensis	*E. coli*	*O. ssp. Maris*	*Alcaligenes sp.*
B. denticum	*G.caldoxylosilyticus*	*P. napthalaenovorans*	*S. aureus*
B. burgdorferi	*H. halmophila*	*P. dentrificans*	*S. pneumoniae*
B. capacia	*H. influenza*	*P sanguineus*	*S. scabiei*
C. vibriodes	*H. seropedicae*	*P. mirabillis*	*T. maceachernii*
C. michganensis	*L. garviease*	*P aeruginosae*	*V. paraheamolyticus*
			X. translucens

TABLE 228. Results of signatures tested with LLNL VSV target RNA at LLNL in Real-time RT- PCR. Results are recorded as the average of triplicate Ct values. Each reaction was performed in triplicate as per the manufacturer's suggested protocol; in a 25ul volume with a total of 200pg TRIZOL extracted RNA.

	VSV	
	Indiana	**New Jersey**
VSV_1798941	25.9	35.9
VSV_1798942	26.5	36.8
VSV_1798943	24.8	34.5
VSV_1798944	28.3	37.2
VSV_1798945	28.9	37.5
VSV_1798946	24.9	35
VSV_1798947	24.8	35.1
VSV_1798948	26.5	35.9
VSV_1798949	24.3	35.3
VSV_1811405	27.7	N
VSV_1811406	22.3	33.1
VSV_1811407	21.8	31.3
VSV_1811408	N	19.5
VSV_1811409	N	25.5

TABLE 229. Results of signatures screened against available VSV isolates at Plum Island in real-time RT- PCR. Results are listed as the average of triplicate Ct values. Each reaction was performed in triplicate as per the manufacturer's suggested protocol in a 25ul volume with a total of 200pg TRIZOL extracted RNA. "N" indicates no detectable PCR product after 35 cycles of PCR. **IN98NME was later determined to have no VSV RNA in the sample.**

	VSV Indiana-1				**VSV New Jersey**			
	IN94GUB	**IN97CMB**	**IN97COE**	**IN98NME**	**NJ89GAS**	**NJ95COB**	**NJ118HDB**	**NJ0406NME**
VSV_1798941	21.5	23.6	20.1	N	N	N	N	N
VSV_1798942	26.2	24.8	21.5	N	N	N	N	N
VSV_1798943	23.7	25.6	21.5	N	N	N	N	N
VSV_1798944	22.9	24	20.9	N	N	N	N	N
VSV_1798945	31.1	25.6	22.2	N	N	N	N	N
VSV_1798946	22.7	23.8	20.4	N	N	N	N	N
VSV_1798947	23.9	24.5	21.5	N	N	N	N	N
VSV_1798948	25.8	21.9	19.1	N	N	N	N	N
VSV_1798949	23	23.1	20.5	N	N	N	N	N

VSV_1811405	24.9	25.6	21.4	N	N	N	N	N
VSV_1811406	20	22.9	19	N	N	N	N	N
VSV_1811407	22.5	23.4	19.6	N	N	N	N	N
VSV_1811408	N	N	N	N	21.1	21.2	N	25.8
VSV_1811409	N	N	N	N	N	25.4	N	23.4

Note: VSV isolates Salt, Maipu, Cocal, Parana (118), Alagoas (146), and Alagoas (174) were also screened against all 14 VSV signatures at Plum Island. None of the signatures produced a PCR product with any of these isolates. This result can be understood given that there were no complete genome sequences for IN serotypes 2&3 used in our signature generation for VSV so signatures for these serotypes of IN would only be developed by chance cross reaction between regions of IN serotype 1 and 2 & 3..

Vesicular Stomatitis Virus (VSV) Limit of Detection:

The VSV isolates used in this target screening were not purified viral RNA, but rather total RNA extracted from cell culture, therefore the limit of detection (LOD), for each signature reported is not the absolute LOD. Rather, it is the relative LOD, comparing one signature with another. In order to determine the relative limit of detection for each Real-time RT- PCR signature developed, a dilution series of available targets was made [6 logs, from 1 fg to 10^5 fg per reaction]. The limit of detection is recorded as the weight of total RNA detectable. The previously described real-time RT-PCR protocol was followed in triplicate and average Ct values were reported for each dilution.

TABLE 230. Results of limit of detection screening of VSV Indiana and VSV New Jersey RNA at LLNL. In order to determine relative limits of detection for each Real-time RT- PCR signature developed, a 6 log dilution series of each target was made. The diluted targets were then tested with each signature using the standard Real-time RT- PCR protocol in triplicate and average Ct values are reported for each dilution. "N" indicates no detectable PCR product.

	VSV Indiana						VSV New Jersey					
	100pg	10pg	1pg	100fg	10fg	1fg	100pg	10pg	1pg	100fg	10fg	1fg
VSV_1798941	29.2	30.8	34.3	36.9	37.2	N	N	N	N	N	N	N
VSV_1798942	37.3	31.3	34.2	36.5	N	N	N	N	N	N	N	N
VSV_1798943	25.2	28.5	32.6	34.6	36	34.5	N	N	N	N	N	N
VSV_1798944	28.5	31.7	36	36.5	N	N	N	N	N	N	N	N
VSV_1798945	28.9	33.2	35.9	N	N	N	N	N	N	N	N	N
VSV_1798946	23	29.1	32.6	35.6	N	35	N	N	N	N	N	N
VSV_1798947	29.4	30.2	33.8	36.7	37.7	35.1	38.9	N	N	N	N	N
VSV_1798948	25.9	29.7	33.4	35.9	N	35.9	N	N	N	N	N	N
VSV_1798949	26	29.1	32.3	35.4	37.2	35.3	37.1	N	N	N	N	N
VSV_1811405	28.2	31.9	34.8	36.5	N	N	N	N	N	N	N	N
VSV_1811406	25.4	28.2	30.1	34.1	N	35.6	N	N	N	N	N	N
VSV_1811407	23	26.1	29.5	31.8	N	33.9	N	N	N	N	N	N
VSV_1811408	N	N	N	N	N	N	19.2	22.5	26.1	29.5	32.6	36.2

VSV_1811409	N	N	N	N	N	N	26.2	28.3	32	35.5	N	N

TABLE 231. Limit of detection results summary for all signatures screened against VSV Indiana and VSV New Jersey strains. The first 12 signatures listed in the table have a lower detection limit, or exclusively detect the Indiana strain, while the last two signatures, VSV_1811408 and VSV_1811409 exclusively detects the New Jersey strain. For optimal genome coverage a combination of the last two signatures and most sensitive signatures from the remaining 12 would work as a minimal set.

Signature	Target Nucleic Acid	
	VSV Indiana strain	VSV New Jersey strain
VSV_1798941	10 fg	N
VSV_1798942	100 fg	N
VSV_1798943	10 fg	N
VSV_1798944	100 fg	N
VSV_1798945	1 pg	N
VSV_1798946	100 fg	N
VSV_1798947	10 fg	100 pg
VSV_1798948	100 fg	N
VSV_1798949	10 fg	100 pg
VSV_1811405	100 fg	N
VSV_1811406	100 fg	N
VSV_1811407	100 fg	N
VSV_1811408	N	1 fg
VSV_1811409	N	100 fg

Vesicular Stomatitis Virus (VSV) TaqMan Conclusions:

TABLE 232. All 14 of the VSV signatures made the appropriate PCR product with at least one VSV species and none of the signatures were eliminated due to producing PCR product with backgrounds. Further screening is on going at Plum Island. From our preliminary data, 8 signatures were chosen to be tested in the multiplex assay format. The decision was based on the isolate the signature was designed to detect and the sensitivity it expressed in LOD screening. Below is the list of signatures moved onto MUX testing.

Signatures designed to detect VSV Indiana:	LOD against VSV Indiana
1798941	10fg
1798943	10fg
1798947	10fg
1798949	10fg
1811405	100fg
1811406	100fg

Signatures designed to detect VSV New Jersey:	LOD against VSV New Jersey
1811408	1fg
1811409	100fg

**Additional screening is in process at PIADC to re-test VSV IN-1 strains that were have thought to have degraded, to complete near-neighbor screening, and to broaden the set of VSV targets tested.

7.5. VSV MULTIPLEXED PCR SCREENING REPORT

SCREENING AND DOWN-SELECTION TECHNICAL APPROACH:

Signatures that pass gel and real-time PCR screening are candidates for multiplexed assays. If the number of candidate signatures is large than a subset of the available signatures are selected based on measured sensitivity and specificity observed in real-time screening. In preliminary screening the background of individual signatures is measured by running "blank" (no sample added) controls and observing signature response. In some cases a primer-to-primer non-specific interference will eliminate candidate signatures. Signatures are then added iteratively to each multiplexed panel, titrated, and with each signature addition, collectively, all signatures are re-titrated to determine if the presence of one signature will interfere with the performance of another. During this process signatures are added or subtracted from the multiplexed panel depending on individual outcomes until all desirable signatures have been added.

Criterion for down-selection of multiplexed signatures

Signature specificity. In initial real-time PCR screening signatures undergo target and near neighbor screening and screening against environmental confounders which assess individual signature specificity. In multiplex format this is repeated to verify that a more complex reaction system gives the same level of specificity.

Signature sensitivity. In initial real-time PCR screening signatures undergo limit of detection testing where a dilution curve is generated for each signature. This determines individual signature sensitivity. In multiplex format this is repeated to verify that a more complex reaction system gives acceptable signature sensitivity.

Optimal signal to noise ratio. In the multiplex assay system the potential for cross reactivity is larger than in single reaction testing, this may cause unwanted PCR reaction products or non-specific cross-reactivity. Signatures are preferred to have a high signal to noise ratio, where desired signal is significantly greater than the background noise of the signatures when there is no target nucleic acid in the reaction.

Compatibility with other signatures in multiplex. In some cases a primer set may interfere with other primers or probes in the multiplex and there by cause limit of detection shifting for one or more signature in the multiplex. For each scenario it must

be evaluated whether the shift in detection level is significant enough to remove (what could be a very desirable signature) from the panel.

TABLE 233. Order details for VSV signatures ordered for multiplexed signature screening and development.

ID	Modification details	Vendor
VSV_11409.BF	5'-/5Bio/CTCACAACATGGGTCCTGAA-3'	Biosearch
VSV_11409.FCP	5'-/5AmMC6/iSp18/GGCACAGCTCATCTGCGACTTCCCT-3'	Biosearch
VSV_11409.R	5'-TTCTTGCCCCGGATACATCAT -3'	Biosearch
VSV_1405.BF	5'-/5Bio/AAGAGATGGTCACGAGTGAC-3'	Biosearch
VSV_1405.FCP	5'-/5AmMC6/iSp18/TGGGTATTTGGTCATTGGTGACACA-3'	Biosearch
VSV_1405.R	5'-GAGCATTTGTGGAAACCGAGC-3'	Biosearch
VSV_1406.BF	5'-/5Bio/GCTTGCACAGTTCTACTT-3'	Biosearch
VSV_1406.FCP	5'-/5AmMC6/iSp18/CCTCCAATGGAAGGGTCCAAATACAACA-3'	Biosearch
VSV_1406.R	5'-GACAAAGACATGCCTGACAC-3'	Biosearch
VSV_1408.BF	5'-/5Bio/CTCACAACATGGGTCCTGAA-3'	Biosearch
VSV_1408.FCP	5'-/5AmMC6/iSp18/GGCATAGYTCGTCTGCRACTTCCCT-3'	Biosearch
VSV_1408.R	5'-TTCTTGACCTGGATACATCAT-3'	Biosearch
VSV_8941.BF	5'-/5Bio/AGAACCAGCGCAGATGACAA-3'	Biosearch
VSV_8941.FCP	5'-/5AmMC6/iSp18/TCAGGCATTTGTGTTCTGCCCACTCTGTATA-3'	Biosearch
VSV_8941.R	5'-CAGCCCATCCATGAGCTTTT-3'	Biosearch
VSV_8943.BF	5'-/5Bio/CGCCACAAGGCAGAGATGT-3'	Biosearch
VSV_8943.FCP	5'-/5AmMC6/iSp18/GCATACTGCATCATATCAGGAGTCGGTTTTCTG-3'	Biosearch
VSV_8943.R	5'-TGTCAAATTCTGACTTAGCATACTTGC-3'	Biosearch
VSV_8947.BF	5'-/5Bio/CCCAATCAATGCCATGATACA-3'	Biosearch
VSV_8947.FCP	5'-/5AmMC6/iSp18/TTTGAAAGTAGAACTGTGCAAGCCCGGTATC-3'	Biosearch
VSV_8947.R	5'-CTCCAATGGAAGGGTCCAAA-3'	Biosearch
VSV_8949.BF	5'-/5Bio/GGCGCTCATTATAAAATTCGGA-3'	Biosearch
VSV_8949.FCP	5'-/5AmMC6/iSp18/GAAGTCCCTGTAATGGATTCCCATTCCATGT-3'	Biosearch
VSV_8949.R	5'-ACATTTTCTCGTAGTAATGCAGCAG-3'	Biosearch

TABLE 234. Signature design specificity of the VSV signatures. Signatures are designed to detect all VSV isolates in a paired “compliment set” of signatures; requiring that at least one signature from each specificity is in the multiplexed detection panel.

Signatures designed to detect VSV Indiana:
VSV_1798941
VSV_1798943
VSV_1798947
VSV_1798949
VSV_1811405
VSV_1811406
Signatures designed to detect VSV New Jersey:

VSV_1811408
VSV_1811409

Multiplex Data Analysis -Technical approach

Disease signature thresholds

Thresholds were initially determined using all available data from known negative samples (blanks) by plotting a histogram which showed the probability of a given sample generating a particular MFI. In the absence of cross reactivity, each signature in the multiplex assay could be treated as an individual signature result. For example, for a sample spiked with RPV, data for other disease signatures could contribute towards negative data, providing that cross reaction between disease signature sets was not observed. This is a way to increase the number of samples that can be used to establish thresholds.

TABLE 235. Individual signature thresholds and ranges for VSV signatures in each specific panel. For FY06, threshold determinations have not yet been made as they require a significant number of tests (>500) to generate this information. This information will be updated when available.

Signature Name	Mux Assay name	Panel Membership	Signature background in Tris NaCl Buffer (MFI +/-)	Signature background in Clinical matrices (MFI +/-)	Threshold
VSV_1811409	VSV_1811409	Porcine	TBD	TBD	TBD
VSV_1811405	VSV_1811405	Porcine	TBD	TBD	TBD
VSV_1811408	VSV_1811408	Bovine/Porcine	TBD	TBD	TBD
VSV_1798943	VSV_1798943	Bovine/Porcine	TBD	TBD	TBD
VSV_1798947	VSV_1798947	Bovine/Porcine	TBD	TBD	TBD
VSV_1798949	VSV_1798949	Bovine/Porcine	TBD	TBD	TBD

TABLE 236. List of Targets screened at LLNL.

Virus	Species-serotype	Isolate	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	Use stock titer	Titer Method
VSV	New Jersey	N/A	NVSL	10^7 $TCID_{50}$/mL	Unknown	9/14/06	Trizol	10^7 $TCID_{50}$/mL	Reed & Muench
VSV	Indiana-1	N/A	NVSL	10^8 $TCID_{50}$/mL	Unknown	9/14/06	Trizol	10^6 $TCID_{50}$/mL	Reed & Muench

TABLE 237. List of targets screened at Plum Island.

Virus	Species-serotype	Isolate	Source	Original Titer (Log10 TCID50)	Passage History	Extraction date/ID	Extraction Method	Use stock titer	Titer Method
VSV	Indiana - 2	Salt	PIADC	Unknown	Unknown	Unknown	Qiagen Rneasy Mini Kit	Unknown	N/A

VSV	Indiana - 2	Maipu	PIADC	Unknown	Unknown	Unknown	Qiagen Rneasy Mini Kit	Unknown	N/A
VSV	Indiana - 2	Cocal	PIADC	Unknown	Unknown	Unknown	Qiagen Rneasy Mini Kit	Unknown	N/A
VSV	Indiana - 2	Parana (118)	PIADC	Unknown	Unknown	Unknown	Qiagen Rneasy Mini Kit	Unknown	N/A
VSV	Indiana - 3	Alagoas (146)	PIADC	Unknown	Unknown	Unknown	Qiagen Rneasy Mini Kit	Unknown	N/A
VSV	Indiana - 3	Alagoas (174)	PIADC	Unknown	Unknown	Unknown	Qiagen Rneasy Mini Kit	Unknown	N/A
VSV	New Jersey	NJ95COB	PIADC: Luis Rodriguez	1 x E9	Unknown	Unknown	Qiagen Rneasy Mini Kit	1 x E9	Spearman-Karber
VSV	New Jersey	NJ89GAS	PIADC: Luis Rodriguez	1 x E9.25	Unknown	Unknown	Qiagen Rneasy Mini Kit	1 x E9.25	Spearman-Karber
VSV	New Jersey	NJ0604NME	PIADC: Luis Rodriguez	1 x E7.75	Unknown	Unknown	Qiagen Rneasy Mini Kit	1 x E7.75	Spearman-Karber
VSV	New Jersey	NJ1184HDB	PIADC: Luis Rodriguez	1 x E7.5	Unknown	Unknown	Qiagen Rneasy Mini Kit	1 x E7.5	Spearman-Karber
VSV	Indiana-1	IN97COE	PIADC: Luis Rodriguez	Unknown	Unknown	Unknown	Qiagen Rneasy Mini Kit	Unknown	N/A
VSV	Indiana-1	IN98NME	PIADC: Luis Rodriguez	Unknown	Unknown	Unknown	Qiagen Rneasy Mini Kit	Unknown	N/A
VSV	Indiana-1	IN97CMB	PIADC: Luis Rodriguez	1 x E7.5	Unknown	Unknown	Qiagen Rneasy Mini Kit	1 x E7.5	Spearman-Karber
VSV	Indiana-1	IN94GUB	PIADC: Luis Rodriguez	1 x E9.59	Unknown	Unknown	Qiagen Rneasy Mini Kit	1 x E9.59	Spearman-Karber

TABLE 238. List of additional near-neighbors screened against the Bovine Panel. Most near-neighbors samples were extracted nucleic acids with unknown titers.

Virus	Strain[1]	Unique ID	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	NA conc. used or stock titer	Titer Method
Border Disease virus	Coos Bay	4-6-92	NADC, Julia Ridpath	1.5×10^8 TCID50/mL	unknown	12/14/06	Trizol	1.18×10^6 TCID50/mL	Reed & Muench
Bovine Herpes Virus-1	California	NVSL 20032 111903	CAHFS	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Cooper	NVSL 86741	CAHFS	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A

		111903							
Bovine Herpes Virus-1	Los Angeles	Los Angeles ATCC VR188	ATCC (CAHFS)	1 x 10^5 TCID50/mL	(CAHFS) MDBK2(LLNL)MDBK1	5/17/07	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	RLB-106	ATCC VR793	ATCC (CAHFS)	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	A033640072	CAHFS, R. Mock, #5 Swab, 1BDFK1, 1/28/2004, A030020072	unknown	1BDFK1,	4/23/04	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	A040130066	CAHFS, R. Mock, #4 Lung, 1BFK1, 1/28/2004, A040130066	unknown	1BFK1	4/23/04	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	Texas A030020072	CAHFS, R. Mock, #80 Swab, 1BDFK1, 1/28/2004, A030020072	unknown	1BDFK1	4/23/2004	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	California	NVSL 200032	NVSL, CAHFS	unknown	MDBK CAHFS	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Virginia	NVSL 231221	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	California	NVSL 51619	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Minnesota	NVSL 86741	NVSL, CAHFS	unknown	MDBK CAHFS	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Minnesota	NVSL 97-10720	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Cooper	NVSL-Cooper RA 309	NVSL, CAHFS	unknown	MDBK CAHFS	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes-5	DN-599	A032540006	R. Mock, from lung tissue, 2BFK2, 2/3/2004	Unknown	CAHFS Lab 2BFK2 4/30/2004	6/3/2004	Phenol/Chloroform	40 pg/uL	N/A
Bovine Herpes-5	DN-599	A040150085	R. Mock, #2 Lung, 5BFK5, 2/3/04	Unknown	CAHFS Lab 5BFK5 4/23/2004	7/7/2004	Phenol/Chloroform	40 pg/uL	N/A
Bovine Herpes-5	D9402133	D9402133	Sonoma Co. 3/94 Severe necrotizing encephalitis, 1 week old calf	Unknown	CAHFS Lab (MDBK), (1 flasks) 11/19/2003	11/19/2003	Phenol/Chloroform	40 pg/uL	N/A
Bovine Herpes Virus	BHV 5	D9403153	CAHFS	unknown	unknown	unknown	unknown	40 pg/uL	unknown
Caprine Herpes Virus		VR462	ATCC	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Caprine Herpes Virus	D0201157	D0201157	CAHFS, Isolate D0201157 History: San Joaquin Co. 2/02 Aborted fetus-third trimester	unknown	CAHFS Lab (MDBK)	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Caprine Herpes Virus	S0201998	S0201998	CAHFS, Isolate S0201998 History: San Diego Co. 2/02 Aborted fetus-	unknown	CAHFS Lab (MDBK)	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A

			third trimester						
Epizootic Hemorrhagic Disease virus-1	Santa Barbara	N/A	Bridget McLaughlin CAHFS	unknown	unknown	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-1	Georgia	N/A	Bridget McLaughlin CAHFS	unknown	unknown	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-2	Alberta	041 ODV 0401	NVSL	unknown	BHK	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-1 (SV123)	New Jersey (3TD) Deer, NJ 1955	041 ODV 0401	NVSL	1 x $10^{5.125}$ $TCID_{50}$/0.1 ml	+BHK-3 (12/7/83); (12/11/86); (12/18/86)	Unknown	Trizol	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A011120004	CAHFS, R. Mock, from brain tissue, 2BFK2, 2/3/2004, A011120004	unknown	CAHFS, 2BFK2	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A99043047	CAHFS, R. Mock, Spleen, 1BFK1, 2/3/2004, A99043047	unknown	CAHFS, 1BFK1	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A9904309	CAHFS	unknown	CAHFS, unknown	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Bovine 1247	Bovine 1247 ATCC VR2003	ATCC	unknown	CAHFS, unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	NVSL 002	NVSL 002 1/28/04	NVSL	unknown	CAHFS, unknown	1/29/03	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	LK	LK ATCC VR701	ATCC	unknown	CAHFS, unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	D990	D990	CAHFS	unknown	CAHFS, unknown	1/29/03	Phenol/ Chloroform	40 pg/uL	N/A
Feline Herpes	C-27	ATCC VR636	ATCC	unknown	CAHFS, unknown	unknown	unknown	40 pg/uL	N/A
Fowl Pox	Vaccine strain	Poxine™	Jeffers Livestock	unknown	ECE	5/17/07	Phenol/ Chloroform	40 pg/uL	N/A
Parainfluenza Virus type 3	C-243	TC-81335	LLNL	1 x 10^5 TCID50/0.1 mL	(VL)MK14(LLNL)H292-1	5/17/07	Trizol	40 pg/uL	Reed and Muench
Porcine Coronavirus	AR310	ATCC VR-2384	LLNL	1 x $10^{4.25}$ $TCID_{50}$/0.1 ml	ST2				Reed and Muench
Porcine Herpes Pseudorabies	Shope	RA 180	CAHFS	1 x 10^5 TCID50/mL		5/17/07	Trizol	40 pg/uL	N/A
Pseudorabies		92-12013	NVSL 92-12013 bovine isolate Iowa December, 1991	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies		93-11745	NVSL 93-11745 Illinois December, 1992	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies	Shope	RA180	NVSL Shope (PRV) RA 180	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies	Shope	N/A	NVSL	unknown		unknown	Phenol/		Reed &

Titered							Chloroform		Muench
Respiratory Syncytial virus	Long	N/A		unknown	(VL)KB6 L2KB2HF DL2A549-1(LLNL)H eLa3	5/17/07	Trizol	40 pg/uL	N/A
Transmissible Gastroenteritis virus of hogs	Perdue	NVSL 9801	LLNL	1 x $10^{4.75}$ $TCID_{50}$/0.1 ml	ST 1		Trizol	40 pg/ul	Reed and Muench

TABLE 239. Panel membership for signature. Four VSV signatures were included in the **Bovine-specific multiplexed assay panel** for FMDV rule-out diagnostics. The below table describes the constituents of that multiplexed assay panel in which these signatures were tested.

Bead ID	Signature/ Target Name	Mux Group ID	Virus	Source	Forward Primer	Reverse Primer	Luminex Probe (-strand, FCP)
113	emcf_94975.F, emcf_94976.R, emcf_94977.P	MCF_1	Malignant Catarrhal Fever	LLNL	ATGCCAGTCACTGGCTC TCA	GGGTGTTGTAGAATCCTGA AATGG	GTTGATCACGGTGGCACC CTGG
114	emcf_95059.F, emcf_95060.R, emcf_95061.P	MCF_2	Malignant Catarrhal Fever	LLNL	GTTCTGGAAACTGACCA AACAGTGT	AGTGGCACTTGAGTGTAAC TTTTATTG	GCACTCTGGCAGGCATAA GGGAAATACA
115	emcf_95155.F, emcf_95156R, emcf_95157.P	MCF_3	Malignant Catarrhal Fever	LLNL	CCCTGGAAGCTGTCATA CAAAA	AAACATTGGCATATCTTGC AAGGT	CAGTAGAGTCCAGGGCTG CAGTTGTCTCA
117	BVH__94666.F, BVH_94667.R, BVH_94668.P	BHV-94668	Bovine Herpes Virus	LLNL	GTGCCAGCCGCGTAAA AG	GACGACTCCGGGCTCTTTT	TCCTGGTTCCAGAGCGCT AACATGGAG
118	BVH__94738.F, BVH_94739.R, BVH94740.P	BHV-94740	Bovine Herpes Virus	LLNL	TGAGGCCTATGTATGG GCAGTT	GCGCGCCAAACATAAGTAA A	AAATAACACGGTGTGCAC TTAAATAAGATTCGCG
119	BPSV_95719.F, BPSV_95720.R, BPSV_95721.P	PPOX_1	Bovine Papular Stomatitis Virus	LLNL	GCAGATGCGCTCCTGG TT	GCACCTCTGCTGCTGCAA	CCGACTCCGACGTGGAGA ACGTG
120	BPSV_95722.F, BPSV_95723.R, BPSV_95724.P	PPOX_2	Bovine Papular Stomatitis Virus	LLNL	GATGGCCGTGCAGCTC TT	CGTACAAGATCACGGCCAA CT	TGTACGGGCTCATGGGCT TCCG
122	BPSV_95731.F, BPSV_95732.R, BPSV_95733.P	PPOX_4	Bovine Papular Stomatitis Virus	LLNL	GCAGCAGTGCACCACG TAGT	CGCTGAACCCGTACATCCT	GACTTCGAGGCGGACAAC AAGCG
132	FMDV.TC	FMDV.TC	Foot and Mouth Virus	Tetracore	ACTGGGTTTTACAAACC TGTGA	GCGAGTCCTGCCACGGA	GTCCCACGGCGTGCAAAG GA
133	FMDV.Pir	FMDV.Pir	Foot and Mouth Virus	Pirbright	CACYTYAAGRTGACAYT GRTACTGGTAC	CAGATYCCRAGTGWCICIT GTTA	CCTCGGGGTACCTGAAGG GCATCC
134	BVD_1a	BVD_SIG1	Bovine Viral Diarrhea	UCD, CAHFS lab	GGTAGTCGTCAGTGGTT CGAC	CATGTGCCATGTACAGCAG AGAT	CCTCGTCCACGTGGCATC TCGAG
138	BVD_1821165.F, BVD_1821164.R, BVD_1821166.P	BVD_SIG2	Bovine Viral Diarrhea	LLNL	GGGAGTCGTCAATGGT TCGAC	TCCATGTGCCATGTACAGC AGAG	CCTCGTCCACITGGCATCT CGAG
135	BTV_1759932	BTV_1759932	Bluetongue Virus	LLNL	GCACCCTATATGTTTCC AGACCA	CAGCTAACTCTTCAGCCAC ACG	CTAACTCGTGGGCCAATC ATCATCTTCTGT
136	BTV10_1810207	BTV10_1810207	Bluetongue Virus	LLNL	CAAACACAAAAGGCGG AGAAG	GGCGTTTAATCTGTCTTAG TCTTACGT	GAAACGCTTCTGCGTACG ATGCGA
137	BTV10_1810199	BTV10_1810199	Bluetongue Virus	LLNL	CACATGTCGCTTAATTT GTCTTAACC	GCGGAGAAGGCTGCATT	ACGAAACGCTTCCGCGTA CGATG
139	BTV10_1810205	BTV10_1810205	Bluetongue Virus	LLNL	TCAATTTTGGTAGAATTT GTTCATTCA	GCGGAGAAGGCTGCATTC	ACGAAACGCTTCCGCGTA CGATG
163	VSV_1798943	VSV_1798943	Vesicular Stomatitis Virus -Indiana	LLNL	CGCCACAAGGCAGAGA TGT	TGTCAAATTCTGACTTAGC ATACTTGC	GCATACTGCATCATATCA GGAGTCGGTTTTCTG
166	VSV_1798947	VSV_1798947	Vesicular Stomatitis Virus -Indiana	LLNL	CCCAATCAATGCCATGA TACA	CTCCAATGGAAGGGTCCAA A	TTTGAAAGTAGAACTGTG CAAGCCCGGTATC
167	VSV_1798949	VSV_1798949	Vesicular Stomatitis Virus -Indiana	LLNL	GGCGCTCATTATAAAAT TCGGA	ACATTTTCTCGTAGTAATG CAGCAG	GAAGTCCCTGTAATGGAT TCCCATTCCATGT
169	VSV_1811408	VSV_1811408	Vesicular Stomatitis Virus –New Jersey	PIADC	CTCACAACATGGGTCCT GAA	TTCTTGACCTGGATACATC AT	GGCATAGYTCGTCTGCRA CTTCCCT
160	RPV_1814853	RPV_1814853	Rinderpest Virus	LLNL	GGATCGCTGAAATGATC TGTGA	GGAGCCAGTTCACCCATTT G	CTGGCCAACCCTGCCTCC ACTATGTA
161	RPV_1814855	RPV_1814855	Rinderpest Virus	LLNL	TGCATCTTATGTGACTT TGGTTCA	GGCTATCCGCACAGCTGA C	CAGTCCTCTCATCTGTTGT CGATCCGATGTA
162	RPV_1814856	RPV_1814856	Rinderpest Virus	LLNL	AACTCCTGACCTCATTC CTTGC	GGCTCTATAATCCCACTAT GCCA	TGGCTCAGTGCATTCACA AAGACCTTGAATA
170	N/A	NC (MT-7)	Maritima	LLNL	NT	NT	CAAAGTGGGAGACGTCGT TG
171	N/A	b-MT7 (FC)	N/A	LLNL	NT	NT	CAAAGTGGGAGACGTCGT TG

172	N/A	MT7/Cy3 (IC)	N/A	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
173	Alien RNA	arRNA Control	None	Ambion	GACATCAAGGCTCAAACTAATTTTACC	CAAAGGCTGCCAACATAAAATG	CAAGCGTAAATGCAGCGTCCA

[1]FCP indicates that the probe is on the negative strand (Forward Compliment to the Probe).

TABLE 240. Panel membership for signature. Six VSV signatures were included in the **Porcine-specific multiplexed assay panel** for FMDV rule-out diagnostics. The below table describes the constituents of that multiplexed assay panel in which these signatures were tested. Some VSV signatures are found in both the Bovine and Porcine Panels and some are found exclusively in the Porcine panel.

Bead ID	Signature/ Target Name	Mux Group ID	Virus	Source	Forward Primer	Reverse Primer	Luminex Probe (-strand, FCP)
132	FMDV.TC	FMDV.TC	Foot and Mouth Virus	Tetracore	ACTGGGTTTTACAAACCTGTGA	GCGAGTCCTGCCACGGA	GTCCCACGGCGTGCAAAGGA
133	FMDV.Pir	FMDV.Pir	Foot and Mouth Virus	Pirbright	CACYTYAAGRTGACAYTGRTACTGGTAC	CAGATYCCRAGTGWCICITGTTA	CCTCGGGGTACCTGAAGGGCATCC
142	PRRS_1807709	PRRS__1807709	Porcine Reproductive and Respiratory Syndrome	LLNL	GAGCGGCAATTGTGTCTGTC	GCTGAGGGTGATGCTGTGAC	CGCACAGTATGATGCGTAGGCAAACTAAACTC
144	PRRS_1810351	PRRS_1810351	Porcine Reproductive and Respiratory Syndrome	LLNL	TTCTTGTGACCACGATTCGC	GACCCACCGAGTAACTTGCC	GCTCAAGAGCCAAAAGCTCAGCATGACA
145	PRRS_1807706	PRRS_1807706	Porcine Reproductive and Respiratory Syndrome	LLNL	ATTGGTTTGCTCCGCGATAC	AAATGAGCCACCACATCCAA	CGGTACATTCGACGCGACACCATTTC
148	PRRS_1810383	PRRS_1810383	Porcine Reproductive and Respiratory Syndrome	LLNL	CAGTGTGCACGCTTCCATTT	CTCGAATGATGTGTTGCCGT	AAACATAGCGTAGAGCTGGAATTCGAAGCCA
149	PRRS_1810386	PRRS_1810386	Porcine Reproductive and Respiratory Syndrome	LLNL	GCTTTCTGCGTGCCTTTTCT	ACAACGCCAGAGACATTCCC	TGACTTTGAAGCCTTTCTCGCTCATTTCTGA
150	SVD_1727049	SVD_1	Swine Vesicular Disease	LLNL	CAGGATAATTTCTTCCAAGGGC	ACGTGAACATTTCGAGCTTCC	TGCATTGTGTCTGATGGTACAACTTGTGACG
151	SVD_1727050	SVD_2	Swine Vesicular Disease	LLNL	GACTTGTTGTGGCTGGAGGA	CAGCGCCATGGTGAGGTAG	TGACCGTAATGAGGTCATCGTGATTTCTCAC
152	SVD_1727051	SVD_3	Swine Vesicular Disease	LLNL	GACAAAGTGGCCAAGGGAAA	CACGTAAACCACACTGGGCT	CTGGCGTCATAGCCTGAATAGTCAAACGCTA
154	VESV_95653.F, VESV_95654.R, VESV_95655.P	VESV_1	Vesicular Exanthema of Swine Virus	LLNL	GCCTTCTCCCTTCCCAAAA	TGAAGGAATGGTTCCGTCAGT	CATCATCGTTGATAACCTTAGATGTGCAATTTGG
157	VESV_95686.F, VESV_95687.R, VESV_95688.P	VESV_4	Vesicular Exanthema of Swine Virus	LLNL	GGTCGCTCTCACTGATGATGAGTA	GGTGTTATCAGCACCCATTGC	GCTCGGTGCCTGAGTTGGAGGAAG
158	VESV_95692.F, VESV_95693.R, VESV_95694.P	VESV_5	Vesicular Exanthema of Swine Virus	LLNL	ACCACCTCTGGAAACATCTATGG	TTTGTGCACGTGTCACGAAT	CGGGACGGGCATTTGTCACCA
163	VSV_1798943	VSV_1798943	Vesicular Stomatitis Virus -Indiana	LLNL	CGCCACAAGGCAGAGATGT	TGTCAAATTCTGACTTAGCATACTTGC	GCATACTGCATCATATCAGGAGTCGGTTTTCTG
164	VSV_1811409	VSV_1811409	Vesicular Stomatitis Virus –New Jersey	PIADC	CTCACAACATGGGTCCTGAA	TTCTTGCCCCGGATACATCAT	GGCACAGCTCATCTGCGACTTCCCT
166	VSV_1798947	VSV_1798947	Vesicular Stomatitis Virus -Indiana	LLNL	CCCAATCAATGCCATGATACA	CTCCAATGGAAGGGTCCAAA	TTTGAAAGTAGAACTGTGCAAGCCCGGTATC
167	VSV_1798949	VSV_1798949	Vesicular Stomatitis Virus -Indiana	LLNL	GGCGCTCATTATAAAATTCGGA	ACATTTTCTCGTAGTAATGCAGCAG	GAAGTCCCTGTAATGGATTCCCATTCCATGT
168	VSV_1811405	VSV_1811405	Vesicular Stomatitis Virus -Indiana	PIADC	AAGAGATGGTCACGAGTGAC	GAGCATTTGTGGAAACCGAGC	TGGGTATTTGGTCATTGGTGACACA
169	VSV_1811408	VSV_1811408	Vesicular Stomatitis Virus –New	PIADC	CTCACAACATGGGTCCTGAA	TTCTTGACCTGGATACATCAT	GGCATAGYTCGTCTGCRACTTCCCT

			Jersey				
170	N/A	NC (MT-7)	Maritima	LLNL	NT	NT	CAAAGTGGGAGACGTCG TTG
171	N/A	b-MT7 (FC)	N/A	LLNL	NT	NT	CAAAGTGGGAGACGTCG TTG
172	N/A	MT7/Cy3 (IC)	N/A	LLNL	NT	NT	CAAAGTGGGAGACGTCG TTG
173	Alien RNA	arRNA Control	None	Ambion	GACATCAAGGCTCAAACT AATTTTACC	CAAAGGCTGCCAACATAAA ATG	CAAGCGTAAATGCAGCG TCCA

7.5.1. BOVINE PANEL MULTIPLEXED PCR DATA

Preliminary screening: Preliminary screening can be broken down into several steps. The signatures are preliminarily screened by first assessing how the signatures will perform under multiplex PCR conditions and whether or not the addition of the new signatures will adversely effect other signatures, these preliminary screening methods are described as "signature baseline screening" and "multiplex addition screening" respectively. First signature baseline screening tests the performance of the signature in a no-target reaction (blank), a "passing" score is indicated by a low (typically less than 100) MFI. Two VSV signatures failed the signature baseline screening. Next in multiplex addition screening the new signature primers are added one-by –one to the other panel signatures to determine if there would be any cross-reactions between primer pairs in the panel. Further assessment of the signatures is done through target and near-neighbor screening and the determination of relative sensitivities. The signature down-selection for the VSV signature is further described in Table 26 below

TABLE 241. Preliminary screening of VSV signatures by adding step-wise into the **Bovine panel**. Data is reported in Median Fluorescent Intensity (MFI) units. All samples below were non template controls (NTCs) using different primer mixes by step-wise adding a new primer set to each run. VSV1811409 showed an elevated **background** throughout. The addition of VSV-1811405 caused low-level cross-reactions with signatures BVD-Sig1 and BVD-Sig2 and moderate cross-reactions with BTV-9932 (data not shown).

Description	VSV-1408 (46)	VSV-1409 (15)	VSV-8943 (65)	VSV-8949 (38)	VSV-8947 (66)	VSV-1405 (43)
Mix 1:Bovine Panel 18 Plex	37	226	38	13	9	10
Mix 1:Bovine Panel 18 Plex	39	249	32	12	8	9
Mix 1:Bovine Panel 18 Plex	38	247	37	11	8	8
Mix 1:Bovine Panel 18 Plex	32	203	24	9	6	7
Mix 2: Mix 1 + VSV-1408	28.5	188	40	9	9	14
Mix 2: Mix 1 + VSV-1408	34	197	49	12	12	16
Mix 2: Mix 1 + VSV-1408	28	181	41	8	9	12
Mix 2: Mix 1 + VSV-1408	37	210	64	11	13	18
Mix 3: Mix 2 + VSV-1409	42	259	67.5	12	21.5	18
Mix 3: Mix 2 + VSV-1409	36	206	73	13	19	21
Mix 3: Mix 2 + VSV-1409	26	185	54	8	10	15
Mix 3: Mix 2 + VSV-1409	28	176	58	9	12	18

Mix 4: Mix 3 + VSV-8943	30	196	58	8	10	21
Mix 4: Mix 3 + VSV-8943	29	170	63	8	12	23
Mix 4: Mix 3 + VSV-8943	24	164	49	8	9	20
Mix 4: Mix 3 + VSV-8943	33.5	192	64	11	19	22
Blank Mix 5: Mix 4 + VSV-8949	36.5	206	65	12	21.5	25
Blank Mix 5: Mix 4 + VSV-8949	42	233	73	11	18	28
Blank Mix 5: Mix 4 + VSV-8949	34	189	63	11	17	26
Blank Mix 5: Mix 4 + VSV-8949	33	201	56	10	14	24
Blank Mix 6: Mix 5 + VSV-8947	27	166.5	51	8	9	29
Blank Mix 6: Mix 5 + VSV-8947	34	195.5	59.5	10	20	33
Blank Mix 6: Mix 5 + VSV-8947	33	190	61	11	20	33.5
Blank Mix 6: Mix 5 + VSV-8947	30	185	45	9	13	28
Blank Mix 7: Mix 6 + VSV-1405	32	196	48	10	17	29
Blank Mix 7: Mix 6 + VSV-1405	28	201	47	8	13	30
Blank Mix 7: Mix 6 + VSV-1405	24	171	36.5	7	9	22
Blank Mix 7: Mix 6 + VSV-1405	23	157.5	44	8	10	23

TABLE 242. Multiplexed assay down-selection summary for VSV in the **Bovine Panel**. Signature baseline screening indicates the performance of the signature in a no-sample reaction (blank), a "passing" score is indicated by a low MFI. All signatures passed the signature baseline screening. In the multiplex addition screening the primers are added one-by –one to the other panel constituents to determine if there would be any cross-reactions between primer pairs in the panel. VSV-1798941, VSV-1811405, VSV-1811406, VSV-1811406 failed in multiplexed screening as described below and thus were not carried forth in the Bovine panel.

Signature	Mux Screening: Assay Down Selection					
	Signature baseline screening	**Multiplex addition screening**	**Target screening**	**Near-neighbor screening**	**Limit of detection screening**	**Noted cross-reactions**
VSV_1798941	Fail: Based on testing in the Porcine this signature not tested here	No further testing	No further testing	No further testing	No further testing	None
VSV_1798943	Pass	Pass	Pass	Pass	Pass	Pass
VSV_1798947	Pass	Pass	Pass	Pass	Pass	Pass
VSV_1798949	Pass	Pass	Pass	Pass	Pass	Pass

VSV_1811405	Pass	Fail (4-25-07): Crossreacts with BVD-1a,2 and BTV_1759932	No further testing	No further testing	No further testing	VSV_1811405 primers crossreact with BVD1a,2 and BTV_1759932 probes
VSV_1811406	Fail: Based on testing in the Porcine this signature not tested here	No further testing	No further testing	No further testing	No further testing	None
VSV_1811408	Pass	Pass	Pass	Pass	Pass	Pass
VSV_1811409	Pass	Fail (5-1-07): Crossreacts with BVD-1a,2	No further testing	No further testing	No further testing	VSV_1811409 probe crossreacts with BVD-1a and 2 primers

Near neighbor and Target screening: Four VSV signatures were added to the Bovine panel. The signatures exhibited a reasonably low background response (<50 MFI) in the Bovine panel. Target screening for VSV was conducted at Plum Island Animal Disease Center. Preliminary (non-target or nucleic acid only) screening was conducted at LLNL.

TABLE 243. Backgrounds screening in multiplexed format for VSV at LLNL and PIADC. Additional target screening is pending at PIADC, Aug 2007.

	Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors	Targets
Mux PCR Screening	54	135	16	0	48	2(7pending)

[1]-There are 752 pooled samples in each Aerosol Block.

TABLE 244. Bovine panel backgrounds screening in **multiplexed** format for down-selected VSV signatures against the below listed **eukaryotes**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. VSV-1811408 cross-reacted moderately with several of the eukaryotes (cat, monkey and tick). None of the other 3 signatures cross-reacted with any of the samples listed below.

Description	VSV-8943 (63)	VSV-8947 (66)	VSV-8949 (67)	VSV-1408 (69)
BOVINE	16	10	4	42
CAT	14	9	4	154
CHICKEN	14	8	4	55
DOG	12	7	3	35
DROSOPHILA MELANOGASTER	13	8	13	17

EQUINE	13	7	3	60
FLEA	11	7	3	26
HUMAN	8	6	3	23
MONKEY	13	9	10	325
MOSQUITO	12	8	3	18
MOUSE	13	8	11	51
PIG / PORCINE	14	9	3	25
RABBIT	14	9	4	17
RAT	10	6	3	15
SHEEP	9	6	3	37
TICK	10	7	3	111

TABLE 245. Bovine panel backgrounds screening in **multiplexed** format for the four VSV signatures against the below listed **prokaryotes**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. None of the signatures cross-reacted with any of the samples listed below.

Description	VSV-8943 (63)	VSV-8947 (66)	VSV-8949 (67)	VSV-1408 (69)
Erwinia amylovora	23	12	5	25
Actinobacillus suis	21	12	4	23
Aneurinbacillus migulanus	20	10	5	21
Bacillus cereus	29	11	5	24
Bacillus globigii	28	11	5	26
Bacillus subtilis	20	10	5	19
Bacillus thuringiensis	29	12	5	27
Bifidobacterium denticum	21	10	5	22
Borrelia burgdorferi	31	12	5	24
Burkholderia capacia	29	12	5	23
Caulobacter vibriodes	17	10	5	19
Clavibacter michganensis	19	11	5	19
Clostridium butyricum	24	12	5	24
Corynebacterium pseudodipthericum	25	12	5	22
Cytophaga marinoflava	25	11	5	19
Erwinia herbicola	24	11	5	23
Escherichia coli	29	13	6	27
Geobacillus caldoxylosilyticus	22	11	5	21
Halomonas halmophila	19	10	5	23
Haemophilus influenza	23	11	5	33
Herbaspirillium seropedicae	31	11	13	22
Lactobacillus garvieae	17	9	4	20
Lactobacillus gasseri	21	11	5	22
Listeria monocytogenes	22	10	5	21
Listeria seeligeri	24	11	5	24
Micrococcus luteus	22	11	4	23
Moraxella lacunatica	23	12	5	28
Oceanospirillium ssp. Maris	25	13	5	22
Paenibacillus napthalaenovorans	21	12	5	22
Paracoccus dentrificans	23	11	6	23
Porphyrobacter sanguineus	21	10	3	21
Proteus mirabillis	21	10	4	20

Pseudomonas aeruginosae	24	10	5	22
Pseudomonas oleovorans	18	9	5	17
Rhizobium leguminosarum	26	12	4	22
Rhodococcus rhodochrous	16	9	5	18
Salmonella typhimurium	24	12	5	25
Simonsiella muelleri	20	10	5	22
Sphingomonas sp. (Alcaligenes sp)	20	11	5	20
Staphylococcus aureus	24	11	6	22
Streptococcus pneumoniae	24	12	5	23
Streptomyces scabiei	18	10	5	20
Tatlockia maceachernii	26	14	4	23
Vibrio paraheamolyticus	19	11	5	20
Xanthomonas translucens	22	11	5	20

TABLE 246. Bovine panel backgrounds screening in **multiplexed** format for the four VSV signatures against the below listed **soils**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. None of the signatures cross-reacted with any of the samples listed below.

Description	VSV-8943 (63)	VSV-8947 (66)	VSV-8949 (67)	VSV-1408 (69)
D 000107-49	34	16	7	27
D 000109 # 50	33	15	7	25
D 000402 # 53	34	16	7	28
D 000500 - 26 - 1	36	16	7	25
D 000501-14-1	30	15	7	23
D 000505 - 11 - 4	34	16	7	29
D 000521 - 23	35	18	8	24
D 000527 - 3	32	16	7	27
D 000531 - 21	30	15	8	24
D 000533 - 17 -1	35	16	8	21
D 000542 - 6	29	15	9	28
D 000550 - 20	28	15	8	30
D 000551 - 5	34	16	8	25
D 000561 - 8 - 6	29	15	8	23
D 000562 - 30 - 5	30	16	8	23
S 251	31	16	8	23
S 252	26	15	7	22
S 253	25	14	8	22
S 254	28	16	8	23
S 255	27	15	7	21
S 256	27	16	7	22
S 257	32	15	8	20
S 259	32	14	7	19
S 260	27	15	8	20
S 271	30	15	7	18
S 272	26	16	8	21
S 273	24	15	8	24

S 274	26	15	7	23
S 275	26	15	7	20
S 276	28	16	7	21
S 277	26	16	8	24
S 279	23	13	7	22
S 280	24	15	8	21
S 282	18	12	7	19
S 283	26	14	8	21
S 284	26	15	7	22
S 286	25	16	8	28
S 287	14	9	6	13
S 288	24	15	7	23
S 289	26	15	8	20
S 290	21	14	7	20
S 291	23	14	7	18
S 292	26	15	7	26
S 295	26	15	7	20
S 296	24	15	8	22
S 297	21	13	7	21
S 298	27	15	8	20
S 299	25	13	7	17
S 300	30	11	4	16
S 301	30	11	4	20
S 303	30	11	3	17
S 304	30	13	4	18
S 305	29	12	4	19
S 307	30	11	4	16

TABLE 247. Bovine Panel **Near-Neighbor** screening (Data from 20070601) against the 4 VSV signatures. Values shown are Median Flourescence Intensity (MFI) units. “Blanks” are buffer-only samples (no nucleic acid added to reaction), shown below in red. Extracted nucleic acid was added to each PCR reaction in triplicate totaling 200pg/rxn. The data shows that the VSV signatures did not cross- reacted with any listed near-neighbors of the Bovine panel constituents.

Description	VSV-8943 (63)			VSV-8947 (66)			VSV-8949 (67)			VSV-1408 (69)		
Replicate	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Blank	31	26	28	16	13	16	4	4	4	44	36	41
Blank	30	28	23	13	14	11	4	4	4	40	37	34
BHV A040150085	40	21	42	19	9	17	4	3	4	60	29	51
BHV (BFK)	34	29	30	15	15	11	4	4	4	45	40	44
BHV-1 A040130066	42	27	28	18	13	12	4	3	3	50	39	39
BHV-1 A033640072	40	26	28	16	12	12	4	3	3	50	38	38
BHV-1 ATCC VR 793	39	28	29	16	12	12	3	3	4	71	37	42
IBR CA 111903	41	7	34	18	4	15	4	3	4	45	16	36
IBR MN 111903	47	26	33	12	11	14	3	4	4	27	37	41
BHV-1 NVSL 231221	30	37	28	12	18	13	4	4	4	41	49	39
BHV-1 RA309	32	29	26	14	12	12	4	5	5	37	32	37

BHV-1 NVSL 97-10720	31	26	37	14	11	18	4	4	3	40	38	47
BHV-1 NVSL 51619	41	28	37	15	12	15	3	4	3	41	31	42
BHV-1 NVSL 86741	31	30	30	14	14	13	4	4	3	40	37	43
BHV-1 NVSL 200032	32	32	30	14	14	13	4	4	3	46	34	38
BHV-1 LA ATCC VR188	41	30	27	15	14	12	4	4	3	46	41	37
BHV-1 (IBR) Texas CAHFS A030020072	34	37	24	15	18	12	3	3	4	43	45	37
EHV-1 ATCC VR2003	29	28	30	14	14	13	5	4	4	49	48	47
EHV-1 A9904309	26	35	28	14	8	15	4	3	3	38	33	54
EHV-1 A011120004 CAHFS	34	24	23	15	12	11	4	3	3	72	38	34
EHV-1 NVSL 00002	32	29	24	14	13	12	4	4	4	48	43	41
EHV-2 ATCC VR701	29	31	23	15	15	12	4	4	4	58	43	35
EHV-1 A99043047 CAHFS	29	26	19	14	13	10	4	4	3	44	33	29
EHV-2 D990 CAHFS	31	28	25	16	15	11	4	4	3	79	41	50
Pseudorabies Titered	33	27	27	15	13	11	4	4	3	54	37	38
Pseudorabies NVSL 93-11745	33	24	26	15	13	13	4	4	4	58	31	38
Pseudorabies NVSL 92-12013	36	25	23	15	14	11	4	3	3	42	35	31
Pseudorabies RA180 CAHFS	32	24	23	15	13	11	4	3	4	42	34	34
Porcine Herpes Pseudorabies Shope	29	25	23	13	14	11	4	4	3	36	38	31
Feline Herpes ATCC VR636	28	25	22	13	14	11	4	4	4	37	33	33
Caprine Herpes ATCC VR462	28	22	22	13	14	11	4	3	4	39	34	36
Caprine Herpes S0201998 CAHFS	28	31	23	14	16	12	3	4	3	40	40	37
Caprine Herpes D0201157 CAHFS	34	28	25	16	16	12	4	3	4	47	46	33
BHV-5 A040150085 CAHFS	31	29	21	16	18	11	4	4	3	60	62	35
BHV-5 A032540006 CAHFS	26	24	27	12	14	15	4	4	3	35	33	35
BHV-5 D9403153 CAHFS	31	26	21	15	15	11	4	4	4	40	36	31
BHV-5 D9402133 CAHFS	26	27	21	14	14	11	3	5	4	46	33	28
BDV Coos Bay	31	26	20	14	15	11	4	3	4	35	35	29
EHD-1 Georgia	35	26	24	17	14	15	4	4	3	41	37	37
EHD-1 New Jersey	33	26	19	16	15	12	4	4	3	46	35	35
EHD-1 Santa Barbara	31	58	18	14	11	10	4	4	3	66	24	27
EHD-2 Alberta	30	36	19	14	15	10	4	4	4	27	43	30
Fowl Pox	34	37	21	16	14	12	4	4	3	43	46	32
Parainfluenza Type 3	29	36	21	13	13	11	3	3	4	42	50	28
Respiratory Syncytial	30	32	22	14	11	11	4	3	3	40	45	31

Multiplexed PCR Assay Titrations (Bovine panel)

Upon finalization of the assay panel constituents each agent assay is characterized in multiplexed PCR format by running dose-response (titration) curves against titered virus extracted from virus infected cell culture for each available agent strain. Each sample is tested in duplicate and reported as a mean value of the median fluorescence intensity units (MFI) against agent concentration (per reaction volume, 5uL) on a double log plot. Concentration units are reported in TCID50/rxn (assumes 5uL sample volume per reaction) or pfu/rxn depending on virus type and source. In some cases where titered virus was not available data is reported as picogram units (pg). Each assay is evaluated by its ability to detect agent strains and differentiate from near neighbors (specificity) and by its range of sensitivity to each strain relative to each signature tested for that agent. Because this method is not aimed at determination of analytical

sensitivities (requiring a more extensive sample set tested in clinical sample matrices which is not practical for this stage of development) it does however, provide tentative limits of detection that are used to measure the performance of each assay.

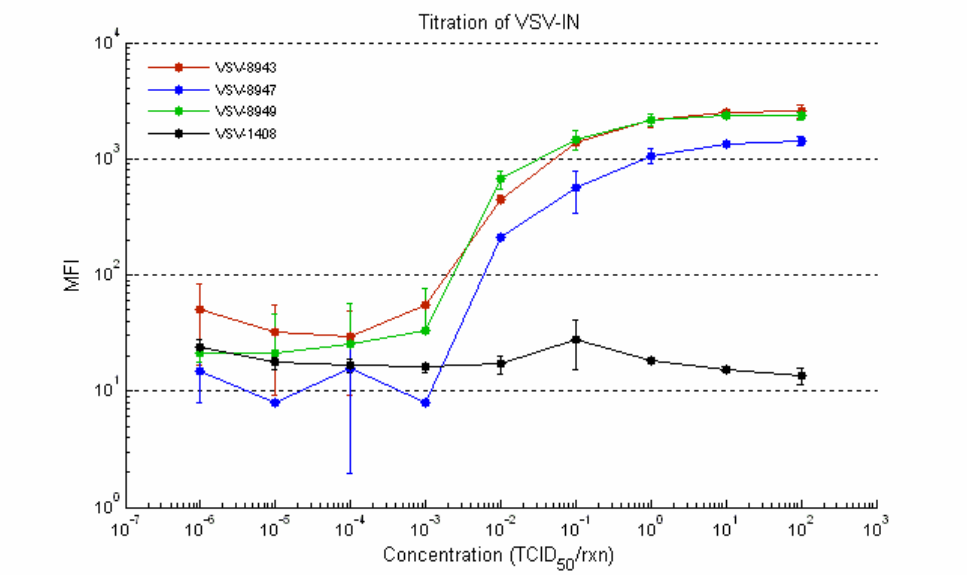


FIG. 30. Bovine multiplex screening data for the four VSV signatures against extracted nucleic acids from isolate VSV-IN. Signature VSV-1408 has design specificity for the New Jersey Strain, so it is not expected to react with the Indiana strain as shown here. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1 of the mean.

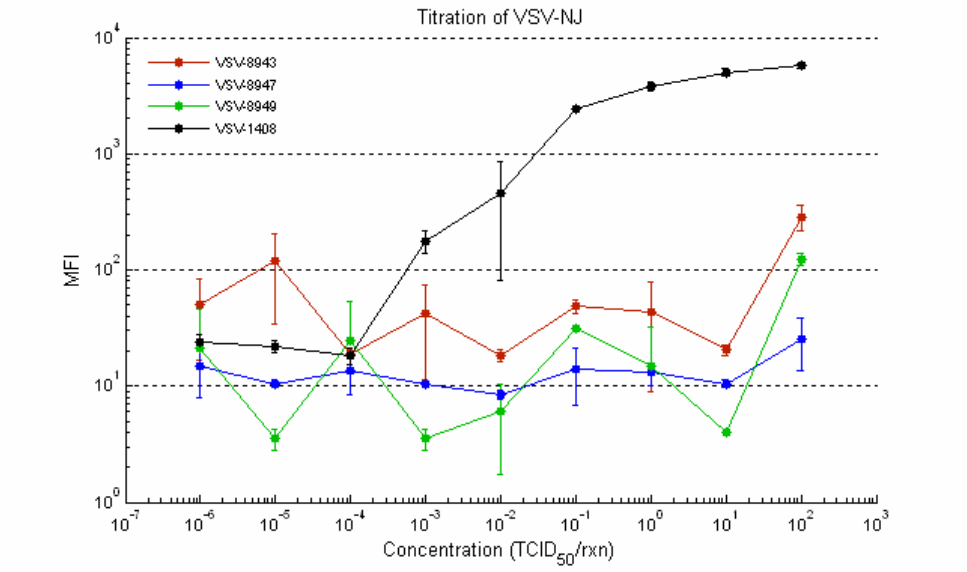


FIG. 31. Bovine multiplex screening data for the four VSV signatures against extracted nucleic acids from isolate VSV-NJ. Signature VSV-1408 has design specificity for the New Jersey Strain, so it is the only signature of the four that is expected to react with the New Jersey strain as shown here. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1 of the mean.

RESULTS: These four signatures have been currently added the Bovine panel and tested against two species of VSV. Additional screening is currently underway at PIADC to test against 7 additional targets of VSV and near neighbors that were not available to test at LLNL. Based on preliminary characterization data, the four VSV signatures selected for the Bovine panel should consist of a complimentary set of signatures for detection of VSV Indiana and New Jersey.

7.5.2. PORCINE PANEL MULTIPLEXED PCR DATA

Preliminary screening: Preliminary screening can be broken down into several steps. The signatures are preliminarily screened by first assessing how the signatures will perform under multiplex PCR conditions and whether or not the addition of the new signatures will adversely effect other signatures, these preliminary screening methods are described as “signature baseline screening” and “multiplex addition screening” respectively. First signature baseline screening tests the performance of the signature in a no-target reaction (blank), a “passing” score is indicated by a low (typically less than 100) MFI. Two VSV signatures failed the signature baseline screening. Next in multiplex addition screening the new signature primers are added one-by –one to the other panel signatures to determine if there would be any cross-reactions between primer pairs in the panel. Further assessment of the signatures is done through target and near-neighbor screening and the determination of relative sensitivities. The signature down-selection for the VSV signature is further described in the table below.

TABLE 248. Preliminary screening of VSV signatures by adding step-wise into the **Porcine panel.** Strong **cross-reactions** observed with VSV-8947 (caused by the addition of VSV-1406), and VSV-1409, VSV-1406 (caused by an unknown panel constituent.

Description	VSV_1798947 (66)	VSV_1811409 (15)	VSV_1811406 (69)	VSV_1811408 (14)	VSV_1811405 (35)	VSV_1798949 (38)	VSV_1798941 (64)	VSV_1798943 (65)
Blank Mix 2 + VSV 1798947 (mix 3)	7	119	90	10	23	5	13	26
Blank Mix 2 + VSV 1798947 (mix 3)	11	121	95	10	22	6	12	25
Blank Mix 2 + VSV 1798947 (mix 3)	6	126	86	11	24	5	12	27
Blank Mix 2 + VSV 1798947 (mix 3)	10	107	89	8	21	5	11	24
Blank Mix 3 + VSV 1811409 (mix 4)	24	83	47	8	18	5	9	20

Blank Mix 3 + VSV 1811409 (mix 4)	14	125	66	10	24	5	11	30
Blank Mix 3 + VSV 1811409 (mix 4)	16	99	70	10	20	6	10	26
Blank Mix 3 + VSV 1811409 (mix 4)	16	129	93	12	24	6	14	31
Blank Mix 4 + VSV 1811406 (mix 5)	7200	95	84	10	22	8	10	25
Blank Mix 4 + VSV 1811406 (mix 5)	8177	117	84	11	23	8	12	27
Blank Mix 4 + VSV 1811406 (mix 5)	7791	117	110	11	24	10	13	26
Blank Mix 4 + VSV 1811406 (mix 5)	8341	112	113	11	23	10	12	27
Blank Mix 5 + VSV 1811408 (mix 6)	8203	108	82	12	22	10	12	34
Blank Mix 5 + VSV 1811408 (mix 6)	8089	116	93	11	23	9	12	33
Blank Mix 5 + VSV 1811408 (mix 6)	8265	106	94	11	20	10	13	28
Blank Mix 5 + VSV 1811408 (mix 6)	8210	107	89	11	21	10	12	30
Blank Mix 6 + VSV 1811405 (mix 7)	7171	100	68	11	18	8	10	26
Blank Mix 6 + VSV 1811405 (mix 7)	6799	106	71	11	18	9	11	29
Blank Mix 6 + VSV 1811405 (mix 7)	6992	117	69	12	20	10	12	28
Blank Mix 6 + VSV 1811405 (mix 7)	6673	100	56	10	19	9	12	26
Blank Mix 7 + VSV 1798949 (mix 8)	6452	100	72	11	21	8	11	24
Blank Mix 7 + VSV 1798949 (mix 8)	6264	91	73	10	17	10	12	23
Blank Mix 7 + VSV 1798949 (mix 8)	6998	96	70	10	16	10	11	23
Blank Mix 7 + VSV 1798949 (mix 8)	8697	109	77	12	18	10	12	24
Blank Mix 8 + VSV 1798941 (mix 9)	7267	**1470**	76	22	16	9	14	25
Blank Mix 8 + VSV 1798941 (mix 9)	7169	**1460**	70	22	16	8	15	24
Blank Mix 8 + VSV 1798941 (mix 9)	7099	**1323**	61	21	16	8	13	21
Blank Mix 8 + VSV 1798941 (mix 9)	6717	**1428**	74	22	16	9	15	22
Blank Mix 9 + VSV 1798943 (mix 10)	7654	**1380**	61	22	17	11	16	22
Blank Mix 9 + VSV 1798943 (mix 10)	8099	**1475**	66	22	17	9	14	24
Blank Mix 9 + VSV 1798943 (mix 10)	7278	**1355**	55	22	15	9	15	20
Blank Mix 9 + VSV	8053	**1435**	72	22	17	9	15	22

1798943 (mix 10)								

TABLE 249. Additive and subtractive approach in determining the source of cross reaction with VSV-1409 and suspected VESV panel constituents. The addition of VESV-3 significantly increased the background MFI of the VSV-1409 signatures, with no additive gain per subsequent VESV signatures; therefore VESV-3 was determined to be the causative factor and was removed from the Porcine Panel.

Description	VSV_1811405 (43)	VSV_1811408 (46)	VSV_1811409 (15)	VSV_1798943 (65)	VSV_1798947 (66)	VSV_1798949 (38)
blank Porcine no VESV (mix 1)	38	8	48	45	16	3
blank Porcine no VESV (mix 1)	45	9	46	56	22	3
blank Porcine no VESV (mix 1)	50	10	50	54	20	3
blank Porcine no VESV (mix 1)	48	10	44	47	21	3
blank mix 1 + VESV_3	44	11	259	41	22	3
blank mix 1 + VESV_3	39	11	235	38	21	3
blank mix 1 + VESV_3	48	12	242	42	23	4
blank mix 1 + VESV_3	41	13	230	42	25	3
blank mix 1 + VESV_3 + 5	32	11	172	30	17	3
blank mix 1 + VESV_3 + 5	39	12	213	36	19	3
blank mix 1 + VESV_3 + 5	39	13	218	36	23	4
blank mix 1 + VESV_3 + 5	43	12	214	35	23	3
blank mix 1 + VESV_3 + 5 + 1	37	11	198	36	18	4
blank mix 1 + VESV_3 + 5 + 1	41	13	194	33	20	4
blank mix 1 + VESV_3 + 5 + 1	37	11	193	30	19	3
blank mix 1 + VESV_3 + 5 + 1	29	11	168	27	18	4
blank mix 1 + VESV_3 + 5 + 1 + 4	24	11	170	33	18	4
blank mix 1 + VESV_3 + 5 + 1 + 4	30	11	182	41	19	4
blank mix 1 + VESV_3 + 5 + 1 + 4	29	10	170	36	18	3
blank mix 1 + VESV_3 + 5 + 1 + 4	30	12	181	38	20	4

TABLE 250. Multiplexed assay down-selection summary for VSV in the Porcine Panel. Signature baseline screening indicates the performance of the signature in a no-sample reaction (blank), a “passing” score is indicated by a low MFI. All signatures passed the signature baseline screening. In the multiplex addition screening the primers are added one-by –one to the other panel constituents to determine if there would be any cross-reactions between primer pairs in the panel. VSV-1798941, VSV-1811406 VSV-1811409 cross-reacted with other panel constituents during the multiplex addition screening. VSV-1798941 was eliminated from the panel, as was VSV-1811406, however, further experiments were conducted to troubleshoot VSV-1811409 and was determined to cross react with VESV-3. It was decided to remove VESV-3 for preference to the VSV-New Jersey –specific signature.

Signature	Mux Screening: Assay Down Selection

	Signature baseline screening	Multiplex addition screening	Target screening	Near-neighbor screening	Limit of detection screening	Noted cross-reactions
VSV_1798941	N/A	Fail (4-4-07): Crossreacts with VESV-3 and VSV_1811409	No further testing	No further testing	No further testing	Crossreacts with VESV-3 probe and VSV_1811409 probe
VSV_1798943	N/A	Pass	Pass	Pass	TBD	None
VSV_1798947	N/A	Pass	Pass	Pass	TBD	cross-reaction with VESV-1406; that signature was removed
VSV_1798949	N/A	Pass	Pass	Pass	TBD	None
VSV_1811405	N/A	Pass	Pass	Pass	TBD	None
VSV_1811406	Fail: cross-reaction	Fail (4-11-07) Crossreacts with VSV_1811406	No further testing	No further testing	No further testing	Crossreacts with VSV_1811406 probe; VSV 1811406 primers with VSV 1798947 probe
VSV_1811408	N/A	Pass	Pass	Pass	TBD	None
VSV_1811409	Fail: cross-reaction; decided to troubleshoot	Pass	Pass	Pass	TBD	cross-reacts with one of the core 14-plex primers: determined to be VESV-3 primers:VESV-3 removed from panel to keep VSV-1409

Near-neighbor and Target screening: Six VSV signatures were added to the **Porcine panel** and further characterization against targets and near-neighbors was conducted. With the exception of VSV-1409 (background MFI of 100-150), the signatures exhibited a reasonably low background response (<50 MFI) in the Porcine panel. Target screening for VSV was conducted in part at LLNL and further screening is pending at Plum Island Animal Disease Center [PIADC], New York.

TABLE 251. Backgrounds screening in multiplexed format for VSV at LLNL and PIADC.

	Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors	Targets
Mux PCR Screening	54	135	16	0	48	2(7 pending)

[1]-There are 752 pooled samples in each Aerosol Block.

TABLE 252. Backgrounds screening in **multiplexed** format for down-selected VSV signatures against the below listed **eukaryotes**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. A few "suspect" cross-reactions were observed with VSV-1409, but due to the high background of this signature those results are not considered significant. No other cross-reactions were observed.

Description	VSV_1798 943 (63)	VSV_1811 409 (64)	VSV_1798 947 (66)	VSV_1798 949 (67)	VSV_1811 405 (68)	VSV_1811 408 (69)
BOVINE	21	204	17	10	17	28
CAT	21	202	14	10	19	50
CHICKEN	19	112	13	9	18	24
DOG	19	127	15	10	17	28
DROSOPHILA MELANOGASTER	21	118	18	10	20	26
EQUINE	23	177	17	10	20	33
FLEA	19	114	17	10	19	24
HUMAN	18	110	15	9	16	22
MONKEY	27	190	18	11	39	40
MOSQUITO	25	110	14	9	25	23
MOUSE	28	126	16	10	46	26
PIG / PORCINE	31	133	19	11	32	41
RABBIT	30	121	17	11	31	26
RAT	35	138	23	11	32	29
SHEEP	32	157	21	11	28	28
TICK	26	150	20	11	25	40

TABLE 253. Backgrounds screening in **multiplexed** format for the six VSV signatures against the below listed **prokaryotes**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. None of the signatures cross-reacted with any of the samples listed below.

Description	VSV_1798 943 (63)	VSV_1811 409 (64)	VSV_1798 947 (66)	VSV_1798 949 (67)	VSV_1811 405 (68)	VSV_1811 408 (69)
Erwinia amylovora	31	123	18	10	32	27
Actinobacillus suis	23	90	12	8	21	20
Aneurinbacillus migulanus	23	95	13	9	23	21
Bacillus cereus	51	142	21	11	49	29
Bacillus globigii	47	119	18	9	42	26

Bacillus subtilis	49	136	19	10	47	29
Bacillus thuringiensis	50	141	20	10	52	29
Bifidobacterium denticum	28	111	15	10	29	24
Borrellia burgdorferi	51	149	24	12	51	30
Burkholderia capacia	28	112	17	11	29	24
Caulobacter vibriodes	19	94	14	9	19	21
Clavibacter michganensis	25	118	19	10	25	26
Clostridium butyricum	40	131	20	12	39	26
Corynebacterium pseudodipthericum	33	124	18	11	33	27
Cytophaga marinoflava	31	123	21	11	34	27
Erwina herbicola	39	130	19	11	40	28
Escherichia coli	45	139	21	11	47	30
Geobacillus caldoxylosilyticus	30	123	21	11	33	27
Halomonas halmophila	26	110	15	10	25	23
Haemophilus influenza	36	125	17	10	36	25
Herbaspirillium seropedicae	21	101	13	10	20	22
Lactobacillus garvieae	20	83	9	8	16	19
Lactobacillus gasseri	20	92	14	9	19	20
Listeria monocytogenes	36	118	18	10	35	25
Listeria seeligeri	36	135	22	12	41	28
Micrococcus luteus	23	92	14	8	19	20
Moraxella lacunatica	26	112	17	10	28	24
Oceanospirillium ssp. Maris	24	113	17	10	24	24
Paenibacillus napthalaenovorans	26	112	17	10	27	23
Paracoccus dentrificans	27	110	18	11	28	24
Porphyrobacter sanguineus	17	91	12	10	15	34
Proteus mirabillis	25	115	21	11	29	25
Pseudomonas aeruginosae	33	107	16	10	36	24
Pseudomonas oleovorans	22	96	14	9	20	23
Rhizobium leguminosarum	23	115	20	10	23	25
Rhodococcus rhodochrous	21	109	16	9	21	23
Salmonella typhimurium	37	125	18	10	36	26
Simonsiella muelleri	22	104	13	10	21	21
Sphingomonas sp. (Alcaligenes sp)	24	104	15	10	23	23
Staphylococcus aureus	39	127	18	11	39	27
Streptococcus pneumoniae	38	132	21	11	41	28
Streptomyces scabiei	24	120	20	11	25	25
Tatlockia maceachernii	22	113	18	11	23	26
Vibrio paraheamolyticus	25	118	17	10	26	26
Xanthomonas translucens	26	114	18	11	27	25

TABLE 254. Porcine panel **backgrounds** screening in **multiplexed** format for the six VSV signatures against the below listed **soils**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. None of the signatures cross-reacted with any of the soil samples listed below.

Description	VSV-8943 (63)	VSV-1409 (64)	VSV-8947 (66)	VSV-8949 (67)	VSV-1405 (68)	VSV-1408 (69)
D 000107-49	24	103	17	11	24	23
D 000109 # 50	25	111	18	12	26	25
D 000402 # 53	25	105	19	12	27	26
D 000500 - 26 - 1	24	105	18	12	26	24
D 000501-14-1	19	91	18	12	21	22
D 000505 - 11 - 4	24	101	18	12	24	25
D 000521 - 23	25	110	21	12	26	25
D 000527 - 3	23	112	20	12	22	26
D 000531 - 21	22	101	20	12	23	24
D 000533 - 17 -1	20	99	17	11	22	22
D 000542 - 6	21	98	18	11	22	22
D 000550 - 20	20	95	17	12	20	23
D 000551 - 5	23	109	20	12	24	25
D 000561 - 8 - 6	20	96	20	11	21	24
D 000562 - 30 - 5	21	102	20	13	20	24
S 251	30	97	17	11	19	23
S 252	18	92	17	11	17	21
S 253	22	90	18	12	18	22
S 254	17	93	17	11	18	22
S 255	17	87	17	11	18	21
S 256	17	93	18	12	17	22
S 257	16	88	16	11	16	21
S 259	15	85	17	11	15	20
S 260	15	91	17	12	17	21
S 271	15	87	17	11	15	21
S 272	15	84	16	11	15	21
S 273	16	90	18	11	16	23
S 274	16	88	19	12	16	23
S 275	16	93	18	11	17	22
S 276	16	92	18	12	17	23
S 277	16	92	18	12	16	22
S 279	15	85	17	10	14	21
S 280	14	83	17	12	14	21
S 282	16	92	18	12	16	23
S 283	15	84	17	11	14	21
S 284	15	85	17	12	15	21
S 286	13	80	17	11	14	20
S 287	15	85	19	12	15	21
S 288	14	88	18	12	14	23
S 289	13	85	17	11	14	22
S 290	15	89	18	12	13	21
S 291	13	81	17	11	13	20
S 292	13	85	17	11	12	20
S 295	13	88	18	11	14	21
S 296	15	87	19	11	13	21

S 297	15	84	18	13	13	22
S 298	13	85	17	11	13	21
S 299	12	88	16	11	12	21
S 300	7	89	13	8	7	17
S 301	8	88	15	9	8	18
S 303	6	54	11	9	6	16
S 304	7	74	16	9	7	16
S 305	7	74	15	10	7	18
S 307	8	80	16	10	7	18

TABLE 255. Porcine Panel **Near-Neighbor** Screening (Data from 20070601) against VSV signatures. Values shown are Median Fluorescence Intensity (MFI) units. “Blanks” are buffer-only samples (no nucleic acid added to reaction), shown below in red. Extracted nucleic acid was added to each PCR reaction in triplicate totaling 200pg/rxn. The data shows that the VSV signatures did not cross- reacted with any listed near-neighbors of the Porcine panel constituents.

Description	**VSV_1798943 (63)**	**VSV_1811409 (64)**	**VSV_1798947 (66)**	**VSV_1798949 (67)**	**VSV_1811405 (68)**	**VSV_1811408 (69)**
Blank	26	79	7	7	21	17
Blank	44	143	35	15	48	36
BDV Coos Bay	21	86	12	10	22	22
BHV (BFK) A03250006 DN-599	36	113	19	11	45	27
BHV A040150085	37	109	21	11	36	27
BHV-1 (IBR) Texas A030020072 CAHFS	29	108	18	12	34	26
BHV-1 A033640072	34	115	16	11	38	26
BHV-1 A040130066	39	128	20	12	47	29
BHV-1 ATCC VR 793	38	110	14	11	42	26
BHV-1 NVSL 10720	31	110	17	11	32	27
BHV-1 NVSL 200032	30	104	18	10	33	26
BHV-1 NVSL 231221	32	119	22	12	36	27
BHV-1 NVSL 51619	29	109	16	11	33	24
BHV-1 NVSL 86741	31	107	19	11	35	26
BHV-1 or IBR LA ATCC VR188	30	118	21	11	36	28
BHV-1 RA309	29	106	15	10	30	25
BHV-5 A032540006 CAHFS	22	92	13	10	25	22
BHV-5 A040150085 CAHFS	20	184	13	11	23	25
BHV-5 D9402133 CAHFS	22	98	11	10	23	21
BHV-5 D9403153 CAHFS	23	104	17	10	26	25
Caprine Herpes D0201157 CAHFS	20	106	14	11	21	24
Caprine Herpes-2 ATCC VR 462	23	109	15	11	27	25
Caprine Herpes-2 S0201998 CAHFS	22	90	13	9	19	22
EHD-1 A9904309	22	99	17	10	26	24
EHD-1 Georgia	22	93	15	10	23	21
EHD-1 New Jersey	18	72	12	8	17	18

EHD-1 Santa Barbara	18	83	12	9	17	23
EHD-2 Alberta	19	86	15	10	19	22
EHV-1 A011120004 CAHFS	28	117	17	13	32	27
EHV-1 A99043047	26	98	18	11	29	25
EHV-1 ATCC VR2003	29	103	15	10	30	24
EHV-2 ATCC VR701	30	101	16	10	33	25
EHV-2 D990 CAFHS	24	93	14	10	23	25
EHV-2 NVSL 0002	26	96	16	11	28	24
Feline Herpes ATCC VR 636	24	94	14	10	26	23
Fowl Pox	21	97	14	11	22	23
IBR CA 111903	34	120	20	12	43	28
IBR MN 111903	33	111	15	11	37	25
Parainfluenza Type 3	21	102	18	11	23	24
Porcine Herpesvirus or Pseudorabies Shope	22	91	14	10	25	22
Pseudorabies NVSL 92-12013	23	96	14	11	24	23
Pseudorabies NVSL 93-11745	25	110	18	11	29	27
Pseudorabies RA 180 CAHFS	24	94	13	10	26	22
Pseudorabies Titered	23	115	17	10	26	25
Respiratory Syncytial	20	97	18	11	23	24

Multiplexed PCR Assay Titrations (Porcine panel)

Upon finalization of the assay panel constituents each agent assay is characterized in multiplexed PCR format by running dose-response (titration) curves against titered virus extracted from virus infected cell culture for each available agent strain. Each sample is tested in duplicate and reported as a mean value of the median fluorescence intensity units (MFI) against agent concentration (per reaction volume, 5uL) on a double log plot. Concentration units are reported in TCID50/rxn (assumes 5uL sample volume per reaction) or pfu/rxn depending on virus type and source. In some cases where titered virus was not available data is reported as picogram units (pg). Each assay is evaluated by its ability to detect agent strains and differentiate from near neighbors (specificity) and by its range of sensitivity to each strain relative to each signature tested for that agent. Because this method is not aimed at determination of analytical sensitivities (requiring a more extensive sample set tested in clinical sample matrices which is not practical for this stage of development) it does however, provide tentative limits of detection that are used to measure the performance of each assay.

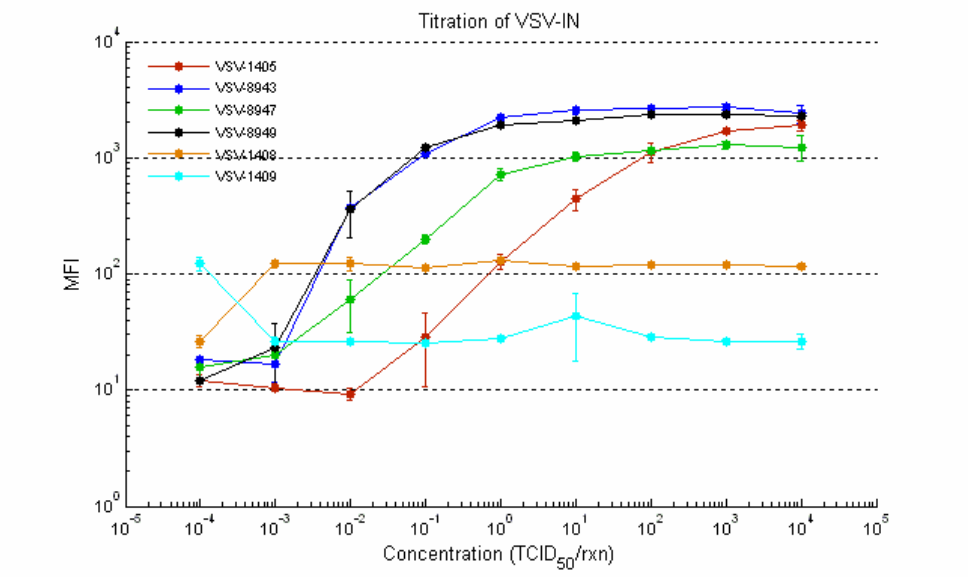


FIG. 32. Porcine multiplex screening data for the six VSV signatures against extracted nucleic acids from isolate VSV-IN. Signatures VSV-1408 and VSV-1409 have design specificity for the New Jersey Strain, so they are not expected to react with the Indiana strain as shown here. Serial dilution of nucleic acid Trizol extracted from titered virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1 of the mean.

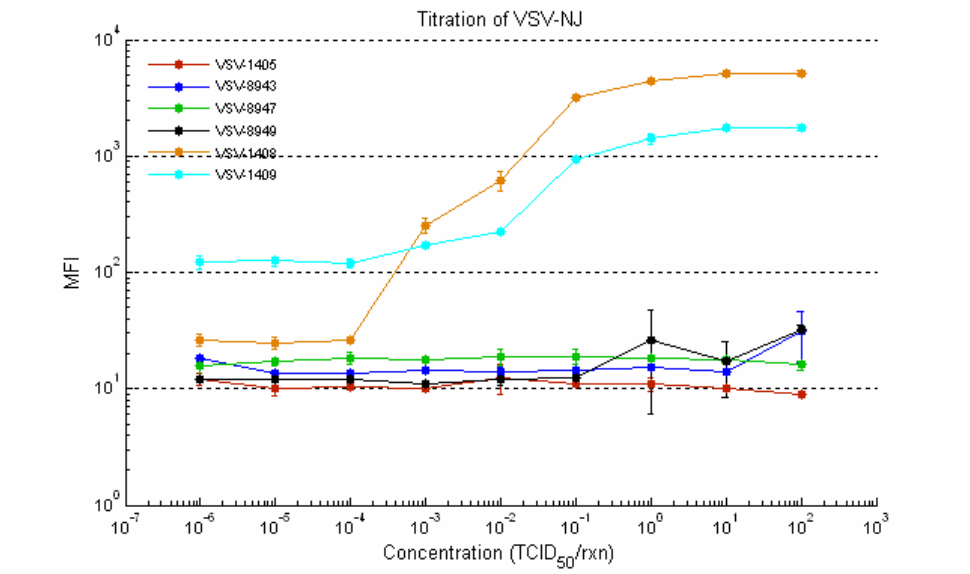


FIG. 33. Porcine multiplex screening data for the six VSV signatures against extracted nucleic acids from isolate VSV-NJ. Signatures VSV-1408 and VSV-1409 have design specificity for the New Jersey Strain, so they are the only signature of the six that is expected to react with the New

Jersey strain as shown here. Serial dilution of nucleic acid Trizol extracted from titered virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1 of the mean.

RESULTS: Six VSV signatures have been currently added the Porcine panel and tested against two species of VSV. Additional screening is currently underway at PIADC to test against 7 additional target strains of VSV and near neighbors that were not available to test at LLNL. Based on preliminary characterization data, the six VSV signatures selected for the porcine panel should consist of a complimentary set of signatures for detection of VSV Indiana and New Jersey.

8. FOOT-AND-MOUTH DISEASE (BOVINE AND PORCINE PANELS)

OBJECTIVE: In 2005 we were tasked by the Department of Homeland Security to develop specific and reliable Real-time RT- PCR signatures for Foot-and-mouth Disease virus, that could be incorporated into a Foot-and-Mouth Disease multiplex rule-out panel (completed Version 1.0 in 2005). In 2006 the goal of these assays (collectively with other virus-specific signatures developed in parallel) is to develop a species specific (i.e. bovine, porcine) multiplexed detection assay for Foot-and-Mouth disease rule-out for use in veterinary diagnostic laboratories. The two signatures that were incorporated into the Version 1.0 FMDV Rule-out panel have been re-evaluated for use in a bovine and porcine-specific FMDV rule-out panel. This document describes the historic data and information as well as preliminary 2006 data screening the 2 FMDV signatures for inclusion into both panels. Screening for FMDV signatures in both panels against FMDV targets was still occurring at the writing of this report.

8.1. BACKGROUND AND ETIOLOGY OF FMDV

Foot-and-mouth disease (FMD) is a highly infectious viral disease of cattle, pigs, sheep, goats, buffalo, and artiodactyl wildlife species. It is characterized by fever and vesicles in the mouth and on the muzzle, teats, and feet. In a susceptible population, morbidity approaches 100%. The disease is rarely fatal except in young animals. All species of deer and antelope, elephant, and giraffe are susceptible to FMD, but Old World camels are resistant to natural infection. South American camelids such as alpacas and llamas, although susceptible, are probably of no epidemiologic significance. Rats, mice, and guinea pigs can be infected experimentally.

FMD is endemic in the Middle East, Iran, the southern countries of the former Soviet Union, India, and southeast Asia. Sporadic outbreaks occurred in South Korea in 2000 and 2002, Japan in 2000, and in peninsular Malaysia. FMD is restricted to Luzon island in the Philippines. Australasia and Indonesia are free of FMD, as are Central and North America. In South America, Chile, southern Argentina, Guyana, Surinam, and the region of Colombia bordering Panama are free; large outbreaks of FMD in Uruguay and central Argentina during 2001 were

brought under control, and these areas together with Paraguay and large parts of Brazil are now considered free areas in which vaccination is still used. Most of sub-Saharan Africa has endemic FMD, and also Egypt, Ethiopia, and Eritrea; FMD has returned to Zimbabwe associated with economic and social changes, and sporadic outbreaks have also occurred in the previously FMD-free zones of South Africa, Namibia and Botswana. In Europe, an outbreak in Greece on the border with Turkey in 2000 was quickly eliminated, but in 2001, FMD was introduced into the UK, from where it spread to the Republic of Ireland, the Netherlands, and France. The strain causing the outbreak was the same as that found throughout Asia, and was eventually brought under control in the UK following the slaughter of >4 million animals, without the use of vaccination. Vaccination was used in the Netherlands, and all vaccinated animals were subsequently slaughtered. Europe is currently free of FMD. FMD is caused by an aphthovirus of the family Picornaviridae. There are 7 immunologically distinct serotypes:

A, O, C, Asia 1, and SAT (Southern African Territories) 1, 2, and 3. Within each serotype, there are a large number of strains that exhibit a spectrum of antigenic characteristics; therefore, more than one vaccine strain for each serotype, particularly O and A, is required to cover the antigenic diversity. Strains are characterized by their genomic relationships and their antigenic similarities with established vaccine strains. (Previous classification into subtypes became untenable as the number of subtypes rapidly increased[8].

8.2. FMDV COMPREHENSIVE ASSAY SUMMARY

Bioinformatics

Virus name: Foot-and-Mouth Disease Virus
Signature generation reference: not available
Level of discrimination: Species; Tetracore signature hits 115 targets, primarily complete genomes for A, C, O, Sat 1,2 and Asian strains.
Number of Initial Signatures: 1 (LLNL/UCD, 3 externally developed)
Number of Signatures forwarded to bench-screening: 4

Real-time PCR Screening Summary

TABLE 256. Final signatures set screening (4).

LLNL Signature Designation[3]	Sequence
FMDV64761F (aka CAHFS-FMD-F)	CGACAAAGGTTTTGTTCTTGGTC
FMDV64762R (aka CAHFS-FMD-R)	CACCCAWCGCAGGTAAAGTG
FMDV64762P (aka CAHFS-FMD-P)[1]	CCGTGGGACCATMCAGGAGAAGTTGA
FMDV.TC_F	ACTGGGTTTTACAAACCTGTGA
FMDV.TC_R	GCGAGTCCTGCCACGGA

[8] *Source: The Merck Veterinary Manual*

http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/51000.htm&word=foot%2cand%2cmouth%2cdisease

FMDV.TC_P	TCCTTTGCACGCCGTGGGAC
CanadianFMDV.BF	ACTGGGTTTTACAAACCTGTGATG
CanadianFMDV.R	TCAACTTCTCCTGKATGGTCCCA
CanadianFMDV.P	ATCCTCTCCTTTGCACGC
FMDV.Pirbright.BF[2]	CACYTYAAGRTGACAYTGRTACTGGTAC
FMDV.Pirbright.R[2]	CAGATYCCRAGTGWCICITGTTA
FMDV.Pir.P	CCTCGGGGTACCTGAAGGGCATCC

[1]The original probe was shortened for synthesis as a beacon probe: 5'-CCGTGGGACCATACAGGAGA-3'. [2]The signature from Pirbright laboratories was noted to have several degeneracies. [3]TC: Tetracore/USDA Signature; Canadian: Signature developed at the Canadian Science Centre for Human and Animal Health ;Pirbright signature developed at the Institute for Animal Health (IAH) Pirbright Laboratory in Pirbright, United Kingdom.

TABLE 257. Summary of bench screening **(applies only to FMDV.LLNL).** No cross reactivity seen in gel screening. In TaqMan screening, Cross reactivity was seen with Soil D000086 but not reproducibly.

	Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors	Target
Gel Screening	17	12	22	none	none	none
Real-time RT- PCR Screening	18	16	14	1 Aerosol block	1	1

[1] There are 752 pooled samples in each Aerosol Block.

Multiplexed PCR Screening Summary

TABLE 258. Backgrounds screening in multiplexed format for the bovine and porcine panels FMDV at LLNL and PIADC.

	Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors[2]	Targets
Mux PCR Screening	54	135	16	0	51	7 (7 pending)

[1-]There are 752 pooled samples in each Aerosol Block. [2]Near-neighbors indicated (43) apply to the near-neighbor panel that is not uniquely specific for FMDV, but for the other panel constituents that were screened concurrently.

TABLE 259. Signature summary for FMDV signatures in the bovine panel.

LLNL Signature Designation	Panel Designation	MUX Group ID	Gene ID	Relative Limit of detection[1]	Targets Screened
CAHFS-FMD	Bovine /Porcine	FMDV.LLNL	FdvCgp1; 3D polymerase [RdRp]	pending	7 (all serotypes)[2]
FMDV.TC	Bovine /Porcine	CAHFS-FMD	FdvCgp1; 3D polymerase [RdRp]	pending	7 (all serotypes) [2]

[1] The relative "Limit of detection" is described as a range of lowest and highest sensitivity determined by **multiple** target strains screened and is described further in the Multiplex results summary report section. [2] Targets

TABLE 260. Signature summary for FMDV signatures in the porcine panel.

LLNL Signature Designation	Panel Designation	MUX Group ID	Gene ID	Relative Limit of detection[1]	Targets Screened
CAHFS-FMD	Bovine /Porcine	FMDV.LLNL	FdvCgp1; 3D polymerase [RdRp]	pending	7 (all serotypes) [2]
FMDV.TC	Bovine /Porcine	CAHFS-FMD	FdvCgp1; 3D	pending	7 (all serotypes) [2]

			polymerase [RdRp]		

[1] The relative "Limit of detection" is described as a range of lowest and highest sensitivity determined by **multiple** target strains screened and is described further in the Multiplex results summary report section.

8.3. FMDV SIGNATURE BIOINFORMATICS AND GENERATION INFORMATION (LLNL/UCD)

NOTE: SIGNATURE DESIGN SECTION APPLIES ONLY TO SIGNATURES GENERATED AT LLNL; THE TWO FMDV SIGNATURES DESCRIBED HERE WERE GENERATEDY EXTERNALLY.

TABLE 261. External FMDV Source Information.

Signature Internal Name	Name	Sequence	Source of Primers/Probes
FMD.TC-1	FMD.TC.P (Tetracore)	TCCTTTGCACGCCGTGGGAC	JAVMA, Vol. 220, No.11, June 1, 2002
FMD.TC-1	FMD.TC.BF (Tetracore)	ACTGGGTTTTACAAACCTGTGA	
FMD.TC-1	FMD.TC.R (Tetracore)	GCGAGTCCTGCCACGGA	
FMD.Pir-1	FMDV.Pirbright.P	CCTCGGGGTACCTGAAGGGCATCC	Journal of Virological Methods 105, (2002), 67-80.
FMD.Pir-1	FMDV.Pirbright.BF	CACYTYAAG RTGACAYTGRTACTGGTAC	
FMD.Pir-1	FMDV.Pirbright.R	CAGATYCCRAGTGWCICI TGTTA	

Sequence Bioinformatics:

TABLE 262. Comparison of Bioinformatics of the 2 candidate signatures:

Target Virus	FMDV	FMDV
Forward Primer	FMDV.TC_F	FMDV.Pir.F
FWD Primer Length (bp)	22	28
FWD Primer TM (^{o}C)	53	57
FWD Primer GC Content (%)	41	43
Forward Sequence	ACTGGGTTTTACAAACCTGTGA	CACTTCAAGGTGACATTGATACTGGTAC
Forward Location	7826	857
Reverse Primer	FMDV.TC_R	FMDV.PIR.R
Rev Primer Length (bp)	17	23
Rev Primer TM (^{o}C)	56	57
Rev Primer GC Content (%)	71	52
Reverse Sequence	GCGAGTCCTGCCACGGA	CAGATCCCGAGTGTCGCTTGTTA
Reverse location	7916	931
Probe Name	FMDV.TC_FCP	FMDV.Pir.FCP
Probe Length (bp)	20	24
Probe TM (^{o}C)	65	63

Probe GC Content (%)	60	67
Probe Sequence	GTCCCACGGCGTGCAAAGGA	CCTCGGGGTACCTGAAGGGCATCC
Probe location	7877	907
Probe strand	minus	minus
Predicted Product Size	107	97

Target Region Gene Information

TABLE 263a-b. (a) Reference genomes used for gene information. (b) Gene information for each signature

(a)

Genome Description	GI Number	Sequence Length (bp)
Foot-and-mouth disease virus A, complete genome	48429536/NC_011450.1	8161

(b)

			Gene Location		Target Region Location	
Signature	**Gene/ID**	**Description**	**Start**	**End**	**Start**	**End**
FMDV.TC	FdvCgp1	3D polymerase [RdRp]	6610	8019	7795	7901
FMDV_Pirbright	No gene in target region	5' UTR	1	1038	839	936

8.4. FMDV GEL AND TAQMAN SCREENING REPORT (LLNL SIGNATURE)

Not performed.

Note: Externally developed signatures were not subjected to the same level of screening as internally developed signatures. It was assumed that these signatures were thoroughly evaluated using methods similar to those employed by LLNL.

8.5. FMDV MULTIPLEXED PCR SCREENING REPORT

SCREENING AND DOWN-SELECTION TECHNICAL APPROACH:

Signatures that pass gel and real-time PCR screening are candidates for multiplexed assays. If the number of candidate signatures is large than a subset of the available signatures are selected based on measured sensitivity and specificity observed in real-time screening. In preliminary screening the background of individual assays is measured by running “blank” (no sample added) controls and observing signature response. In some cases a primer-to-primer non-specific interference will eliminate candidate signatures. Signatures are then added iteratively to each multiplexed panel, titrated, and with each signature addition, collectively, all signatures are re-titrated to determine if the presence of one signature will interfere with the performance of

another. During this process signatures are added or subtracted from the multiplexed panel depending on individual outcomes until all desirable signatures have been added.

Criterion for down-selection of multiplexed signatures

Signature specificity. In initial real-time PCR screening signatures undergo target and near neighbor screening and screening against environmental confounders which assess individual signature specificity. In multiplex format this is repeated to verify that a more complex reaction system gives the same level of specificity.

Signature sensitivity. In initial real-time PCR screening signatures undergo limit of detection testing where a dilution curve is generated for each signature. This determines individual signature sensitivity. In multiplex format this is repeated to verify that a more complex reaction system gives acceptable signature sensitivity.

Optimal signal to noise ratio. In the multiplex assay system the potential for cross reactivity is larger than in single reaction testing, this may cause unwanted PCR reaction products or non-specific cross-reactivity. Signatures are preferred to have a high signal to noise ratio, where desired signal is significantly greater than the background noise of the signatures when there is no target nucleic acid in the reaction.

Compatibility with other signatures in multiplex. In some cases a primer set may interfere with other primers or probes in the multiplex and there by cause limit of detection shifting for one or more signature in the multiplex. For each scenario it must be evaluated whether the shift in detection level is significant enough to remove (what could be a very desirable signature) from the panel.

TABLE 264. Order details for FMDV signatures ordered for multiplexed assay screening and development.

Signature ID	Modification details	Vendor
SA-IR-219-246F.BF	5'-/5Bio/CAC YTY AAG R/iBiodT/G ACA YTG RTA C/iBiodT/G GTA C	IDT DNA
SA-IR-315-293R.R	5'-CAG ATY CCR AGT GWC ICI TGT TA-3'	IDT DNA
SAmulti-P-IR-292-269R.FCP	5'-/5AmMC6//iSp18/CCT CGG GGT ACC TGA AGG GCA TCC-3'	IDT DNA
FMD.TC.BF	5' -/5Bio/ACTGGG/iBiodT/TTTACAAACC/iBiodT/GTGA-3'	IDT DNA
FMD.TC.R	5'- GCGAGTCCTGCCACGGA- 3'	IDT DNA
FMD.TC.FCP	5'- /5AmMC6//iSp18/GTCCCACGGCGTGCAAAGGA-3'	IDT DNA

Multiplex Data Analysis -Technical approach

Disease signature thresholds

Thresholds were initially determined using all available data from known negative samples (blanks) by plotting a histogram which showed the probability of a given sample generating a particular MFI. In the absence of cross reactivity, each signature in the multiplex assay could be treated as an individual signature result. For example, for a sample spiked with FMDV, data for other disease signatures could contribute towards negative data, providing that cross reaction

between disease signature sets was not observed. This is a way to increase the number of samples that can be used to establish thresholds.

TABLE 265. Individual signature thresholds and ranges for FMDV signatures in each species-specific panel. For FY06, threshold determinations have not yet been made as they require a significant number of tests (>500) to generate this information. This information will be updated when available.

Signature Name	Mux Assay name	Panel Membership	Signature background in Tris NaCl Buffer (MFI +/-)	Signature background in Clinical matrices (MFI +/-)	Threshold
FMDV-TC	FMDV-TC	Bovine/Porcine	TBD	TBD	TBD
FMDV-Pir	FMDV-Pir	Bovine/Porcine	TBD	TBD	TBD

TABLE 266. Summary of FMDV target nucleic acid available for multiplexed screening at PIADC.

Virus	Serotype	Strain	Source[1]	V#	Passage History	Extraction date/ID	Extraction Method	Titer (pfu/mL)	Titer Method
FMDV	A	Argentina 2001	PIADC	V02764	BHK P56	unknown	Ambion MagMax 96	2.05 x 10 ^ 7	Spearman-Karber
FMDV	O	O1, South Korea	PIADC	V02722	1 LK, 1 BHK	unknown	Ambion MagMax 96	3.05 x 10 ^ 7	Spearman-Karber
FMDV	C	C4, Tierra Del Fuego	PIADC	V02367	1 LK, 1 BHK	unknown	Ambion MagMax 96	1.0 x 10 ^ 7	Spearman-Karber
FMDV	Asia 1	LEB '83	PIADC	V02594	2 BOV, 1 BHK	unknown	Ambion MagMax 96	6.5 x 10 ^ 4	Spearman-Karber
FMDV	SAT 1	Sat 1/6 SWA 40/61	PIADC	V02412	1 LK, 1 BHK	unknown	Ambion MagMax 96	2.1 x 10 ^ 7	Spearman-Karber
FMDV	SAT 2	Sat 2 Zim 5/81	PIADC	V02403	3 BTY, 2LK, 7BHK,	unknown	Ambion MagMax 96	1.5 x 10 ^ 5	Spearman-Karber
FMDV	SAT 3	Sat 3/3 Bech 1Nov05	PIADC	V02376	3 LK, 1 BHK	unknown	Ambion MagMax 96	2.25 x 10 ^ 7	Spearman-Karber

[1]PIADC (Plum Island Animal Disease Center, Plum Island, NY)

TABLE 267. List of FMDV extracted RNA screened at LLNL.

Agent	Serotype	Isolate	Source	Original Titer (Log10 TCID50)	Passage History	Extraction date/ID	Extraction Method	Use stock titer	Titer Method
FMDV	A	A18	PIADC	N/A	BHK P56	2/2006	Trizol	ng/uL	N/A
FMDV	O	O1 Brugge	PIADC	N/A	1 LK, 1 BHK	2/2006	Trizol	ng/uL	N/A
FMDV	C	C1 Noville	PIADC	N/A	1 LK, 1 BHK	2/2006	Trizol	ng/uL	N/A
FMDV	Asia 1	N/A	PIADC	N/A	2 BOV, 1 BHK	2/2006	Trizol	ng/uL	N/A

FMDV	SAT 1	N/A	PIAD C	N/A	1 LK, 1 BHK	2/2006	Trizol	ng/uL	N/A
FMDV	SAT 2	N/A	PIAD C	N/A	3 BTY, 2LK, 7BHK,	2/2006	Trizol	ng/uL	N/A
FMDV	SAT 3	N/A	PIAD C	N/A	3 LK, 1 BHK	2/2006	Trizol	ng/uL	N/A

TABLE 268. List of additional near-neighbors screened against the Bovine Panel. All near-neighbors samples were extracted nucleic acids with unknown titers.

Virus	Strain[1]	Unique ID	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	NA conc. used or stock titer	Titer Method
Border Disease virus	Coos Bay	4-6-92	NADC, Julia Ridpath	1.5 x 10^8 TCID50/mL	unknown	12/14/06	Trizol	1.18 x 10^6 TCID50/mL	Reed & Muench
Bovine Herpes Virus-1	California	NVSL 20032 111903	CAHFS	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Cooper	NVSL 86741 111903	CAHFS	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Los Angeles	Los Angeles ATCC VR188	ATCC (CAHFS)	1 x 10^5 TCID50/mL	(CAHFS) MDBK2(LLNL)MDBK1	5/17/07	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	RLB-106	ATCC VR793	ATCC (CAHFS)	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	A033640072	CAHFS, R. Mock, #5 Swab, 1BDFK1, 1/28/2004, A030020072	unknown	1BDFK1,	4/23/04	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	A040130066	CAHFS, R. Mock, #4 Lung, 1BFK1, 1/28/2004, A040130066	unknown	1BFK1	4/23/04	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	Texas A030020072	CAHFS, R. Mock, #80 Swab, 1BDFK1, 1/28/2004, A030020072	unknown	1BDFK1	4/23/2004	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	California	NVSL 200032	NVSL, CAHFS	unknown	MDBK CAHFS	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Virginia	NVSL 231221	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	California	NVSL 51619	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Minnesota	NVSL 86741	NVSL, CAHFS	unknown	MDBK CAHFS	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Minnesota	NVSL 97-10720	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Cooper	NVSL-Cooper RA 309	NVSL, CAHFS	unknown	MDBK CAHFS	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes-5	DN-599	A032540006	R. Mock, from lung tissue, 2BFK2, 2/3/2004	Unknown	CAHFS Lab 2BFK2 4/30/2004	6/3/2004	Phenol/Chloroform	40 pg/uL	N/A
Bovine Herpes-5	DN-599	A040150085	R. Mock, #2 Lung, 5BFK5,	Unknown	CAHFS Lab	7/7/2004	Phenol/Chloroform	40 pg/uL	N/A

			2/3/04		5BFK5 4/23/2004				
Bovine Herpes-5	D9402133	D9402133	Sonoma Co. 3/94 Severe necrotizing encephalitis, 1 week old calf	Unknown	CAHFS Lab (MDBK), (1 flasks) 11/19/2003	11/19/2003	Phenol/Chloroform	40 pg/uL	N/A
Bovine Herpes Virus	BHV 5	D9403153	CAHFS	unknown	unknown	unknown	unknown	40 pg/uL	unknown
Caprine Herpes Virus		VR462	ATCC	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Caprine Herpes Virus	D0201157	D0201157	CAHFS, Isolate D0201157 History: San Joaquin Co. 2/02 Aborted fetus-third trimester	unknown	CAHFS Lab (MDBK)	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Caprine Herpes Virus	S0201998	S0201998	CAHFS, Isolate S0201998 History: San Diego Co. 2/02 Aborted fetus-third trimester	unknown	CAHFS Lab (MDBK)	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-1	Santa Barbara	N/A	Bridget McLaughlin CAHFS	unknown	unknown	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-1	Georgia	N/A	Bridget McLaughlin CAHFS	unknown	unknown	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-2	Alberta	041 ODV 0401	NVSL	unknown	BHK	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-1 (SV123)	New Jersey (3TD) Deer, NJ 1955	041 ODV 0401	NVSL	1 x $10^{5.125}$ $TCID_{50}$/0.1 ml	+BHK-3 (12/7/83); (12/11/86); (12/18/86)	Unknown	Trizol	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A011120004	CAHFS, R. Mock, from brain tissue, 2BFK2, 2/3/2004, A011120004	unknown	CAHFS, 2BFK2	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A99043047	CAHFS, R. Mock, Spleen, 1BFK1, 2/3/2004, A99043047	unknown	CAHFS, 1BFK1	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A9904309	CAHFS	unknown	CAHFS, unknown	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Bovine 1247	Bovine 1247 ATCC VR2003	ATCC	unknown	CAHFS, unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	NVSL 002	NVSL 002 1/28/04	NVSL	unknown	CAHFS, unknown	1/29/03	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	LK	LK ATCC VR701	ATCC	unknown	CAHFS, unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	D990	D990	CAHFS	unknown	CAHFS, unknown	1/29/03	Phenol/ Chloroform	40 pg/uL	N/A
Feline Herpes	C-27	ATCC VR636	ATCC	unknown	CAHFS, unknown	unknown	unknown	40 pg/uL	N/A

Fowl Pox	Vaccine strain	Poxine™	Jeffers Livestock	unknown	ECE	5/17/07	Phenol/ Chloroform	40 pg/uL	N/A
Parainfluenza Virus type 3	C-243	TC-81335	LLNL	1 x 10^5 TCID50/0.1 mL	(VL)MK14(LLNL)H292-1	5/17/07	Trizol	40 pg/uL	Reed and Muench
Porcine Coronavirus	AR310	ATCC VR-2384	LLNL	1 x $10^{4.25}$ $TCID_{50}$/0.1 ml	ST2				Reed and Muench
Porcine Herpes Pseudorabies	Shope	RA 180	CAHFS	1 x 10^5 TCID50/mL		5/17/07	Trizol	40 pg/uL	N/A
Pseudorabies		92-12013	NVSL 92-12013 bovine isolate Iowa December, 1991	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies		93-11745	NVSL 93-11745 Illinois December, 1992	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies	Shope	RA180	NVSL Shope (PRV) RA 180	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies Titered	Shope	N/A	NVSL	unknown		unknown	Phenol/ Chloroform		Reed & Muench
Respiratory Syncytial virus	Long	N/A		unknown	(VL)KB6L2KB2HFDL2A549-1(LLNL)HeLa3	5/17/07	Trizol	40 pg/uL	N/A
Transmissible Gastroenteritis virus of hogs	Perdue	NVSL 9801	LLNL	1 x $10^{4.75}$ $TCID_{50}$/0.1 ml	ST 1		Trizol	40 pg/ul	Reed and Muench

TABLE 269. Panel membership for signature. The two FMDV signatures were included in the **Bovine-specific multiplexed assay panel** for FMDV rule-out diagnostics. The below table describes the constituents of that multiplexed assay panel in which these signatures were tested.

Bead ID	Signature/ Target Name	Mux Group ID	Virus	Source	Forward Primer	Reverse Primer	Luminex Probe (-strand, FCP)
113	emcf_94975.F, emcf_94976.R, emcf_94977.P	MCF_1	Malignant Catarrhal Fever	LLNL	ATGCCAGTCACTGGCTCTCA	GGGTGTTGTAGAATCCTGAAATGG	GTTGATCACGGTGGCACCCTGG
114	emcf_95059.F, emcf_95060.R, emcf_95061.P	MCF_2	Malignant Catarrhal Fever	LLNL	GTTCTGGAAACTGACCAAACAGTGT	AGTGGCACTTGAGTGTAACTTTTATTG	GCACTCTGGCAGGCATAAGGGAAATACA
115	emcf_95155.F, emcf_95156R, emcf_95157.P	MCF_3	Malignant Catarrhal Fever	LLNL	CCCTGGAAGCTGTCATACAAAA	AAACATTGGCATATCTTGCAAGGT	CAGTAGAGTCCAGGGCTGCAGTTGTCTCA
117	BVH__94666.F, BVH_94667.R, BVH_94668.P	BHV-94668	Bovine Herpes Virus	LLNL	GTGCCAGCCGCGTAAAAG	GACGACTCCGGGCTCTTTT	TCCTGGTTCCAGAGCGCTAACATGGAG
118	BVH__94738.F, BVH_94739.R, BVH94740.P	BHV-94740	Bovine Herpes Virus	LLNL	TGAGGCCTATGTATGGGCAGTT	GCGCGCCAAACATAAGTAAA	AAATAACACGGTGTGCACTTAAATAAGATTCGCG
119	BPSV_95719.F, BPSV_95720.R, BPSV_95721.P	PPOX_1	Bovine Papular Stomatitis Virus	LLNL	GCAGATGCGCTCCTGGTT	GCACCTCTGCTGCTGCAA	CCGACTCCGACGTGGAGAACGTG
120	BPSV_95722.F, BPSV_95723.R, BPSV_95724.P	PPOX_2	Bovine Papular Stomatitis Virus	LLNL	GATGGCCGTGCAGCTCTT	CGTACAAGATCACGGCCAACT	TGTACGGGCTCATGGGCTTCCG
122	BPSV_95731.F, BPSV_95732.R, BPSV_95733.P	PPOX_4	Bovine Papular Stomatitis Virus	LLNL	GCAGCAGTGCACCACGTAGT	CGCTGAACCCGTACATCCT	GACTTCGAGGCGGACAACAAGCG
132	FMDV.TC	FMDV.TC	Foot and Mouth Virus	Tetracore	ACTGGGTTTTACAAACCTGTGA	GCGAGTCCTGCCACGGA	GTCCCACGGCGTGCAAAGGA
133	FMDV.Pir	FMDV.Pir	Foot and	Pirbright	CACYTYAAGRTGACAYT	CAGATYCCRAGTGWCICITG	CCTCGGGGTACCTGAAGG

			Mouth Virus		GRTACTGGTAC	TTA	GCATCC
134	BVD_1a	BVD_SIG1	Bovine Viral Diarrhea	UCD, CAHFS lab	GGTAGTCGTCAGTGGTTCGAC	CATGTGCCATGTACAGCAGAGAT	CCTCGTCCACGTGGCATCTCGAG
138	BVD_1821165.F, BVD_1821164.R, BVD_1821166.P	BVD_SIG2	Bovine Viral Diarrhea	LLNL	GGGAGTCGTCAATGGTTCGAC	TCCATGTGCCATGTACAGCAGAG	CCTCGTCCACITGGCATCTCGAG
135	BTV_1759932	BTV_1759932	Bluetongue Virus	LLNL	GCACCCTATATGTTTCCAGACCA	CAGCTAACTCTTCAGCCACACG	CTAACTCGTGGGCCAATCATCATCTTCTGT
136	BTV10_1810207	BTV10_1810207	Bluetongue Virus	LLNL	CAAACACAAAAGGCGGAGAAG	GGCGTTTAATCTGTCTTAGTCTTACGT	GAAACGCTTCTGCGTACGATGCGA
137	BTV10_1810199	BTV10_1810199	Bluetongue Virus	LLNL	CACATGTCGCTTAATTTGTCTTAACC	GCGGAGAAGGCTGCATT	ACGAAACGCTTCCGCGTACGATG
139	BTV10_1810205	BTV10_1810205	Bluetongue Virus	LLNL	TCAATTTTGGTAGAATTTGTTCATTCA	GCGGAGAAGGCTGCATTC	ACGAAACGCTTCCGCGTACGATG
163	VSV_1798943	VSV_1798943	Vesicular Stomatitis Virus -Indiana	LLNL	CGCCACAAGGCAGAGATGT	TGTCAAATTCTGACTTAGCATACTTGC	GCATACTGCATCATATCAGGAGTCGGTTTTCTG
166	VSV_1798947	VSV_1798947	Vesicular Stomatitis Virus -Indiana	LLNL	CCCAATCAATGCCATGATACA	CTCCAATGGAAGGGTCCAAA	TTTGAAAGTAGAACTGTGCAAGCCCGGTATC
167	VSV_1798949	VSV_1798949	Vesicular Stomatitis Virus -Indiana	LLNL	GGCGCTCATTATAAAATTCGGA	ACATTTTCTCGTAGTAATGCAGCAG	GAAGTCCCTGTAATGGATTCCCATTCCATGT
169	VSV_1811408	VSV_1811408	Vesicular Stomatitis Virus –New Jersey	PIADC	CTCACAACATGGGTCCTGAA	TTCTTGACCTGGATACATCAT	GGCATAGYTCGTCTGCRACTTCCCT
160	RPV_1814853	RPV_1814853	Rinderpest Virus	LLNL	GGATCGCTGAAATGATCTGTGA	GGAGCCAGTTCACCCATTTG	CTGGCCAACCCTGCCTCCACTATGTA
161	RPV_1814855	RPV_1814855	Rinderpest Virus	LLNL	TGCATCTTATGTGACTTTGGTTCA	GGCTATCCGCACAGCTGAC	CAGTCCTCTCATCTGTTGTCGATCCGATGTA
162	RPV_1814856	RPV_1814856	Rinderpest Virus	LLNL	AACTCCTGACCTCATTCCTTGC	GGCTCTATAATCCCACTATGCCA	TGGCTCAGTGCATTCACAAAGACCTTGAATA
170	N/A	NC (MT-7)	Maritima	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
171	N/A	b-MT7 (FC)	N/A	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
172	N/A	MT7/Cy3 (IC)	N/A	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
173	Alien RNA	arRNA Control	None	Ambion	GACATCAAGGCTCAAACTAATTTTACC	CAAAGGCTGCCAACATAAAATG	CAAGCGTAAATGCAGCGTCCA

[1]FCP indicates that the probe is on the negative strand (Forward Compliment to the Probe).

TABLE 270. Panel membership for signature. The two FMDV signatures were also included in the **Porcine-specific multiplexed assay panel** for FMDV rule-out diagnostics. The below table describes the constituents of that multiplexed assay panel in which these signatures were tested.

Bead ID	Signature/ Target Name	Mux Group ID	Virus	Source	Forward Primer	Reverse Primer	Luminex Probe (-strand, FCP)
132	FMDV.TC	FMDV.TC	Foot and Mouth Virus	Tetracore	ACTGGGTTTTACAAACCTGTGA	GCGAGTCCTGCCACGGA	GTCCCACGGCGTGCAAAGGA
133	FMDV.Pir	FMDV.Pir	Foot and Mouth Virus	Pirbright	CACYTYAAGRTGACAYTGRTACTGGTAC	CAGATYCCRAGTGWCICITGTTA	CCTCGGGGTACCTGAAGGGCATCC
142	PRRS_1807709	PRRS__1807709	Porcine Reproductive and Respiratory Syndrome	LLNL	GAGCGGCAATTGTGTCTGTC	GCTGAGGGTGATGCTGTGAC	CGCACAGTATGATGCGTAGGCAAACTAAACTC
144	PRRS_1810351	PRRS_1810351	Porcine Reproductive and Respiratory Syndrome	LLNL	TTCTTGTGACCACGATTCGC	GACCCACCGAGTAACTTGCC	GCTCAAGAGCCAAAAGCTCAGCATGACA
145	PRRS_1807706	PRRS_1807706	Porcine Reproductive and Respiratory Syndrome	LLNL	ATTGGTTTGCTCCGCGATAC	AAATGAGCCACCACATCCAA	CGGTACATTCGACGCGACACCATTTC
148	PRRS_1810383	PRRS_1810383	Porcine Reproductive and Respiratory Syndrome	LLNL	CAGTGTGCACGCTTCCATTT	CTCGAATGATGTGTTGCCGT	AAACATAGCGTAGAGCTGGAATTCGAAGCCA
149	PRRS_1810386	PRRS_1810386	Porcine Reproductive and Respiratory Syndrome	LLNL	GCTTTCTGCGTGCCTTTTCT	ACAACGCCAGAGACATTCCC	TGACTTTGAAGCCTTTCTCGCTCATTTCTGA
150	SVD_1727049	SVD_1	Swine Vesicular Disease	LLNL	CAGGATAATTTCTTCCAAGGGC	ACGTGAACATTTCGAGCTTCC	TGCATTGTGTCTGATGGTACAACTTGTGACG
151	SVD_1727050	SVD_2	Swine Vesicular Disease	LLNL	GACTTGTTGTGGCTGGAGGA	CAGCGCCATGGTGAGGTAG	TGACCGTAATGAGGTCATCGTGATTTCTCAC
152	SVD_1727051	SVD_3	Swine Vesicular Disease	LLNL	GACAAAGTGGCCAAGGGAAA	CACGTAAACCACACTGGGCT	CTGGCGTCATAGCCTGAATAGTCAAACGCTA

154	VESV_95653.F, VESV_95654.R, VESV_95655.P	VESV_1	Vesicular Exanthema of Swine Virus	LLNL	GCCTTCTCCCTTCCCAAAA	TGAAGGAATGGTTCCGTCAGT	CATCATCGTTGATAACCTTAGATGTGCAATTTGG
157	VESV_95686.F, VESV_95687.R, VESV_95688.P	VESV_4	Vesicular Exanthema of Swine Virus	LLNL	GGTCGCTCTCACTGATGATGAGTA	GGTGTTATCAGCACCCATTGC	GCTCGGTGCCTGAGTTGGAGGAAG
158	VESV_95692.F, VESV_95693.R, VESV_95694.P	VESV_5	Vesicular Exanthema of Swine Virus	LLNL	ACCACCTCTGGAAACATCTATGG	TTTGTGCACGTGTCACGAAT	CGGGACGGGCATTTGTCACCA
163	VSV_1798943	VSV_1798943	Vesicular Stomatitis Virus -Indiana	LLNL	CGCCACAAGGCAGAGATGT	TGTCAAATTCTGACTTAGCATACTTGC	GCATACTGCATCATATCAGGAGTCGGTTTTCTG
164	VSV_1811409	VSV_1811409	Vesicular Stomatitis Virus –New Jersey	PIADC	CTCACAACATGGGTCCTGAA	TTCTTGCCCCGGATACATCAT	GGCACAGCTCATCTGCGACTTCCCT
166	VSV_1798947	VSV_1798947	Vesicular Stomatitis Virus -Indiana	LLNL	CCCAATCAATGCCATGATACA	CTCCAATGGAAGGGTCCAAA	TTTGAAAGTAGAACTGTGCAAGCCCGGTATC
167	VSV_1798949	VSV_1798949	Vesicular Stomatitis Virus -Indiana	LLNL	GGCGCTCATTATAAAATTCGGA	ACATTTTCTCGTAGTAATGCAGCAG	GAAGTCCCTGTAATGGATTCCCATTCCATGT
168	VSV_1811405	VSV_1811405	Vesicular Stomatitis Virus -Indiana	PIADC	AAGAGATGGTCACGAGTGAC	GAGCATTTGTGGAAACCGAGC	TGGGTATTTGGTCATTGGTGACACA
169	VSV_1811408	VSV_1811408	Vesicular Stomatitis Virus –New Jersey	PIADC	CTCACAACATGGGTCCTGAA	TTCTTGACCTGGATACATCAT	GGCATAGYTCGTCTGCRACTTCCCT
170	N/A	NC (MT-7)	Maritima	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
171	N/A	b-MT7 (FC)	N/A	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
172	N/A	MT7/Cy3 (IC)	N/A	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
173	Alien RNA	arRNA Control	None	Ambion	GACATCAAGGCTCAAACTAATTTTACC	CAAAGGCTGCCAACATAAAATG	CAAGCGTAAATGCAGCGTCCA

8.5.1. BOVINE PANEL MULTIPLEXED PCR DATA

Preliminary screening: Preliminary screening can be broken down into several steps. The signatures are preliminarily screened by first assessing how the signatures will perform under multiplex PCR conditions and whether or not the addition of the new signatures will adversely effect other signatures, these preliminary screening methods are described as “signature baseline screening” and “multiplex addition screening” respectively. First signature baseline screening tests the performance of the signature in a no-target reaction (blank), a “passing” score is indicated by a low (typically less than 100) MFI. All FMDV signatures passed the signature baseline screening. Next in multiplex addition screening the new signature primers are added one-by –one to the other panel signatures to determine if there would be any cross-reactions between primer pairs in the panel. Further assessment of the signatures is done through target and near-neighbor screening and the determination of relative sensitivities. Preliminary screening of the FMDV signatures was addressed in previous (FY2005) work.

Version 1.0 Panel –Historic Data

(a) (b)

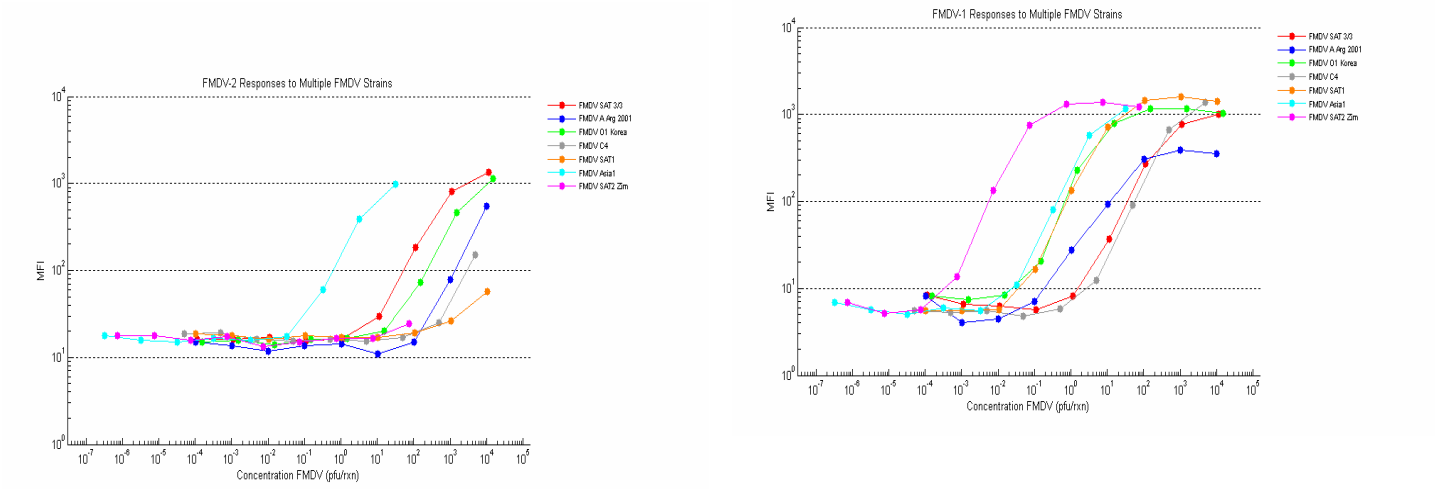


FIG. 34a-b. Version 1.0 panel data for FMDV signatures plotted against different FMDV serotypes at PIADC. (a) FMDV-1 (TC) (b) FMDV-2 (Pir).

TABLE 271. Version 1.0 Panel Historic Data. Summary of limits of detection for FMDV across various strains tested. An "X" indicates that the limit of detection was not reached (unable to be assessed) for that particular strain.

Serotype	FMDV-1 (TC)	FMDV-2 (Pirbright)	units
SAT3	2892.017	6106.7	pfu / ml
A	743.7446	164071	pfu / ml
O	53.57789	34555	pfu / ml
C	4067.236	337335	pfu / ml
SAT1	61.76444	1E+07	pfu / ml
Asia1	32.29428	73.41	pfu / ml
SAT2	0.203846	X	pfu / ml

Near-neighbor and Target screening: The two signatures were added to the Bovine panel. The signatures exhibited very low background response (<10 MFI) in the Bovine panel. Target screening for FMDV was conducted at both LLNL and Plum Island Animal Disease Center [PIADC], New York. Preliminary (non-target or nucleic acid only) screening was conducted at LLNL.

TABLE 272. Backgrounds screening in multiplexed format for FMDV at LLNL and PIADC. Additional target screening is pending at PIADC, Aug 2007

	Soils	**Prokaryotes**	**Eukaryotes**	**Aerosols**	**Near Neighbors**	**Targets**
Mux PCR Screening	54	135	16	0	51(+)	7(7pending)

[1-]There are 752 pooled samples in each Aerosol Block.

TABLE 273. Bovine panel backgrounds screening in **multiplexed** format for down-selected FMDV signatures against the below listed **eukaryotes**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate and the average MFI is reported below. Neither FMDV reacted with the below eukaryotes.

Description	FMD-TC (32)	FMD-PIR (33)
BOVINE	15	4
CAT	13	3
CHICKEN	7	4
DOG	6	3
DROSOPHILA MELANOGASTER	9	3
EQUINE	5	3
FLEA	5	3
HUMAN	7	3
MONKEY	16	4
MOSQUITO	18	4
MOUSE	9	4
PIG / PORCINE	13	4
RABBIT	16	4
RAT	8	3
SHEEP	4	2
TICK	9	3

TABLE 274. Bovine panel backgrounds screening in **multiplexed** format for the four FMDV signatures against the below listed **prokaryotes**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. None of the signatures cross-reacted with any of the samples listed below.

Description	FMDV-TC (32)	FMDV-PIR (33)
Erwinia amylovora	8	4
Actinobacillus suis	6	5
Aneurinbacillus migulanus	8	5
Bacillus cereus	5	5
Bacillus globigii	9	5
Bacillus subtilis	13	4
Bacillus thuringiensis	7	5
Bifidobacterium denticum	6	4
Borrelia burgdorferi	7	5
Burkholderia capacia	6	5
Caulobacter vibriodes	4	4
Clavibacter michganensis	5	4
Clostridium butyricum	11	5

*Corynebacterium pseudodiptheric*um	6	5
Cytophaga marinoflava	6	4
Erwinia herbicola	5	4
Escherichia coli	7	5
Geobacillus caldoxylosilyticus	6	4
Halomonas halmophila	17	4
Haemophilus influenza	14	4
Herbaspirillium seropedicae	12	5
Lactobacillus garvieae	8	4
Lactobacillus gasseri	9	5
Listeria monocytogenes	6	4
Listeria seeligeri	9	4
Micrococcus luteus	9	4
Moraxella lacunatica	7	4
Oceanospirillium ssp. Maris	5	5
Paenibacillus napthalaenovorans	5	5
Paracoccus dentrificans	5	5
Porphyrobacter sanguineus	13	5
Proteus mirabillis	9	4
Pseudomonas aeruginosae	5	4
Pseudomonas oleovorans	4	4
Rhizobium leguminosarum	6	5
Rhodococcus rhodochrous	4	4
Salmonella typhimurium	7	4
Simonsiella muelleri	7	4
Sphingomonas sp. (Alcaligenes sp)	5	5
Staphylococcus aureus	10	4
Streptococcus pneumoniae	12	4
Streptomyces scabiei	11	4
Tatlockia maceachernii	12	5
Vibrio parahaemolyticus	8	4
Xanthomonas translucens	9	4

TABLE 275. Bovine panel backgrounds screening in **multiplexed** format for the four FMDV signatures against the below listed **soils**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. None of the signatures cross-reacted with any of the samples listed below.

Description	FMDV-TC (32)	FMDV-PIR (33)
D 000107-49	6	5
D 000109 # 50	11	6

D 000402 # 53	7	6
D 000500 - 26 - 1	6	6
D 000501-14-1	6	6
D 000505 - 11 - 4	7	6
D 000521 - 23	7	5
D 000527 - 3	7	6
D 000531 - 21	6	6
D 000533 - 17 -1	6	5
D 000542 - 6	5	6
D 000550 - 20	7	6
D 000551 - 5	7	6
D 000561 - 8 - 6	6	6
D 000562 - 30 - 5	7	7
S 251	7	5
S 252	6	5
S 253	6	5
S 254	7	6
S 255	6	5
S 256	7	6
S 257	6	6
S 259	6	5
S 260	6	6
S 271	6	4
S 272	6	5
S 273	7	5
S 274	7	6
S 275	6	5
S 276	6	5
S 277	6	5
S 279	6	5
S 280	6	5
S 282	14	5
S 283	6	5
S 284	6	5
S 286	6	5
S 287	4	3
S 288	6	5
S 289	5	6
S 290	5	5
S 291	6	4
S 292	5	6
S 295	6	5
S 296	6	5
S 297	7	5
S 298	5	4
S 299	5	4
S 300	5	4

S 301	5	4
S 303	6	4
S 304	6	4
S 305	5	4
S 307	5	4

TABLE 276. Bovine Panel **Near-Neighbor** screening (Data from 20070601) against the 4 FMDV signatures. Values shown are Median Fluorescence Intensity (MFI) units. “Blanks” are buffer-only samples (no nucleic acid added to reaction), shown below in red. Extracted nucleic acid was added to each PCR reaction in triplicate totaling 200pg/rxn. The data shows that the FMDV signatures did not cross-react with any listed near-neighbors of the Bovine panel constituents.

Description	**FMDV-TC (32)**			**FMDV-Pir (33)**		
replicate	**1**	**2**	**3**	**1**	**2**	**3**
Blank	5	5	5	4	4	4
Blank	4	4	4	4	3	3
BHV A040150085	5	3	6	4	3	5
BHV (BFK)	5	5	4	4	4	3
BHV-1 A040130066	6	4	5	4	3	4
BHV-1 A033640072	6	4	4	4	3	4
BHV-1 ATCC VR 793	5	4	4	4	4	3
IBR CA 111903	5	2	6	4	1	4
IBR MN 111903	6	4	5	3	3	4
BHV-1 NVSL 231221	5	5	5	3	4	4
BHV-1 RA309	5	6	5	4	4	4
BHV-1 NVSL 97-10720	5	4	7	4	3	5
BHV-1 NVSL 51619	6	4	5	4	3	4
BHV-1 NVSL 86741	5	5	5	4	4	4
BHV-1 NVSL 200032	5	5	6	4	4	5
BHV-1 LA ATCC VR188	4	5	4	4	4	4
BHV-1 (IBR) Texas CAHFS A030020072	5	5	5	4	4	4
EHV-1 ATCC VR2003	5	5	4	5	3	4
EHV-1 A9904309	6	3	5	4	2	5
EHV-1 A011120004 CAHFS	5	4	4	6	5	5
EHV-1 NVSL 00002	4	4	4	3	4	4
EHV-2 ATCC VR701	5	4	4	4	3	3
EHV-1 A99043047 CAHFS	5	4	4	4	4	3
EHV-2 D990 CAHFS	4	4	5	4	3	4
Pseudorabies Titered	5	4	4	4	3	4
Pseudorabies NVSL 93-11745	5	4	4	4	2	3
Pseudorabies NVSL 92-12013	6	4	4	3	3	3
Pseudorabies RA180 CAHFS	5	5	4	4	3	4
Porcine Herpes Pseudorabies Shope	5	4	4	4	3	3
Feline Herpes ATCC VR636	5	4	4	6	6	5
Caprine Herpes ATCC VR462	5	5	4	4	3	3
Caprine Herpes S0201998 CAHFS	5	7	5	4	4	3

Caprine Herpes D0201157 CAHFS	6	6	5	3	4	4
BHV-5 A040150085 CAHFS	9	8	6	5	4	4
BHV-5 A032540006 CAHFS	4	5	4	4	3	3
BHV-5 D9403153 CAHFS	5	4	4	4	3	3
BHV-5 D9402133 CAHFS	5	4	4	3	4	3
BDV Coos Bay	5	4	4	4	3	3
EHD-1 Georgia	6	4	5	5	2	4
EHD-1 New Jersey	5	4	4	3	4	3
EHD-1 Santa Barbara	5	5	4	4	3	3
EHD-2 Alberta	5	4	4	4	4	4
Fowl Pox	5	5	4	4	4	3
Parainfluenza Type 3	4	5	4	3	4	3
Respiratory Syncytial	5	8	4	4	3	3

Multiplexed PCR Assay Titrations (Bovine Panel)

Upon finalization of the assay panel constituents each agent assay is characterized in multiplexed PCR format by running dose-response (titration) curves against titered virus extracted from virus infected cell culture for each available agent strain. Each sample is tested in duplicate and reported as a mean value of the median fluorescence intensity units (MFI) against agent concentration (per reaction volume, 5uL) on a double log plot. Concentration units are reported in TCID50/rxn (assumes 5uL sample volume per reaction) or pfu/rxn depending on virus type and source. In some cases where titered virus was not available data is reported as picogram units (pg). Each assay is evaluated by its ability to detect agent strains and differentiate from near neighbors (specificity) and by its range of sensitivity to each strain relative to each signature tested for that agent. Because this method is not aimed at determination of analytical sensitivities (requiring a more extensive sample set tested in clinical sample matrices which is not practical for this stage of development) it does however, provide tentative limits of detection that are used to measure the performance of each assay.

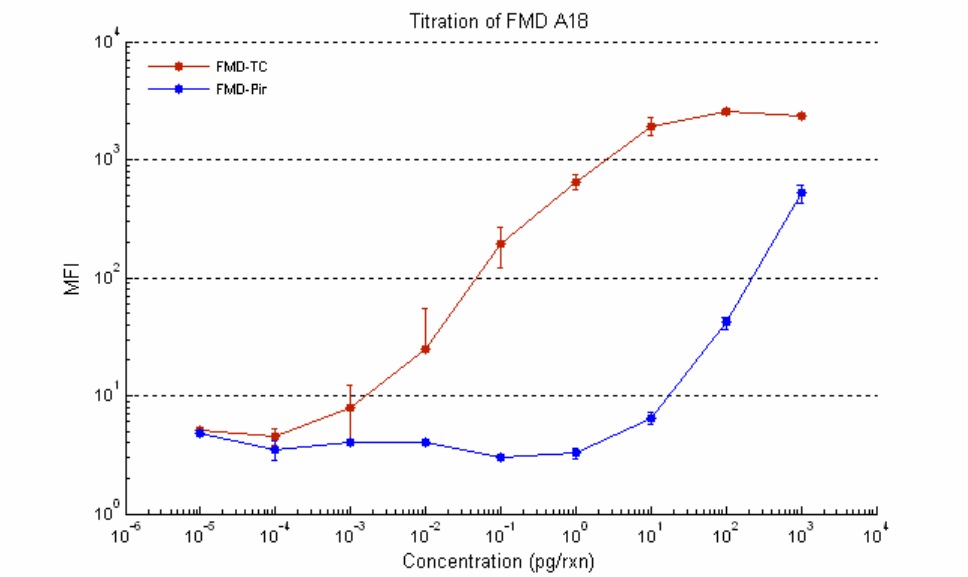


FIG. 35. LLNL bovine multiplex screening data for the two FMDV signatures against extracted nucleic acids from isolate FMDV serotype A strain 18. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1 of the mean.

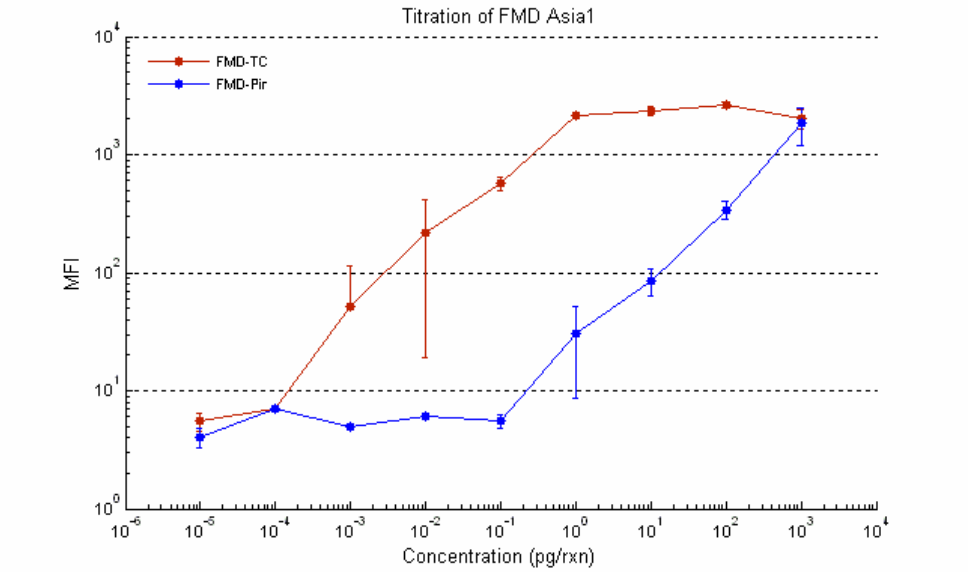


FIG. 36. LLNL bovine multiplex screening data for the two FMDV signatures against extracted nucleic acids from isolate FMDV serotype Asia strain 1. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Error bars indicate ±1 of the mean.

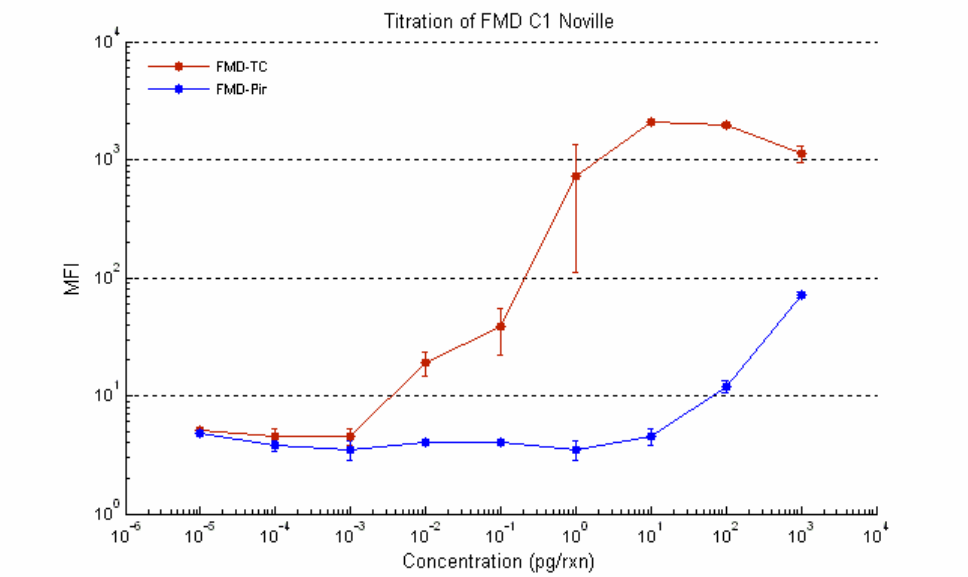


FIG. 37. LLNL bovine multiplex screening data for the two FMDV signatures against extracted nucleic acids from isolate FMDV serotype C strain 1 Noville. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1 of the mean.

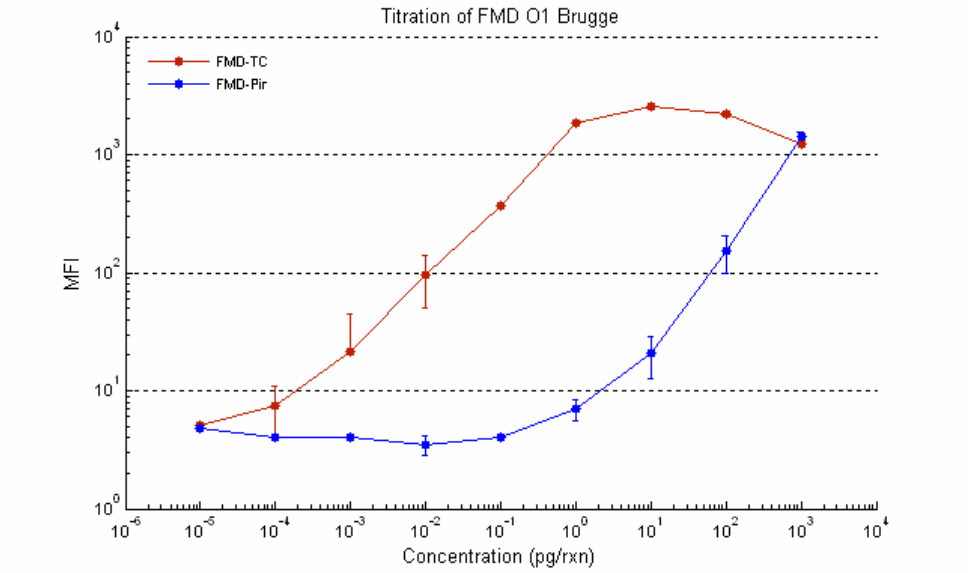


FIG. 38. LLNL bovine multiplex screening data for the two FMDV signatures against extracted nucleic acids from isolate FMDV serotype O strain 1 Brugge. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1 of the mean.

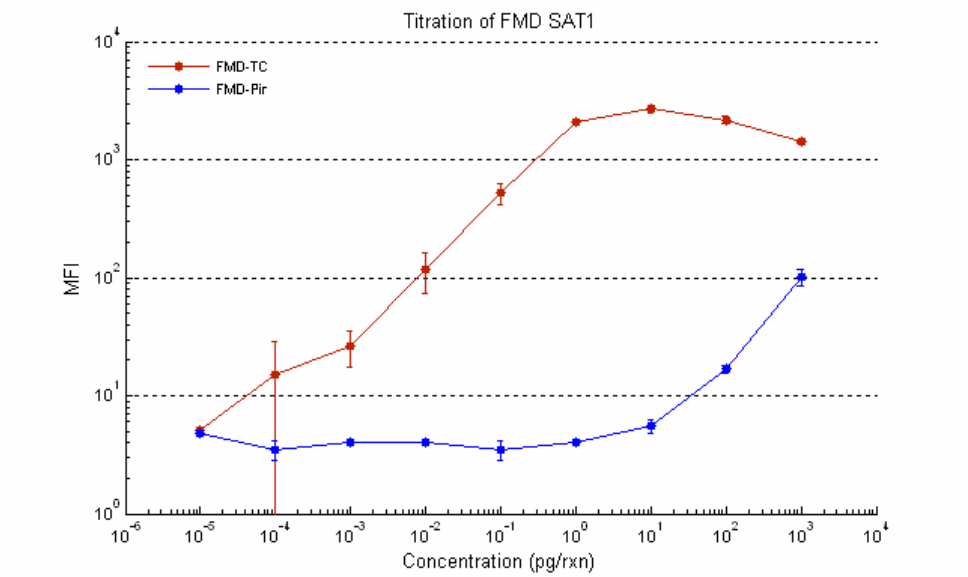


FIG. 39. LLNL bovine multiplex screening data for the two FMDV signatures against extracted nucleic acids from isolate FMDV serotype SAT1. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1 of the mean.

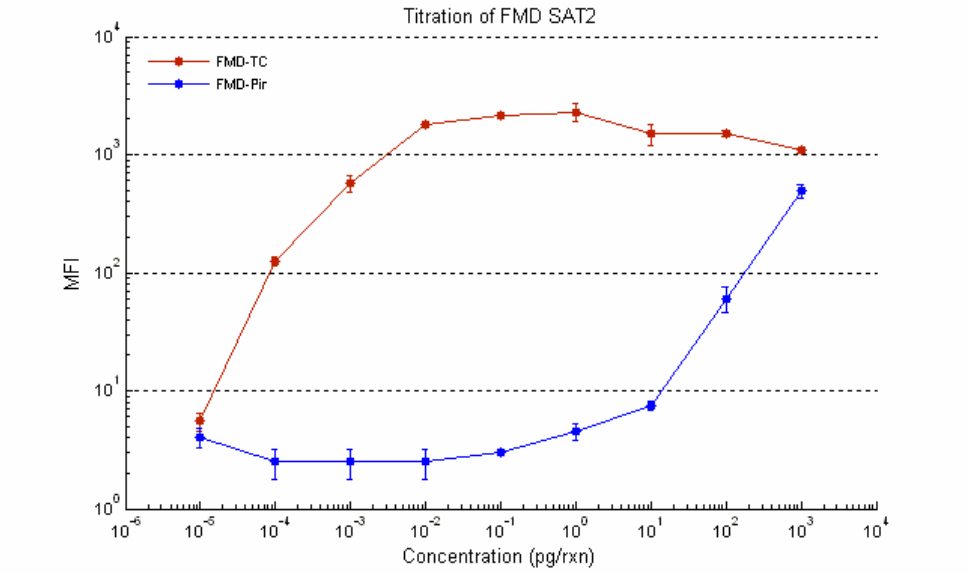


FIG. 40. LLNL bovine multiplex screening data for the two FMDV signatures against extracted nucleic acids from isolate FMDV serotype SAT2. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1 of the mean.

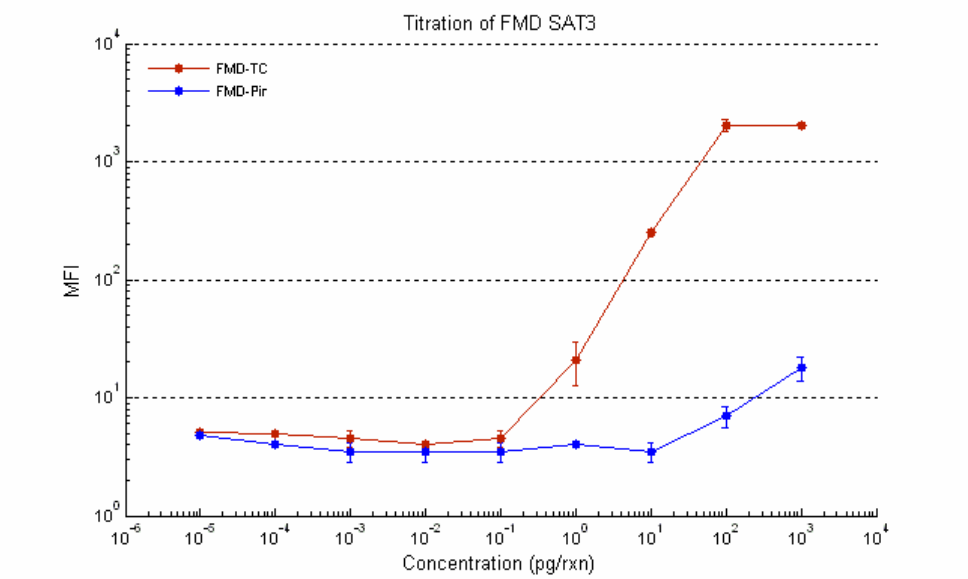


FIG. 41. LLNL bovine multiplex screening data for the two FMDV signatures against extracted nucleic acids from isolate FMDV serotype SAT3. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1 of the mean.

Pending additional Target Screening at PIADC (in process)

RESULTS: The two FMDV signatures were incorporated into the Bovine panel with no consequence to the other panel constituents. The performance of the FMDV signatures is shown above, and similarly to the performance of the signatures in the Version 1.0 panel, the Tetracore signature is consistently more sensitive in detecting all seven FMDV serotypes. Additional testing is in process at PIADC.

8.5.2. PORCINE PANEL MULTIPLEXED PCR DATA

.**Preliminary screening:** Preliminary screening can be broken down into several steps. The signatures are preliminarily screened by first assessing how the signatures will perform under multiplex PCR conditions and whether or not the addition of the new signatures will adversely effect other signatures, these preliminary screening methods are described as “signature baseline screening” and “multiplex addition screening” respectively. First signature baseline screening tests the performance of the signature in a no-target reaction (blank), a “passing” score is indicated by a low (typically less than 100) MFI. All FMDV signatures passed the signature baseline screening. Next in multiplex addition screening the new signature primers are added one-by –one to the other panel signatures to determine if there would be any cross-reactions between primer pairs in the panel. Further assessment of the signatures is done through target

and near-neighbor screening and the determination of relative sensitivities. Preliminary screening of the FMDV signatures was addressed in previous (FY2005) work and is not detailed here.

Near-neighbor and Target screening: The two signatures were added to the Porcine panel. The signatures exhibited very low background response (<10 MFI) in the Porcine panel. Target screening for FMDV was conducted at both LLNL and Plum Island Animal Disease Center [PIADC], New York. Preliminary (non-target or nucleic acid only) screening was conducted at LLNL.

TABLE 277. Backgrounds screening in multiplexed format for FMDV at LLNL and PIADC. Additional target screening is pending at PIADC, July 2007

	Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors	Targets
Mux PCR Screening	54	135	16	0	51 (+)	7(7 pending)

[1]-There are 752 pooled samples in each Aerosol Block.

TABLE 278. Backgrounds screening in **multiplexed** format for down-selected FMDV signatures against the below listed **eukaryotes**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. No cross-reactions were observed.

Description	FMDV-TC (32)	FMDV-Pir (33)
BOVINE	18	8
CAT	17	7
CHICKEN	14	7
DOG	18	8
DROSOPHILA MELANOGASTER	19	8
EQUINE	19	8
FLEA	20	9
HUMAN	17	8
MONKEY	21	9
MOSQUITO	15	7
MOUSE	19	8
PIG / PORCINE	20	10
RABBIT	20	9
RAT	22	10
SHEEP	21	9
TICK	20	9

TABLE 279. Backgrounds screening in **multiplexed** format for the two FMDV signatures against the below listed **prokaryotes**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median

Fluorescence Intensity (MFI) units. Neither signature cross-reacted with any of the samples listed below.

Description	FMDV.TC (32)	FMDV.PIR (33)
Erwinia amylovora	20	10
Actinobacillus suis	14	6
Aneurinbacillus migulanus	15	6
Bacillus cereus	19	9
Bacillus globigii	14	7
Bacillus subtilis	17	8
Bacillus thuringiensis	20	9
Bifidobacterium denticum	19	7
Borrelia burgdorferi	22	10
Burkholderia capacia	19	9
Caulobacter vibriodes	15	7
Clavibacter michganensis	20	9
Clostridium butyricum	22	10
Corynebacterium pseudodipthericum	20	10
Cytophaga marinoflava	21	10
Erwiniaa herbicola	19	9
Escherichia coli	22	10
Geobacillus caldoxylosilyticus	22	10
Halomonas halmophila	17	8
Haemophilus influenza	17	8
Herbaspirillium seropedicae	17	7
Lactobacillus garvieae	10	5
Lactobacillus gasseri	14	7
Listeria monocytogenes	17	8
Listeria seeligeri	22	10
Micrococcus luteus	15	7
Moraxella lacunatica	19	8
Oceanospirillium ssp. Maris	20	9
Paenibacillus napthalaenovorans	19	9
Paracoccus dentrificans	19	8
Porphyrobacter sanguineus	14	6
Proteus mirabillis	22	9
Pseudomonas aeruginosae	15	7
Pseudomonas oleovorans	13	7
Rhizobium leguminosarum	22	9
Rhodococcus rhodochrous	18	8
Salmonella typhimurium	18	8
Simonsiella muelleri	15	7
Sphingomonas sp. (Alcaligenes sp)	16	8
Staphylococcus aureus	21	9
Streptococcus pneumoniae	23	10
Streptomyces scabiei	22	9
Tatlockia maceachernii	21	9
Vibrio parahaemolyticus	21	10

Xanthomonas translucens	20	9

TABLE 280. Porcine panel **backgrounds** screening in **multiplexed** format for the six FMDV signatures against the below listed **soils**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. Neither signature cross-reacted with any of the soil samples listed below.

Description	FMDV-TC (32)	FMDV-Pir (33)
D 000107-49	15	8
D 000109 # 50	15	7
D 000402 # 53	19	9
D 000500 - 26 - 1	16	7
D 000501-14-1	18	9
D 000505 - 11 - 4	16	8
D 000521 - 23	18	9
D 000527 - 3	17	8
D 000531 - 21	17	8
D 000533 - 17 -1	14	7
D 000542 - 6	18	8
D 000550 - 20	18	8
D 000551 - 5	19	9
D 000561 - 8 - 6	18	9
D 000562 - 30 - 5	19	8
S 251	15	8
S 252	15	8
S 253	15	7
S 254	16	8
S 255	15	7
S 256	17	9
S 257	14	8
S 259	14	7
S 260	15	7
S 271	16	8
S 272	15	8
S 273	18	8
S 274	17	9
S 275	17	8
S 276	17	8
S 277	18	8
S 279	15	7
S 280	17	8
S 282	17	8
S 283	16	8
S 284	18	8
S 286	16	8
S 287	17	8

S 288	17	8
S 289	15	8
S 290	17	8
S 291	16	7
S 292	15	8
S 295	15	7
S 296	15	7
S 297	17	8
S 298	16	8
S 299	13	7
S 300	13	6
S 301	16	7
S 303	14	7
S 304	16	7
S 305	18	8
S 307	16	8

TABLE 281. Porcine Panel **Near-Neighbor** Screening (Data from 20070601) against FMDV signatures. Values shown are Median Fluorescence Intensity (MFI) units. “Blanks” are buffer-only samples (no nucleic acid added to reaction), shown below in red. Extracted nucleic acid was added to each PCR reaction in triplicate totaling 200pg/rxn. The data shows that the FMDV signatures did not cross- reacted with any listed near-neighbors of the Porcine panel constituents.

Description	FMDV.TC (32)	FMDV.PIR (33)
Blank	24	10
Blank	21	7
BDV Coos Bay	18	8
BHV (BFK) A03250006 DN-599	19	9
BHV A040150085	18	8
BHV-1 (IBR) Texas A030020072 CAHFS	21	9
BHV-1 A033640072	18	7
BHV-1 A040130066	20	9
BHV-1 ATCC VR 793	18	8
BHV-1 NVSL 10720	19	8
BHV-1 NVSL 200032	18	8
BHV-1 NVSL 231221	18	8
BHV-1 NVSL 51619	17	7
BHV-1 NVSL 86741	19	8
BHV-1 or IBR LA ATCC VR188	20	9
BHV-1 RA309	14	7
BHV-5 A032540006 CAHFS	16	7
BHV-5 A040150085 CAHFS	17	8
BHV-5 D9402133 CAHFS	15	7
BHV-5 D9403153 CAHFS	17	7
Caprine Herpes D0201157 CAHFS	18	7
Caprine Herpes-2 ATCC VR 462	18	8
Caprine Herpes-2 S0201998 CAHFS	14	6

EHD-1 A9904309	19	7
EHD-1 Georgia	17	8
EHD-1 New Jersey	10	6
EHD-1 Santa Barbara	14	6
EHD-2 Alberta	16	7
EHV-1 A011120004 CAHFS	22	11
EHV-1 A99043047	19	8
EHV-1 ATCC VR2003	17	8
EHV-2 ATCC VR701	17	8
EHV-2 D990 CAFHS	13	6
EHV-2 NVSL 0002	18	8
Feline Herpes ATCC VR 636	18	7
Fowl Pox	18	8
IBR CA 111903	20	9
IBR MN 111903	16	8
Parainfluenza Type 3	20	9
Porcine Herpesvirus or Pseudorabies Shope	17	8
Pseudorabies NVSL 92-12013	16	7
Pseudorabies NVSL 93-11745	20	9
Pseudorabies RA 180 CAHFS	16	7
Pseudorabies Titered	19	8
Respiratory Syncytial	19	9

Multiplexed PCR Assay Titrations (Porcine Panel)

Upon finalization of the assay panel constituents each agent assay is characterized in multiplexed PCR format by running dose-response (titration) curves against titered virus extracted from virus infected cell culture for each available agent strain. Each sample is tested in duplicate and reported as a mean value of the median fluorescence intensity units (MFI) against agent concentration (per reaction volume, 5uL) on a double log plot. Concentration units are reported in TCID50/rxn (assumes 5uL sample volume per reaction) or pfu/rxn depending on virus type and source. In some cases where titered virus was not available data is reported as picogram units (pg). Each assay is evaluated by its ability to detect agent strains and differentiate from near neighbors (specificity) and by its range of sensitivity to each strain relative to each signature tested for that agent. Because this method is not aimed at determination of analytical sensitivities (requiring a more extensive sample set tested in clinical sample matrices which is not practical for this stage of development) it does however, provide tentative limits of detection that are used to measure the performance of each assay.

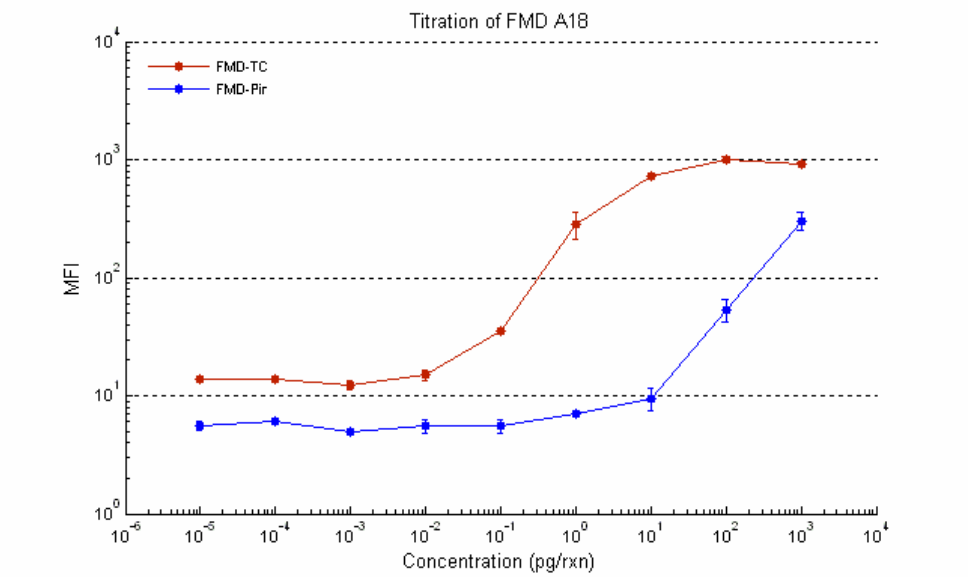


FIG. 42. LLNL procine multiplex screening data for the two FMDV signatures against extracted nucleic acids from isolate FMDV serotype A strain 18. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Error bars indicate ±1 of the mean.

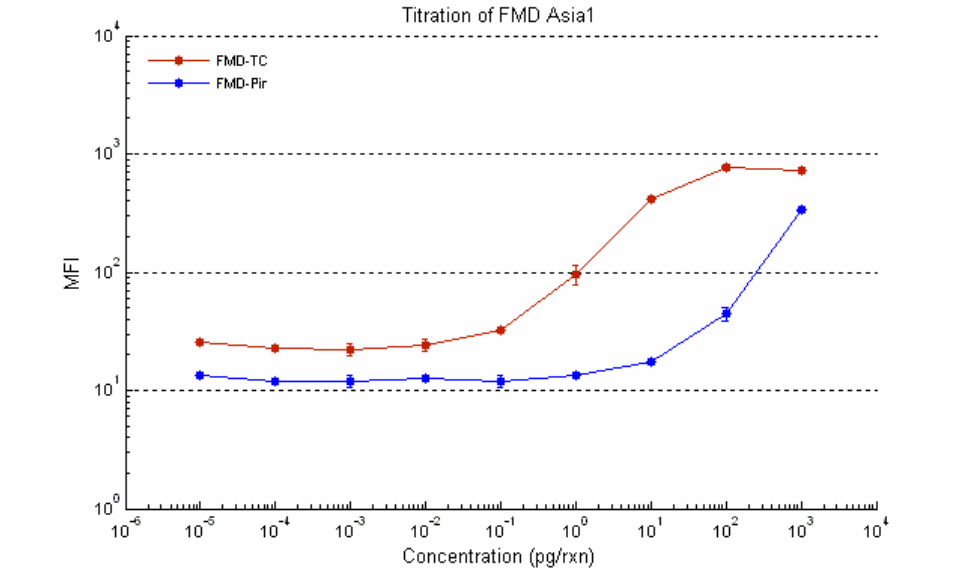


FIG. 43. LLNL porcine multiplex screening data for the two FMDV signatures against extracted nucleic acids from isolate FMDV serotype Asia strain 1. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1 of the mean.

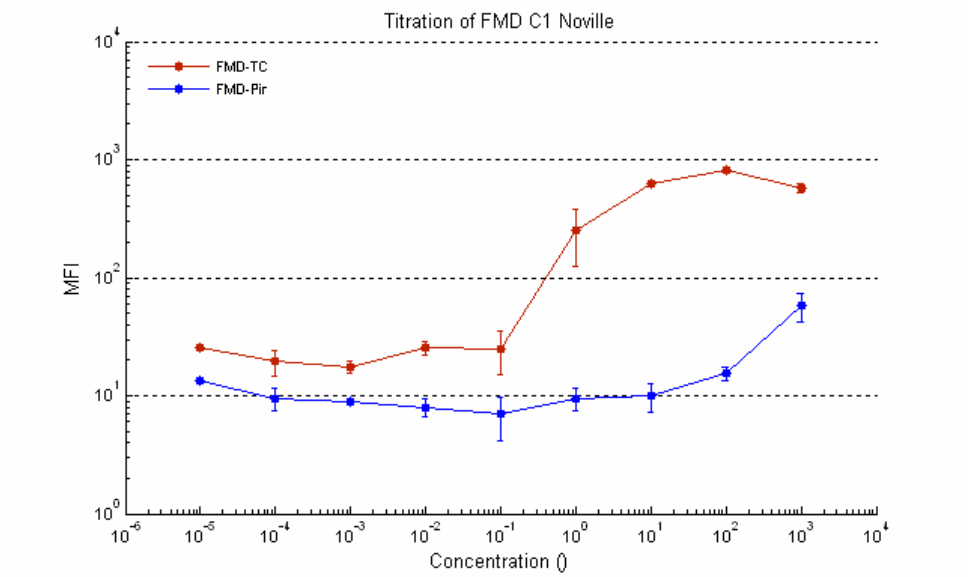


FIG. 44. LLNL porcine multiplex screening data for the two FMDV signatures against extracted nucleic acids from isolate FMDV serotype C strain 1 Noville. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1 of the mean.

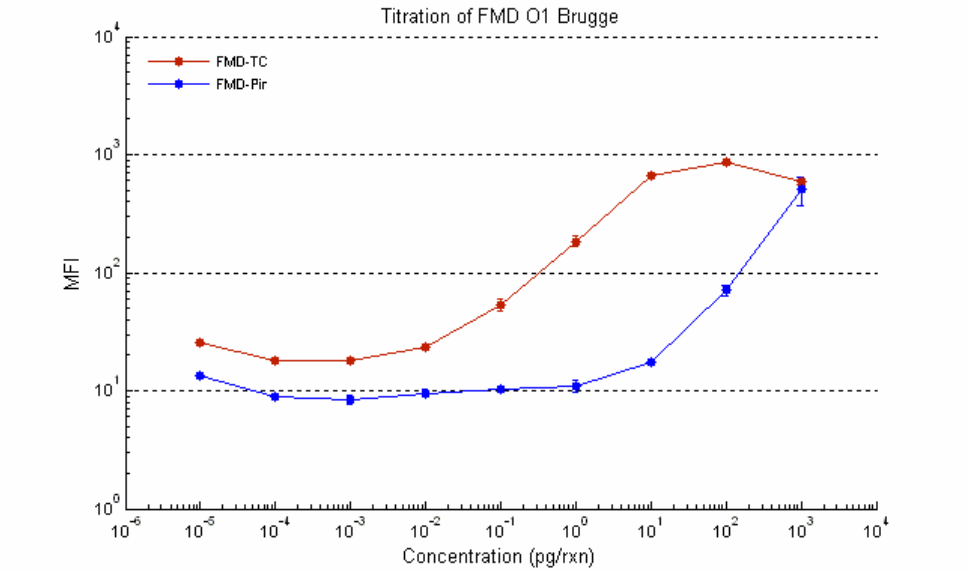


FIG. 45. LLNL porcine multiplex screening data for the two FMDV signatures against extracted nucleic acids from isolate FMDV serotype O strain 1 Brugge. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1 of the mean.

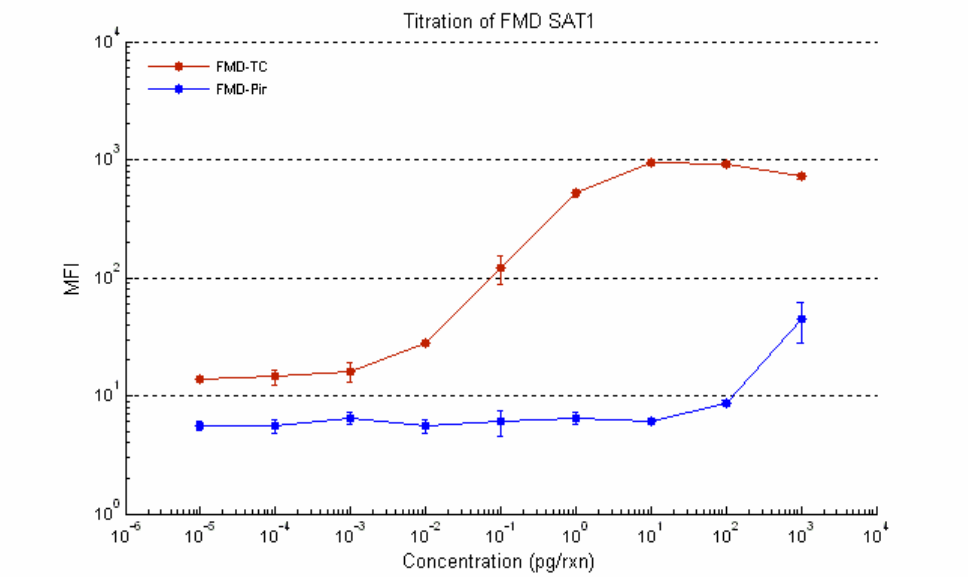


FIG. 46. LLNL porcine multiplex screening data for the two FMDV signatures against extracted nucleic acids from isolate FMDV serotype SAT1. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1 of the mean.

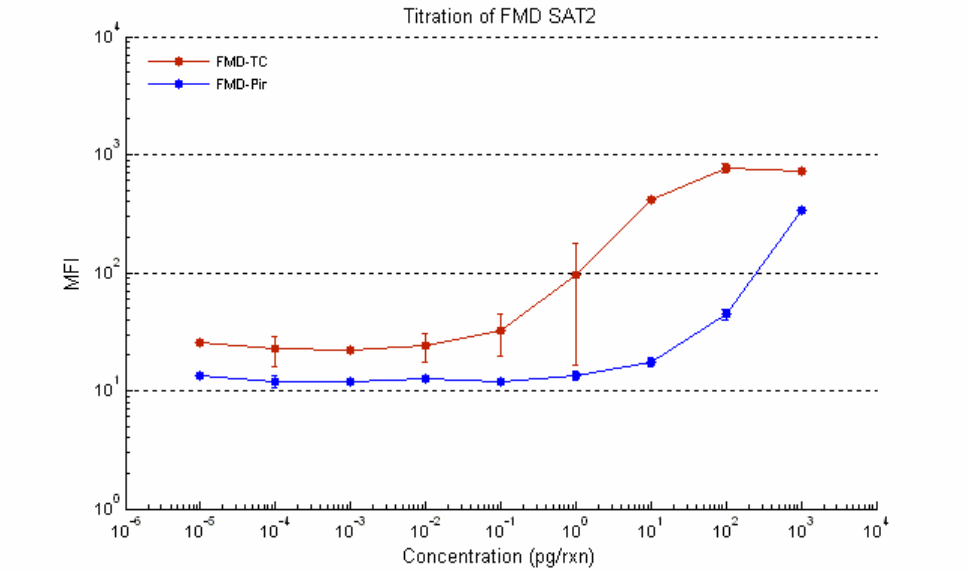


FIG. 47. LLNL porcine multiplex screening data for the two FMDV signatures against extracted nucleic acids from isolate FMDV serotype SAT2. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1 of the mean.

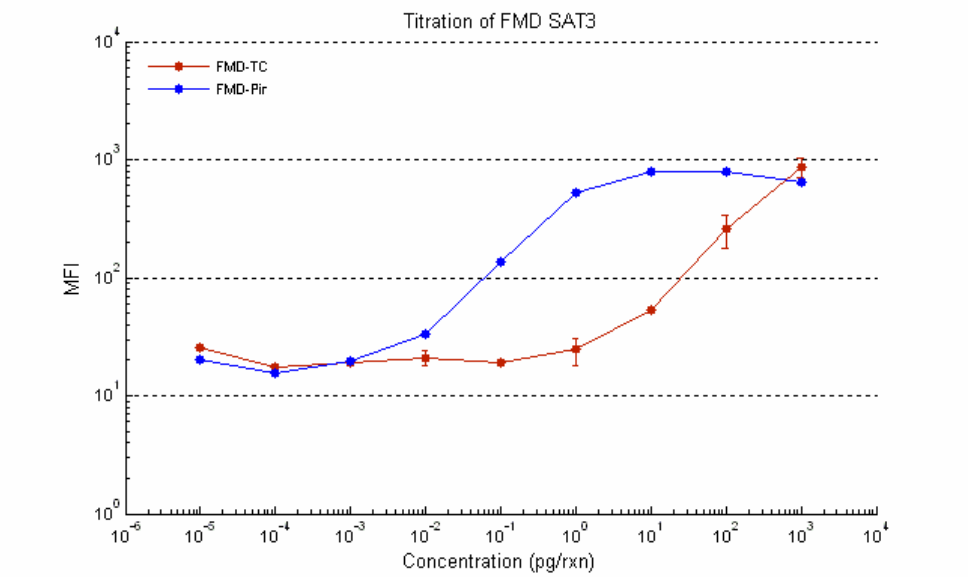


FIG. 48. LLNL procine multiplex screening data for the two FMDV signatures against extracted nucleic acids from isolate FMDV serotype SAT3. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1 of the mean.

Pending additional Target Screening at PIADC (in process)

RESULTS: The two FMDV signatures were incorporated into the Porcine panel with no consequence to the other panel constituents. The performance of the FMDV signatures is shown above, and similarly to the performance of the signatures in the Version 1.0 panel, the Tetracore signature is consistently more sensitive in detecting the various FMDV serotypes. Additional testing is in process at PIADC.

9. SWINE VESICULAR DISEASE (PORCINE PANEL)

OBJECTIVE: In 2005 we were tasked by the Department of Homeland Security to develop specific and reliable Real-time RT- PCR signatures for Swine Vesicular Disease Virus [SVD], among other major agriculturally-impacting viruses for the development of a Foot-and-Mouth Disease rule-out panel. In 2006 the goal of these assays (collectively with other virus-specific signatures developed in parallel) is to develop a species specific (i.e.porcine) multiplexed detection assay for Foot-and-Mouth disease rule-out for use in veterinary diagnostic laboratories. The three signatures that were developed and incorporated into the Version 1.0 FMDV Rule-out panel have been re-evaluated for use in a porcine-specific FMDV rule-out panel. This document describes the historic data and information as preliminary 2006 data screening the 3 SVD signatures for inclusion into the Porcine panel. Screening of the signatures in the porcine panel multiplex against SVD targets was occurring at the writing of this report.

9.1. BACKGROUND AND ETIOLOGY OF SVD

Swine vesicular disease (SVD) is typically a transient disease of pigs in which vesicular lesions appear on the feet and snout and in the mouth. It does not cause severe production losses, and recent outbreaks of infection have been mainly subclinical. However, infection is of major economic importance because it must be differentiated from foot-and-mouth disease, eradication is costly, and embargoes on export of pigs and pork products are often imposed on nations not free of SVD.

Although infection in laboratory workers has occurred, and the virus may be present in sheep or cattle, pigs are said to be the only natural host. The disease was first identified in Italy in 1966 and subsequently in Hong Kong, Japan, Taiwan, and 16 countries in Europe. Although SVD virus was eradicated from Japan in the mid-1970s and most European countries by the mid-1980s, it has remained endemic in Italy and caused sporadic outbreaks of disease in other European countries during the 1990s and in Portugal in 2003 and 2004.

The causal agent is an enterovirus of the family Picornaviridae. It belongs to the species human enterovirus B and is thought to have evolved from the human pathogen coxsackievirus B5, with which it shares a close antigenic and genetic relationship. There is only 1 serotype of SVD virus, although isolates may be differentiated by antigenic or genetic typing and may differ in virulence. SVD virus is transmitted by direct or indirect contact or by feeding infected pork or pork products. Infection can give rise to viremia and generalized vesicles that contain large amounts of virus[9].

9.2. SVD VIRUS COMPREHENSIVE ASSAY SUMMARY

Bioinformatics

Virus name: Swine Vesicular Disease Virus
Signature generation reference: Full genome sequences for 5 isolates of SVD.
Level of Discrimination: Species; in the final panel, SVD_1727049 hits strain 7, SVD_1727050 hits strain 5, SVD_1727051 hits strain 6.
Number of Candidate Signatures: 4
Number of Signatures forwarded to gel/ real-time bench-screening: 4
Number of Signatures forwarded to multiplexed bench-screening: 4

Real-time PCR Screening Summary

TABLE 282. Final four signatures resulting from bench gel and real-time PCR screening.

LLNL Signature Designation	Sequence

[9] *Source: The Merck Veterinary Manual*

http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/54400.htm&word=swine%2cvesicular%2cdisease

SVD_1727049.P	CGTCACAAGTTGTACCATCAGACACAATGCA
SVD_1727049.F	CAGGATAATTTCTTCCAAGGGC
SVD_1727049.R	ACGTGAACATTTCGAGCTTCC
SVD_1727050.P	TGACCGTAATGAGGTCATCGTGATTTCTCAC
SVD_1727050.F	GACTTGTTGTGGCTGGAGGA
SVD_1727050.R	CAGCGCCATGGTGAGGTAG
SVD_1727051.P	CTGGCGTCATAGCCTGAATAGTCAAACGCTA
SVD_1727051.F	GACAAAGTGGCCAAGGGAAA
SVD_1727051.R	CACGTAAACCACACTGGGCT
SVD_1727052.P	TAACCTGGACAAACGTGACCTTCCTGAAAAG
SVD_1727052.F	CCCAGCAGATAAAGGCGAGT
SVD_1727052.R	GCATGACAGGATGGACCAAA

TABLE 283. Summary of wet-bench screening in signature down-selection. No cross reactions were seen in gel or Real-time PCR screening. All 4 signatures were advanced to multiplexed screening.

	Soils	Eukaryotes	Prokaryotes	Aerosols[1]	Near Neighbors	Target
Gel Screening	13	13	10	none	none	none
Real-time PCR Screening	15	16	14	1 Aerosol Block	11	7

[1]There are 752 pooled samples in each Aerosol Block.

Multiplexed PCR Screening Summary

TABLE 284. Historic data from the Version 1.0 panel: SVD signatures from multiplexed down-selection and their relative sensitivity ranges when challenged with extracted nucleic acid from target RNA tested at PIADC.

			Sensitivity Data for final signatures ($TCID_{50}$/mL)			
Agent	Serotype	Strain	SVD-1	SVD-2	SVD-3	Original titer (Log10 TCID50)
SVD	N/A	HKN-12-87	4.38E+02	4.10E+03	7.88E+02	9.11 ± 0.13
SVD	N/A	ITL 1-91	8.34E+05	6.90E+02	5.24E+02	8.49 ± 0.14
SVD	N/A	TAW 1-19	2.39E+02	1.55E+03	3.38E+01	7.43 ± 0.17
SVD	N/A	UKG-72	1.44E+02	3.46E+02	5.81E+01	8.05 ± 0.12
SVD	N/A	ITL 1-66	4.93E+03	2.78E+03	4.85E+03	8.61 ± 0.15
SVD	N/A	ROM 1-87	2.94E+05	X	8.98E+04	7.49 ± 0.14
SVD	N/A	HKN 4-89	1.15E+07	3.36E+02	3.55E+01	8.3 ± 0.14

TABLE 285. Backgrounds screening in multiplexed format for SVD at LLNL and PIADC. Additional screening of SVD targets and near-neighbors is pending at PIADC, Aug, 2007.

	Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors[2]	Targets
Mux PCR Screening	54	135	16	0	pending	pending

[1-]There are 752 pooled samples in each Aerosol Block. [2]Near-neighbors indicated (43) apply to the near-neighbor panel that is not uniquely specific for BHV, but for the other panel constituents that were screened concurrently.

TABLE 286. Summary of multiplexed SVD signatures for Porcine panel.

LLNL Signature Designation	MUX Group ID	Gene ID	Relative Limit of detection[1]	Targets Screened	Near Neighbors Screened
SVD_1727049	SVD-1	ID protein, coat protein	pending	pending	pending
SVD_1727050	SVD-2	Membrane permeability enhancement	pending	pending	pending
SVD_1727051	SVD-3	RdRp	pending	pending	pending

[1] The relative "Limit of detection" is described as a range of lowest and highest sensitivity determined by multiple target strains screened and is described further in the Multiplex results summary report section.

9.3. SVD SIGNATURE BIOINFORMATICS AND GENERATION INFORMATION

Bioinformatics process:
The LLNL Bioinformatics team have developed a computational process that uses available nucleic acid sequence information for a given target organism to generate candidate nucleic acid signature sequences that are conserved and unique. Signatures are primarily used to underlie reliable and specific pathogen detection assays. Evidence of the success of the process is that several of the Real-time PCR signatures that are used in the national BioWatch monitoring system were generated by this process, and to date, has resulted in no false positives.

The general approach is the following: All available complete genomes of different strains of the target species are compared using a multiple genome alignment programs. A *consensus gestalt* is formed from the alignments that contain the sequence conserved amongst all target inputs. (This step is bypassed if only one target sequence is available).

To determine that organism-conserved sequence which does not occur in any other sequenced microbial organism, the consensus gestalt is compared against our immense, continuously updated database of microbial organism. A customized algorithm accomplishes this electronic subtraction, and the result is a *uniqueness gestalt* that is mined for potential signature candidates. A final computational screening is done to verify that cross-reactions are not detected.

Swine Vesicular Disease Virus is an FMDV look alike that occurs in pigs.

Target Virus Information.
Virus name: Swine Vesicular Disease Virus
Type: +RNA
Genome size: 7406 bp

Primer/Probe Set Generation Information
Alignment and All Microbe Database subtraction.

TABLE 287. K-path run id: 61307. Genome Sequences used for alignment.

	Genome Description	GI Number	Sequence Length (bp)	K-path ID
1	Swine vesicular disease virus strain NET/1/92, complete genome	8896132	7406	61307

2	PISVDV Swine vesicular disease virus complete genomic RNA	61167	7400	61307
3	SVDMPS Swine vesicular disease virus gene for polyprotein, complete cds	37993797	7401	61307
4	Swine vesicular disease virus strain HK'70, complete genome	402536	7401	61307
5	Swine vesicular disease virus (STRAIN H/3 '76) genomic RNA, complete genome	1228947	7401	61307

TABLE 288. Primer/Probe Design from Candidates list using Primer3.

Primer3 Main Parameters	
Parameters	**Standard Settings**
PRIMER_OPT_SIZE	**20**
PRIMER_MIN_SIZE	**18**
PRIMER_MAX_SIZE	**27**
PRIMER_PRODUCT_OPT_SIZE	**100**
PRIMER_PRODUCT_SIZE_RANGE	**71-600**
PRIMER_OPT_TM	**62**
PRIMER_MIN_TM	**61**
PRIMER_MAX_TM	**63**
PRIMER_MIN_GC	**20**
PRIMER_MAX_GC	**80**
PRIMER_PICK_INTERNAL_OLIGO	**1**
PRIMER_INTERNAL_OLIGO_OPT_SIZE	**31**
PRIMER_INTERNAL_OLIGO_MIN_SIZE	**18**
PRIMER_INTERNAL_OLIGO_MAX_SIZE	**36**
PRIMER_INTERNAL_OLIGO_OPT_TM	**72**
PRIMER_INTERNAL_OLIGO_MIN_TM	**71**
PRIMER_INTERNAL_OLIGO_MAX_TM	**73**
Number of Primers/Probe Set generated:	**4**

Signature Information

Source: LLNL
Project name: SVD re run for plum
Level of discrimination: Species

Number of Candidate Signatures: 4.
Number of Signatures forwarded to gel and real-time PCR bench-screening: 4
Number of Final Signature forwarded to multiplexed assay development: 4

Signature list

Note: For a listing of computationally predicted product sequences, please see attached document “SVD Taqsim Run Data”.

Taqsim description

We used a computational TaqMan simulator program, "Taqsim", to identify all potential targets for each signature from all sequences available in genbank. Taqsim is a BLAST-based comparison of each signature as a triplet against all sequences in genbank to identify the targets that are predicted to produce a TaqMan reaction at 57 degrees primer annealing and 67 degrees for probe annealing (these temperatures are according to Primer 3 oligo Tm calculations). The first column of the excel spreadsheets list the signatures by the name, and the following columns report the predicted genbank targets, coordinates of signature region, amplicon size, and accession #'s for each target.

TABLE 289a-d. Signature bioinformatics. All sequences are listed in the 5'→ 3' direction.

(a) SVD_1727049

Target Virus	Enterovirus Swine Vesicular Disease Virus NET HK70
Forward Primer	SVD_1727049.F
FWD Primer Length (bp)	22
FWD Primer TM (°C)	61
FWD Primer GC Content (%)	45
Forward Sequence	CAGGATAATTTCTTCCAAGGGC
Reverse Primer	SVD_1727049.R
Rev Primer Length (bp)	21
Rev Primer TM (°C)	61
Rev Primer GC Content (%)	47
Reverse Sequence	ACGTGAACATTTCGAGCTTCC
Probe Name	SVD_1727049.P
Probe Length (bp)	31
Probe TM (°C)	62
Probe GC Content (%)	45
Probe Sequence	CGTCACAAGTTGTACCATCAGACACAATGCA
Probe strand	plus
Predicted Product Size	349

(b) SVD_1727050

Target Virus	Enterovirus Swine Vesicular Disease Virus NET HK70
Forward Primer	SVD_1727050.F
FWD Primer Length (bp)	20
FWD Primer TM (°C)	61
FWD Primer GC Content (%)	55
Forward Sequence	GACTTGTTGTGGCTGGAGGA
Reverse Primer	SVD_1727050.R
Rev Primer Length (bp)	19
Rev Primer TM (°C)	62
Rev Primer GC Content (%)	63
Reverse Sequence	CAGCGCCATGGTGAGGTAG
Probe Name	SVD_1727050.P

Probe Length (bp)	31
Probe TM (^{o}C)	61
Probe GC Content (%)	45
Probe Sequence	TGACCGTAATGAGGTCATCGTGATTTCTCAC
Probe strand	minus
Predicted Product Size	281

(c) SVD_1727051

Target Virus	Enterovirus Swine Vesicular Disease Virus NET HK70
Forward Primer	SVD_1727051.F
FWD Primer Length (bp)	20
FWD Primer TM (^{o}C)	62
FWD Primer GC Content (%)	50
Forward Sequence	GACAAAGTGGCCAAGGGAAA
Reverse Primer	SVD_1727051.R
Rev Primer Length (bp)	20
Rev Primer TM (^{o}C)	61
Rev Primer GC Content (%)	55
Reverse Sequence	CACGTAAACCACACTGGGCT
Probe Name	SVD_1727051.P
Probe Length (bp)	31
Probe TM (^{o}C)	63
Probe GC Content (%)	48
Probe Sequence	CTGGCGTCATAGCCTGAATAGTCAAACGCTA
Probe strand	minus
Predicted Product Size	248

(d) SVD_1727049

Target Virus	Enterovirus Swine Vesicular Disease Virus NET HK70
Forward Primer	SVD_1727052.F
FWD Primer Length (bp)	20
FWD Primer TM (^{o}C)	61
FWD Primer GC Content (%)	55
Forward Sequence	CCCAGCAGATAAAGGCGAGT
Reverse Primer	SVD_1727052.R
Rev Primer Length (bp)	20
Rev Primer TM (^{o}C)	61
Rev Primer GC Content (%)	50
Reverse Sequence	GCATGACAGGATGGACCAAA
Probe Name	SVD_1727052.P
Probe Length (bp)	31
Probe TM (^{o}C)	62

Probe GC Content (%)	41
Probe Sequence	TAACCTGGACAAACGTGACCTTCCTGAAAAG
Probe strand	plus
Predicted Product Size	113

Target Region Gene Information

TABLE 290a-b. (a) Reference genomes used for gene information. (b) Gene information for each signature (a)

(a)

	Genome Description	GI Number	Sequence Length (bp)
1	**Swine vesicular disease virus strain NET/1/92, complete genome**	8896132	7406

(b)

			Gene Location		Target Region Location	
Primer	**Gene**	**Description**	**Start**	**End**	**Start**	**End**
SVD_1727049.F	VP1	ID protein, coat protein	2447	3295	2427	2777
SVD_1727050.F	P2B	Membrane permeability enhancement	3746	4042	3710	3990
SVD_1727051.F	P3D	RdRp	5912	7297	6407	6654
SVD_1727052.F	P3D	RdRp	5912	7297	6979	7091

9.4. SVD GEL AND REAL-TIME PCR SCREENING REPORT

Wet-lab screening process. To ensure extremely high selectivity and sensitivity, we have established a screening protocol that rigorously screens candidate DNA signatures to select the optimal sets for eventual field use. Candidate signature primer pairs that survive computational screening proceed to wet chemistry screening utilizing standard PCR with agarose gel electrophoresis. This step ensures that the primers will react across strains representative of the diversity of the pathogen (avoid false negative), but will not react with the nucleic acids of other organisms that could be present in a sample (avoid false positives). The collection of purified nucleic acid templates used in this screening process is listed below:

1) Strain panels representative of the diversity of the target organism in nature. We have a panel of 7 SVD strains
2) Near neighbors-organisms that are closely related at the genetic level and therefore have the greatest likelihood of cross-reaction. We screened the candidate signatures against eleven near-neighbor isolates
3) Eukaryotic DNA that may carry over from sample collection procedures.
4) Prokaryotic DNA that would be expected to be present in environmental samples and may also be present in Bovine and Porcine oral cavities.
5) Fifteen soil samples from around the country representing diverse climates and contain a complex mixture of organisms from diverse environmental collections
6) Aerosol samples: 752 samples collected from aerosol collectors and pooled for background testing purposes.

Based on data from primer pair screening, a set of **4** specific and reliable signatures were then further tested for suitability for real-time TaqMan fluorogenic PCR detection protocols. The selected signatures showed a robust signal in all target reactions.

TABLE 291. List of Near-neighbors screened

Virus	Strain/ID[1]	Isolate	Source	Passage History	Extraction date/ID	Extraction Method	Use stock titer	Titer Method
Human Entero Virus B	CoxA9	(Bozak) TC-8212	CAHFS	Unknown	5/25/2004	Unknown	N/A	N/A
Human Entero Virus B	CoxB1	(Conn-5) TC-82486	CAHFS	Unknown	5/25/2004	Unknown	N/A	N/A
Human Entero Virus B	CoxB2	(Ohio) TC-229	CAHFS	Unknown	5/25/2004	Unknown	N/A	N/A
Human Entero Virus B	CoxB3	(Nancy) TC-887	CAHFS	Unknown	5/25/2004	Unknown	N/A	N/A
Human Entero Virus B	CoxB4	(JVB) TC-81506	CAHFS	Unknown	5/25/2004	Unknown	N/A	N/A
Human Entero Virus B	CoxB5	(Faulkner) TC-583	CAHFS	Unknown	5/25/2004	Unknown	N/A	N/A
Human Entero Virus B	CoxB6	(Schmitt) TC-223	CAHFS	Unknown	5/25/2004	Unknown	N/A	N/A
Human Entero Virus B	Echo 9	(D'Amoir) TC-80168	CAHFS	Unknown	5/25/2004	Unknown	N/A	N/A
Human Entero Virus B	Echo 11	(Hill) TC-81369	CAHFS	Unknown	5/25/2004	Unknown	N/A	N/A
Human Entero Virus B	Echo 12	(Gregory) TC-3808	CAHFS	Unknown	5/25/2004	Unknown	N/A	N/A
Human Entero Virus B	Echo 24	(Travis) TC-77557	CAHFS	Unknown	5/25/2004	Unknown	N/A	N/A

TABLE 292. List of targets screened at PIADC.

Virus	Strain/ID[1]	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	Stock Titer (Log10 $TCID_{50}$)	Titer Method
SVD	TAW-119-97	PIADC	Unknown	pending (SK6)	Unknown	Ambion MagMax	7.43 ± 0.17	Spearman-Karber
SVD	ITL-1-66	PIADC	Unknown	pending (SK6)	Unknown	Ambion MagMax	8.61 ± 0.15	Spearman-Karber
SVD	HKN-4-89	PIADC	Unknown	pending (SK6)	Unknown	Ambion MagMax	8.3 ± 0.14	Spearman-Karber
SVD	ROM-1-87	PIADC	Unknown	pending (SK6)	Unknown	Ambion MagMax	7.49 ± 0.14	Spearman-Karber
SVD	UKG-72	PIADC	Unknown	pending (SK6)	Unknown	Ambion MagMax	8.05 ± 0.12	Spearman-Karber
SVD	ITL-1-91	PIADC	Unknown	pending (SK6)	Unknown	Ambion MagMax	8.49 ± 0.14	Spearman-Karber

SVD	HKN-12-87	PIADC	Unknown	pending (SK6)	Unknown	Ambion MagMax	9.11 ± 0.13	Spearman-Karber

TABLE 293. List of Signatures screened

Oligonucleotide	Sequence 5′ to 3′
SVD_1727049.P	CGTCACAAGTTGTACCATCAGACACAATGCA
SVD_1727049.F	CAGGATAATTTCTTCCAAGGGC
SVD_1727049.R	ACGTGAACATTTCGAGCTTCC
SVD_1727050.P	TGACCGTAATGAGGTCATCGTGATTTCTCAC
SVD_1727050.F	GACTTGTTGTGGCTGGAGGA
SVD_1727050.R	CAGCGCCATGGTGAGGTAG
SVD_1727051.P	CTGGCGTCATAGCCTGAATAGTCAAACGCTA
SVD_1727051.F	GACAAAGTGGCCAAGGGAAA
SVD_1727051.R	CACGTAAACCACACTGGGCT
SVD_1727052.P	TAACCTGGACAAACGTGACCTTCCTGAAAAG
SVD_1727052.F	CCCAGCAGATAAAGGCGAGT
SVD_1727052.R	GCATGACAGGATGGACCAAA

Swine Vesicular Disease Virus (SVDV) - Gel Screening Report

May 10, 2005 screening at LLNL

List of signatures screened in gel format: Same as above with only F and R primers and no probe

TABLE 294a-c. Nucleic acid extracts used to challenge the initial set of candidate signatures. All soil, Eukaryote, and Prokaryote screening was done in duplicate These select backgrounds are used to determine which signatures will be down-selected against in the screening process. No cross-reactions were observed.

(a)Total of 13 soils screened

D000019	D000036	D000086	D000097	D000402	D000404	D000426
D000052	D000054	D000109	D000117	D000407	D000408	

(b)Total of 13 Eukaryotes screened

Bovine	Cat	Drosophila	Flea	Mouse	Porcine	Tick
Chicken	Dog	Human	Mosquito	Rabbit	Rat	

(c)Total of 10 Prokaryotes screened

E. herbicola	*B. cereus*	*P. aeruginosa*	*B. burgdorferi*	*L. monocytogenes*
B. globigii	*S. aureus*	*S. typhimurium*	*B. subtilis*	*H. influenza*

List of signatures screened in gel format against available targets and near neighbors
No target or near neighbor screening was conducted in gel format.

Swine Vesicular Disease Virus (SVDV) - Real-time PCR Screening Report

TABLE 295. List of signatures screened against Backgrounds in Real-time PCR Format :

Oligonucleotide	Sequence 5′ to 3′
SVD_1727049.P	CGTCACAAGTTGTACCATCAGACACAATGCA
SVD_1727049.F	CAGGATAATTTCTTCCAAGGGC
SVD_1727049.R	ACGTGAACATTTCGAGCTTCC
SVD_1727050.P	TGACCGTAATGAGGTCATCGTGATTTCTCAC
SVD_1727050.F	GACTTGTTGTGGCTGGAGGA
SVD_1727050.R	CAGCGCCATGGTGAGGTAG
SVD_1727051.P	CTGGCGTCATAGCCTGAATAGTCAAACGCTA
SVD_1727051.F	GACAAAGTGGCCAAGGGAAA
SVD_1727051.R	CACGTAAACCACACTGGGCT
SVD_1727052.P	TAACCTGGACAAACGTGACCTTCCTGAAAAG
SVD_1727052.F	CCCAGCAGATAAAGGCGAGT
SVD_1727052.R	GCATGACAGGATGGACCAAA

TABLE 296a-d. Nucleic acid extracts used to challenge the initial set of candidate signatures. These select backgrounds are used to determine which signatures will be down-selected against in the screening process. All reactions were done in duplicate, 200 pg per reaction. No cross-reactions were observed.

(a) Total of 15 soils screened

D000019	D000028	D000054	D000086	D000102	D000117	D000403	D000405
D000036	D000051	D000098	D000101	D000401	D000402	D000407	

(b) Total of 16 Eukaryotes screened

Bovine	Cat	Flea	Human	Rabbit	Rat
Chicken	Dog	Monkey	Mosquito	Sheep	Tick
Drosophila	Equine	Mouse	Porcine		

(c)Total of 14 Prokaryotes screened

E. herbicola	*B. cereus*	*P. aeruginosae*	*B. burgdorferi*	*L. monocytogenes*	*H. influenza*	*E. coli*
B. globigii	*S. aureus*	*S. typhimurium*	*B. subtilis*	*B. thuringiensis*	*C. burnetti*	*S. pneumonia*

(d) Aerosol Block

Aerosol Block	Signatures Screened	Number of Samples in Block
16 053104-051604	4	752
Total:	**4**	**752 samples**

TABLE 297. List of signatures screened in Real-time PCR format against available targets and near neighbors:

Oligonucleotide	Sequence 5’ to 3’
SVD_1727049.P	CGTCACAAGTTGTACCATCAGACACAATGCA
SVD_1727049.F	CAGGATAATTTCTTCCAAGGGC
SVD_1727049.R	ACGTGAACATTTCGAGCTTCC
SVD_1727050.P	TGACCGTAATGAGGTCATCGTGATTTCTCAC
SVD_1727050.F	GACTTGTTGTGGCTGGAGGA
SVD_1727050.R	CAGCGCCATGGTGAGGTAG
SVD_1727051.P	CTGGCGTCATAGCCTGAATAGTCAAACGCTA

SVD_1727051.F	GACAAAGTGGCCAAGGGAAA
SVD_1727051.R	CACGTAAACCACACTGGGCT
SVD_1727052.P	TAACCTGGACAAACGTGACCTTCCTGAAAAG
SVD_1727052.F	CCCAGCAGATAAAGGCGAGT
SVD_1727052.R	GCATGACAGGATGGACCAAA

TABLE 298. Results of real-time PCR target screening of SVD when spiked with 200 pg/ reaction of extracted nucleic acid. SVD_1727049 reacted with all target strains (**bold**) , the other signatures did not react with all strains. SVD_1727052 was noted to cross react with one of the near-neighbors HEV-B (Cox B). "N" indicates that no PCR product was formed after 35 cycles of PCR.

	SVD_1727049	SVD_1727050	SVD_1727051	SVD_1727052
SVDV (HKN-4-89)	**32.8, 34.08**	**27.17, 26.23**	**Not Tested**	**24.44**
SVDV (HKN/12/87)	**20.12, 26.87, 25.23**	**26.69, N, N**	**26.06**	**25.74**
SVDV (ITL/1/91)	**30.43, 32.78, 25.23**	**28.96, 30.03, 30.09**	**N**	**N**
SVDV (RON-1-87)	**27.54, 30.13**	**N**	**Not Tested**	**36.73**
SVDV (UKG72)	**30.53, 29.04**	**30.5, 30.37**	**Not Tested**	**30.43**
SVDV (TAW/119/97)	**20.51, 28.21, 28.74**	**N**	**Not Tested**	**29.46**
SVDV (SVD ITL/1/66)	**24.33, 25.05, 25.66**	**24.31, 25.88, 25.14**	**26.13, N**	**22.2**
Human Entero Virus B (Echo 12)	N	N	Not Tested	N
Human Entero Virus B (Cox B#)	N	N	Not Tested	28.71
Human Entero Virus B (Cox A9)	N	N	Not Tested	N
Human Entero Virus B (Cox B-1)	N	N	Not Tested	N
Human Entero Virus B (Cox B-5)	N	N	Not Tested	N
Human Entero Virus B (Cox B2)	N	N	Not Tested	N
Human Entero Virus B (Cox B4)	N	N	Not Tested	N
Human Entero Virus B (Cox B6)	N	N	Not Tested	N
Human Entero Virus B (Echo 11)	N	N	Not Tested	N
Human Entero Virus B (Echo 24)	N	N	Not Tested	N
Human Entero Virus B (Echo 9 H11)	N	N	Not Tested	N

*Note the number of Ct values denotes the number of replicate screenings against the particular template
** For greater detail on screening data refer to gel pictures and iCycler data files on LiveLink

Swine Vesicular Disease (SVD) - LOD Report

TABLE 299. Summary of real-time PCR titration s for SVD signatures when screened against available targets. A dilution series over 5-logs was prepared from extracted nucleic acid from untitered virus cell culture media. Values presented are in CT, cycle threshold. "N" indicates that no PCR product was detected for the 35 cycles of PCR. The tentative limit of detection is denoted by green highlighted cells. In some cases the LOD was not reached, thus no values are highlighted.

	Signature Avg. Ct[1]			
	1727049	**1727050**	**1727051**	**1727052**
UKG72 1:10	21.6	28.1	26.2	22.0
UKG72 1:100	25.7	**29.8**	28	24.4
UKG72 1:1K	31.1	N	**35.1**	28.9
UKG72 1:10K	34.8	N	N/A	32.1
UKG72 1:100K	37.4	N/A	N/A	34.1
TAW 11997 1:10	24.3	N/A	27.7	23.2
TAW 11997 1:100	28.5	N/A	31.6	24.8
TAW 11997 1:1K	33.1	N/A	**36.8**	29.1
TAW 11997 1:10K	**37.0**	N/A	N/A	32.4
TAW 11997 1:100K	N/A	N/A	N/A	36.4
ITL I-66 1:10	23.8	26.6	29.6	35.2
ITL I-66 1:100	27.5	**30.3**	32.0	**37.2**
ITL I-66 1:1K	32.5	N/A	37.2	N/A
ITL I-66 1:10K	**36.4**	N	**38.9**	N
ITL I-66 1:100K	N	N	N	N
HKN 4-89 1:10	26.7	33.3	26.9	24.2
HKN 4-89 1:100	31.1	**36.2**	30.6	25.0
HKN 4-89 1:1K	33.2	N	35.8	29.6
HKN 4-89 1:10K	**37.4**	N	**38.7**	31.8
HKN 4-89 1:100K	N	N	N	33.8
HKN 12-87 1:10	24.2	N	25.6	22.7
HKN 12-87 1:100	28.5	N	29.1	24.9
HKN 12-87 1:1K	33.9	N	35.1	29.7
HKN 12-87 1:10K	**36.0**	N	**38.0**	31.5
HKN 12-87 1:100K	N	N	N	33.6
ITL I-91 1:10	25.9	29.7	31.8	30.2
ITL I-91 1:100	31.2	**33.6**	**35.3**	33.1
ITL I-91 1:1K	36.3	N	N	**39.2**
ITL I-91 1:10K	**38.3**	N	N	N
ITL I-91 1:100K	N	N	N	N

[1]LOD Ct value for each signature:template combination is highlighted in green and boldface. For some signatures no LOD was reached.
[2]For SVD signatures, the ABI EX RT-PCR kit was used using the standard recipe. The amount of probe used with ABI EZ RT-PCR kit is 50% less than that used for the standard protocol [0.1mM vs. 0.2mM used for Invitrogen RT-PCR kits]].

TABLE 300. Summary of LOD from raw data above. Signature 1727049 proved to be the most sensitive against the strains tested.

Target	**Signature (LOD reported as highest dilution to give Ct Value)**			
	1727049	**1727050**	**1727051**	**1727052**
UKG 72	> 1:100K	1:100	1:1K	>1:100K

TAW 11997	1:10K	[Unreactive]	1:1K	>1:100K
ITL I-66	1:10K	1:100	1:10K	1:100
HKN 4-89	1:10K	1:100	1:10K	>1:100K
HKN 12-87	1:10K	[Unreactive]	1:10K	>1:100K
ITL I-91	1:10K	1:100	1:100	1:1K

9.5. SVD MULTIPLEXED PCR SCREENING REPORT

SCREENING AND DOWN-SELECTION TECHNICAL APPROACH:

Signatures that pass gel and real-time PCR screening are candidates for multiplexed assays. If the number of candidate signatures is large than a subset of the available signatures are selected based on measured sensitivity and specificity observed in real-time screening. In preliminary screening the background of individual signatures is measured by running "blank" (no sample added) controls and observing signature response. In some cases a primer-to-primer non-specific interference will eliminate candidate signatures. Signatures are then added iteratively to each multiplexed panel, titrated, and with each signature addition, collectively, all signatures are re-titrated to determine if the presence of one signature will interfere with the performance of another. During this process signatures are added or subtracted from the multiplexed panel depending on individual outcomes until all desirable signatures have been added.

Criterion for down-selection of multiplexed signatures

Signature specificity. In initial real-time PCR screening signatures undergo target and near neighbor screening and screening against environmental confounders which assess individual signature specificity. In multiplex format this is repeated to verify that a more complex reaction system gives the same level of specificity.

Signature sensitivity. In initial real-time PCR screening signatures undergo limit of detection testing where a dilution curve is generated for each signature. This determines individual signature sensitivity. In multiplex format this is repeated to verify that a more complex reaction system gives acceptable signature sensitivity.

Optimal signal to noise ratio. In the multiplex signature system the potential for cross reactivity is larger than in single reaction testing, this may cause unwanted PCR reaction products or non-specific cross-reactivity. Signatures are preferred to have a high signal to noise ratio, where desired signal is significantly greater than the background noise of the signatures when there is no target nucleic acid in the reaction.

Compatibility with other signatures in multiplex. In some cases a primer set may interfere with other primers or probes in the multiplex and there by cause limit of detection shifting for one or more signature in the multiplex. For each scenario it must be evaluated whether the shift in detection level is significant enough to remove (what could be a very desirable signature) from the panel.

TABLE 301. Order details for SVD signatures ordered for multiplexed assay screening and development.

ID	Modification details	Vendor
SVD_1727049.BF	5'-/5Bio/CAGGA/iBiodT/AATTTCT/iBiodT/CCAAGGGC-3'	IDT DNA
SVD_1727049.FCP	5'-/5AmMC6//iSp18/TGCATTGTGTCTGATGGTACAACTTGTGACG-3'	IDT DNA
SVD_1727049.R	5'-ACGTGAACATTTCGAGCTTCC-3'	IDT DNA
SVD_1727050.BF	5'-/5Bio/GACTTG/iBiodT/TGTGGC/iBiodT/GGAGGA-3'	IDT DNA
SVD_1727050.FCP	5'-/5AmMC6//iSp18/TGACCGTAATGAGGTCATCGTGATTTCTCAC-3'	IDT DNA
SVD_1727050.R	5'-CAGCGCCATGGTGAGGTAG-3'	IDT DNA
SVD_1727051.BF(mod)	5'-/5Bio/GACAAAG/iBiodT/GGCCAAGGGAAA-3'	IDT DNA
SVD_1727051.FCP	5'-/5AmMC6//iSp18/CTGGCGTCATAGCCTGAATAGTCAAACGCTA-3'	IDT DNA
SVD_1727051.R	5'-CACGTAAACCACACTGGGCT-3'	IDT DNA
SVD_1727052.BF(mod)	5'-/5Bio/CCCAGCAGA/iBiodT/AAAGGCGAGT-3'	IDT DNA

Multiplex Data Analysis -Technical approach

Disease signature thresholds

Thresholds were initially determined using all available data from known negative samples (blanks) by plotting a histogram which showed the probability of a given sample generating a particular MFI. In the absence of cross reactivity, each signature in the multiplex assay could be treated as an individual signature result. For example, for a sample spiked with SVD, data for other disease signatures could contribute towards negative data, providing that cross reaction between disease signature sets was not observed. This is a way to increase the number of samples that can be used to establish thresholds.

TABLE 302. Individual signature thresholds and ranges for SVD signatures. For FY06, threshold determinations have not yet been made and require a significant number of tests (>500) to generate this information. This information will be updated when it is available.

Signature Name	Mux Assay name	Panel Membership	Signature background in Tris NaCl Buffer (MFI +/-)	Signature background in Clinical matrices (MFI +/-)	Threshold
SVD_1727049	SVD-1	Porcine	TBD	TBD	TBD
SVD_1727050	SVD-2	Porcine	TBD	TBD	TBD
SVD_1727051	SVD-3	Porcine	TBD	TBD	TBD

TABLE 303. List of targets screened in multiplex at PIADC(pending).

Virus	Serotype	Strain/ID[1]	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	Use stock titer (Log10 $TCID_{50}$	Titer Method
SVD	N/A	HKN-4-89	PIADC	Unknown	Pending (SK6)	Unknown	Ambion MagMax96	8.3 ± 0.14	Spearman-Karber
SVD	N/A	HKN/12/87	PIADC	Unknown	Pending (SK6)	Unknown	Ambion	9.11±0.13	Spearman-

							MagMax96		karber
SVD	N/A	ITL/1/91	PIADC	Unknown	Pending (SK6)	Unknown	Ambion MagMax96	8.49±0.14	Spearman-Karber
SVD	N/A	ROM-1-87	PIADC	Unknown	Pending (SK6)	Unknown	Ambion MagMax96	7.49± 0.14	Spearman-Karber
SVD	N/A	UKG72	PIADC	Unknown	Pending (SK6)	Unknown	Ambion MagMax96	8.05±0.12	Spearman-Karber
SVD	N/A	TAW/119/97	PIADC	Unknown	Pending (SK6)	Unknown	Ambion MagMax96	7.43±0.17	Spearman-Karber
SVD	N/A	ITL/1/66	PIADC	Unknown	Pending (SK6)	Unknown	Ambion MagMax96	8.61±0.15	Spearman-Karber

[1] SVDV isolates from the Winnepeg Canada lab were not titered virus; the data herewith in reference to those targets is expressed as dilutions of a master stock of unknown concentration

TABLE 304. List of additional targets and near-neighbors screened against the porcine Panel. All near-neighbors samples were extracted nucleic acids with unknown titers.

Virus	Strain[1]	Unique ID	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	NA conc used or stock titer	Titer Method
Border Disease virus	Coos Bay	4-6-92	NADC, Julia Ridpath	1.5 x 10^8 TCID50/mL	unknown	12/14/06	Trizol	1.18 x 10^6 TCID50/mL	Reed & Muench
Bovine Herpes Virus-1	California	NVSL 20032 111903	CAHFS	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Cooper	NVSL 86741 111903	CAHFS	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Los Angeles	Los Angeles ATCC VR188	ATCC (CAHFS)	1 x 10^5 TCID50/mL	(CAHFS) MDBK2(LLNL)MDBK1	5/17/07	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	RLB-106	ATCC VR793	ATCC (CAHFS)	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	A033640072	CAHFS, R. Mock, #5 Swab, 1BDFK1, 1/28/2004, A030020072	unknown	1BDFK1,	4/23/04	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	A040130066	CAHFS, R. Mock, #4 Lung, 1BFK1, 1/28/2004, A040130066	unknown	1BFK1	4/23/04	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	Texas A030020072	CAHFS, R. Mock, #80 Swab, 1BDFK1, 1/28/2004, A030020072	unknown	1BDFK1	4/23/2004	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	California	NVSL 200032	NVSL, CAHFS	unknown	MDBK CAHFS	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Virginia	NVSL 231221	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	California	NVSL 51619	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Minnesota	NVSL 86741	NVSL, CAHFS	unknown	MDBK CAHFS	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes	Minnesot	NVSL 97-	NVSL, CAHFS	unknown	MDBK	11/12/03	Phenol/	40 pg/uL	N/A

Virus-1	a	10720			CAHFS		Chloroform		
Bovine Herpes Virus-1	Cooper	NVSL-Cooper RA 309	NVSL, CAHFS	unknown	MDBK CAHFS	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes-5	DN-599	A032540006	R. Mock, from lung tissue, 2BFK2, 2/3/2004	Unknown	CAHFS Lab 2BFK2 4/30/2004	6/3/2004	Phenol/Chloroform	40 pg/uL	N/A
Bovine Herpes-5	DN-599	A040150085	R. Mock, #2 Lung, 5BFK5, 2/3/04	Unknown	CAHFS Lab 5BFK5 4/23/2004	7/7/2004	Phenol/Chloroform	40 pg/uL	N/A
Bovine Herpes-5	D9402133	D9402133	Sonoma Co. 3/94 Severe necrotizing encephalitis, 1 week old calf	Unknown	CAHFS Lab (MDBK), (1 flasks) 11/19/2003	11/19/2003	Phenol/Chloroform	40 pg/uL	N/A
Bovine Herpes Virus	BHV 5	D9403153	CAHFS	unknown	unknown	unknown	unknown	40 pg/uL	unknown
Caprine Herpes Virus		VR462	ATCC	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Caprine Herpes Virus	D0201157	D0201157	CAHFS, Isolate D0201157 History: San Joaquin Co. 2/02 Aborted fetus-third trimester	unknown	CAHFS Lab (MDBK)	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Caprine Herpes Virus	S0201998	S0201998	CAHFS, Isolate S0201998 History: San Diego Co. 2/02 Aborted fetus-third trimester	unknown	CAHFS Lab (MDBK)	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-1	Santa Barbara	N/A	Bridget McLaughlin CAHFS	unknown	unknown	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-1	Georgia	N/A	Bridget McLaughlin CAHFS	unknown	unknown	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-2	Alberta	041 ODV 0401	NVSL	unknown	BHK	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-1 (SV123)	New Jersey (3TD) Deer, NJ 1955	041 ODV 0401	NVSL	1 x $10^{5.125}$ $TCID_{50}$/0.1 ml	+BHK-3 (12/7/83); (12/11/86); (12/18/86)	Unknown	Trizol	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A011120004	CAHFS, R. Mock, from brain tissue, 2BFK2, 2/3/2004, A011120004	unknown	CAHFS, 2BFK2	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A99043047	CAHFS, R. Mock, Spleen, 1BFK1, 2/3/2004, A99043047	unknown	CAHFS, 1BFK1	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A9904309	CAHFS	unknown	CAHFS, unknown	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Bovine 1247	Bovine 1247 ATCC VR2003	ATCC	unknown	CAHFS, unknown	unkown	Phenol/ Chloroform	40 pg/uL	N/A

Equine Herpes 2	NVSL 002	NVSL 002 1/28/04	NVSL	unknown	CAHFS, unknown	1/29/03	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	LK	LK ATCC VR701	ATCC	unknown	CAHFS, unknown	unkown	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	D990	D990	CAHFS	unknown	CAHFS, unknown	1/29/03	Phenol/ Chloroform	40 pg/uL	N/A
Feline Herpes	C-27	ATCC VR636	ATCC	unknown	CAHFS, unknown	unknown	unknown	40 pg/uL	N/A
Fowl Pox	Vaccine strain	Poxine™	Jeffers Livestock	unknown	ECE	5/17/07	Phenol/ Chloroform	40 pg/uL	N/A
Parainfluenza Virus type 3	C-243	TC-81335	LLNL	1×10^5 TCID50/0.1 mL	(VL)MK1 4(LLNL)H 292-1	5/17/07	Trizol	40 pg/uL	Reed and Muench
Porcine Coronavirus	AR310	ATCC VR-2384	LLNL	$1 \times 10^{4.25}$ $TCID_{50}$/0.1 ml	ST2				Reed and Muench
Porcine Herpes Pseudorabies	Shope	RA 180	CAHFS	1×10^5 TCID50/mL		5/17/07	Trizol	40 pg/uL	N/A
Pseudorabies		92-12013	NVSL 92-12013 bovine isolate Iowa December, 1991	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies		93-11745	NVSL 93-11745 Illinois December, 1992	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies	Shope	RA180	NVSL Shope (PRV) RA 180	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies Titered	Shope	N/A	NVSL	unknown		unknown	Phenol/ Chloroform		Reed & Muench
Respiratory Syncytial virus	Long	N/A		unknown	(VL)KB6 L2KB2HF DL2A549-1(LLNL)H eLa3	5/17/07	Trizol	40 pg/uL	N/A
Transmissible Gastroenteritis virus of hogs	Perdue	NVSL 9801	LLNL	$1 \times 10^{4.75}$ $TCID_{50}$/0.1 ml	ST 1		Trizol	40 pg/ul	Reed and Muench

TABLE 305. Panel membership for signature. The 3 SVD signatures from the Version 1.0 panel were included in the **Porcine-specific multiplexed assay panel** for FMDV rule-out diagnostics. The below table describes the constituents of that multiplexed assay panel in which these signatures were tested.

Bead ID	Signature/ Target Name	Mux Group ID	Virus	Source	Forward Primer	Reverse Primer	Luminex Probe (-strand, FCP)
132	FMDV.TC	FMDV.TC	Foot and Mouth Virus	Tetracore	ACTGGGTTTTACAAACCTGTGA	GCGAGTCCTGCCACGGA	GTCCCACGGCGTGCAAAGGA
133	FMDV.Pir	FMDV.Pir	Foot and Mouth Virus	Pirbright	CACYTYAAGRTGACAYTGRTACTGGTAC	CAGATYCCRAGTGWCICITGTTA	CCTCGGGGTACCTGAAGGGCATCC
142	PRRS_1807709	PRRS__1807709	Porcine Reproductive and Respiratory Syndrome	LLNL	GAGCGGCAATTGTGTCTGTC	GCTGAGGGTGATGCTGTGAC	CGCACAGTATGATGCGTAGGCAAACTAAACTC
144	PRRS_1810351	PRRS_1810351	Porcine Reproductive and Respiratory Syndrome	LLNL	TTCTTGTGACCACGATTCGC	GACCCACCGAGTAACTTGCC	GCTCAAGAGCCAAAAGCTCAGCATGACA
145	PRRS_1807706	PRRS_1807706	Porcine Reproductive and Respiratory Syndrome	LLNL	ATTGGTTTGCTCCGCGATAC	AAATGAGCCACCACATCCAA	CGGTACATTCGACGCGACACCATTTC

148	PRRS_1810383	PRRS_1810383	Porcine Reproductive and Respiratory Syndrome	LLNL	CAGTGTGCACGCTTCCATTT	CTCGAATGATGTGTTGCCGT	AAACATAGCGTAGAGCTGGAATTCGAAGCCA
149	PRRS_1810386	PRRS_1810386	Porcine Reproductive and Respiratory Syndrome	LLNL	GCTTTCTGCGTGCCTTTTCT	ACAACGCCAGAGACATTCCC	TGACTTTGAAGCCTTTCTCGCTCATTTCTGA
150	SVD_1727049	SVD_1	Swine Vesicular Disease	LLNL	CAGGATAATTTCTTCCAAGGGC	ACGTGAACATTTCGAGCTTCC	TGCATTGTGTCTGATGGTACAACTTGTGACG
151	SVD_1727050	SVD_2	Swine Vesicular Disease	LLNL	GACTTGTTGTGGCTGGAGGA	CAGCGCCATGGTGAGGTAG	TGACCGTAATGAGGTCATCGTGATTTCTCAC
152	SVD_1727051	SVD_3	Swine Vesicular Disease	LLNL	GACAAAGTGGCCAAGGGAAA	CACGTAAACCACACTGGGCT	CTGGCGTCATAGCCTGAATAGTCAAACGCTA
154	VESV_95653.F, VESV_95654.R, VESV_95655.P	VESV_1	Vesicular Exanthema of Swine Virus	LLNL	GCCTTCTCCCTTCCCAAAA	TGAAGGAATGGTTCCGTCAGT	CATCATCGTTGATAACCTTAGATGTGCAATTTGG
157	VESV_95686.F, VESV_95687.R, VESV_95688.P	VESV_4	Vesicular Exanthema of Swine Virus	LLNL	GGTCGCTCTCACTGATGATGAGTA	GGTGTTATCAGCACCCATTGC	GCTCGGTGCCTGAGTTGGAGGAAG
158	VESV_95692.F, VESV_95693.R, VESV_95694.P	VESV_5	Vesicular Exanthema of Swine Virus	LLNL	ACCACCTCTGGAAACATCTATGG	TTTGTGCACGTGTCACGAAT	CGGGACGGGCATTTGTCACCA
163	VSV_1798943	VSV_1798943	Vesicular Stomatitis Virus -Indiana	LLNL	CGCCACAAGGCAGAGATGT	TGTCAAATTCTGACTTAGCATACTTGC	GCATACTGCATCATATCAGGAGTCGGTTTTCTG
164	VSV_1811409	VSV_1811409	Vesicular Stomatitis Virus –New Jersey	PIADC	CTCACAACATGGGTCCTGAA	TTCTTGCCCCGGATACATCAT	GGCACAGCTCATCTGCGACTTCCCT
166	VSV_1798947	VSV_1798947	Vesicular Stomatitis Virus -Indiana	LLNL	CCCAATCAATGCCATGATACA	CTCCAATGGAAGGGTCCAAA	TTTGAAAGTAGAACTGTGCAAGCCCGGTATC
167	VSV_1798949	VSV_1798949	Vesicular Stomatitis Virus -Indiana	LLNL	GGCGCTCATTATAAAATTCGGA	ACATTTTCTCGTAGTAATGCAGCAG	GAAGTCCCTGTAATGGATTCCCATTCCATGT
168	VSV_1811405	VSV_1811405	Vesicular Stomatitis Virus -Indiana	PIADC	AAGAGATGGTCACGAGTGAC	GAGCATTTGTGGAAACCGAGC	TGGGTATTTGGTCATTGGTGACACA
169	VSV_1811408	VSV_1811408	Vesicular Stomatitis Virus –New Jersey	PIADC	CTCACAACATGGGTCCTGAA	TTCTTGACCTGGATACATCAT	GGCATAGYTCGTCTGCRACTTCCCT
170	N/A	NC (MT-7)	Maritima	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
171	N/A	b-MT7 (FC)	N/A	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
172	N/A	MT7/Cy3 (IC)	N/A	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
173	Alien RNA	arRNA Control	None	Ambion	GACATCAAGGCTCAAACTAATTTTACC	CAAAGGCTGCCAACATAAAATG	CAAGCGTAAATGCAGCGTCCA

[1]FCP indicates that the probe is on the negative strand (Forward Compliment to the Probe).

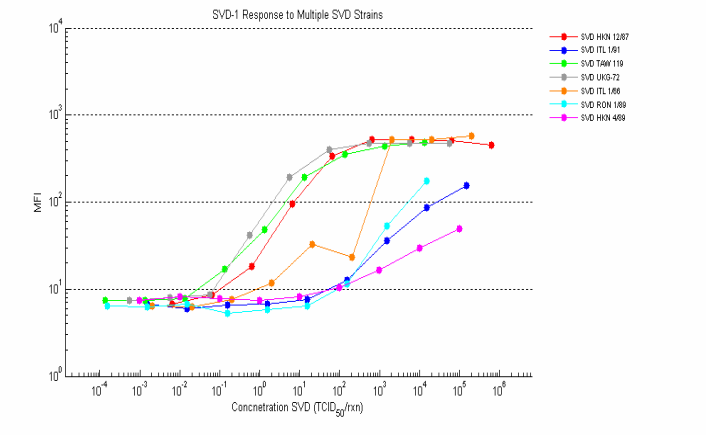
9.5.1. PORCINE PANEL MULTIPLEXED PCR DATA

Preliminary screening: Preliminary screening can be broken down into several steps. The signatures are preliminarily screened by first assessing how the signatures will perform under multiplex PCR conditions and whether or not the addition of the new signatures will adversely effect other signatures, these preliminary screening methods are described as “signature baseline screening” and “multiplex addition screening” respectively. First signature baseline screening tests the performance of the signature in a no-target reaction (blank), a “passing” score is indicated by a low (typically less than 100) MFI. All SVD signatures passed the signature baseline screening. Next in multiplex addition screening the new signature primers are added one-by –one to the other panel signatures to determine if there would be any cross-reactions between primer pairs in the panel. Further assessment of the signatures is done through target and near-neighbor screening and the determination of relative sensitivities. The signature down-selection for the SVD signatures was described in detail in the 2005 Agricultural Assay Report, here only a historical overview is provided.

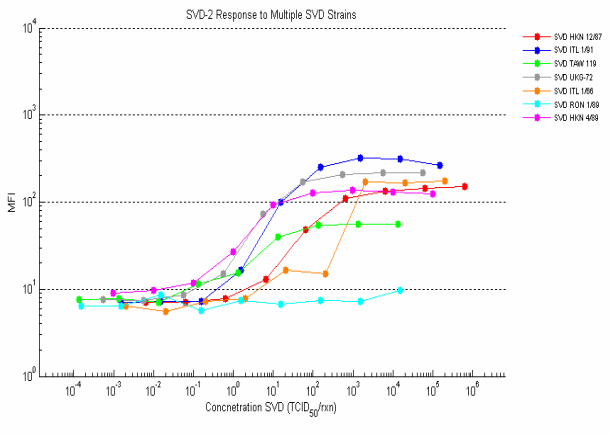
Historic Data Overview: Version 1.0 Panel LLNL 2006

Plotted by each signature against all strains

(a)



(b)



(c)

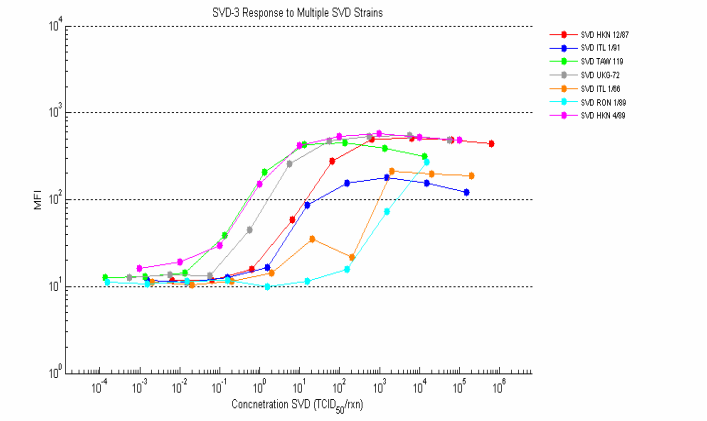


FIG. 49a-c. Historic data from Version 1.0 panel: Each SVD signature plotted against all target strains of SVD at PIADC. (a) SVD-1 (b) SVD-3 (c) SVD-4.

TABLE 306. Summary of limits of detection for SVD across various strains tested. "X" indicates that the limit of detection was not reached (or unable to be assessed) for that strain.

Strain	SVD-1	SVD-2	SVD-3	units
HKN-12-87	4.38E+02	4.10E+03	7.88E+02	TCID50/ml
ITL 1-91	8.34E+05	6.90E+02	5.24E+02	TCID50/ml
TAW 1-19	2.39E+02	1.55E+03	3.38E+01	TCID50/ml
UKG-72	1.44E+02	3.46E+02	5.81E+01	TCID50/ml
ITL 1-66	4.93E+03	2.78E+03	4.85E+03	TCID50/ml

ROM 1-87	2.94E+05	X	8.98E+04	TCID50/ml
HKN 89	1.15E+07	3.36E+02	3.55E+01	TCID50/ml

Near-neighbor and Target screening: The three Version 1.0 panel SVD signatures were added to the Porcine panel. The signatures exhibited a reasonably low background response (<30 MFI) in the Porcine panel.

TABLE 307. Backgrounds screening in multiplexed format for SVD at LLNL and PIADC. Target and near-neighbor testing is in process at PIADC.

	Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors	Targets
Mux PCR Screening	54	135	16	0	43 (8 pending)	7 pending

[1]-There are 752 pooled samples in each Aerosol Block.

TABLE 308. Porcine panel backgrounds screening in **multiplexed** format for down-selected SVD signatures against the below listed **eukaryotes**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. None of the signatures cross-reacted with any of the samples listed below.

Description	SVD-1 (50)	SVD-2 (51)	SVD-3 (52)
BOVINE	21	13	7
CAT	20	13	7
CHICKEN	17	11	6
DOG	21	12	6
DROSOPHILA MELANOGASTER	23	14	8
EQUINE	23	14	8
FLEA	23	14	7
HUMAN	20	12	7
MONKEY	24	16	8
MOSQUITO	20	13	6
MOUSE	23	15	7
PIG / PORCINE	26	17	8
RABBIT	25	16	7
RAT	29	18	8
SHEEP	28	18	8
TICK	24	16	8

TABLE 309. Porcine panel backgrounds screening in **multiplexed** format for the four BHV signatures against the below listed **prokaryotes**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in

Median Fluorescence Intensity (MFI) units. None of the signatures cross-reacted with any of the samples listed below.

Description	SVD_1 (50)	SVD_2 (51)	SVD_3 (52)
Erwinia amylovora	27	17	8
Actinobacillus suis	17	11	6
Aneurinbacillus migulanus	18	12	6
Bacillus cereus	31	20	8
Bacillus globigii	25	15	7
Bacillus subtilis	28	17	7
Bacillus thuringiensis	32	20	8
Bifidobacterium denticum	23	14	7
Borrellia burgdorferi	33	21	8
Burkholderia capacia	24	16	7
Caulobacter vibriodes	19	13	6
Clavibacter michganensis	24	16	8
Clostridium butyricum	29	20	8
Corynebacterium pseudodipthericum	27	17	8
Cytophaga marinoflava	28	19	8
Erwinia herbicola	30	18	7
Escherichia coli	32	22	8
Geobacillus caldoxylosilyticus	29	18	8
Halomonas halmophila	23	15	7
Haemophilus influenza	27	16	7
Herbaspirillium seropedicae	19	13	6
Lactobacillus garvieae	14	9	5
Lactobacillus gasseri	19	12	6
Listeria monocytogenes	25	16	7
Listeria seeligeri	31	20	8
Micrococcus luteus	18	12	6
Moraxella lacunatica	23	16	8
Oceanospirillium ssp. Maris	23	15	8
Paenibacillus napthalaenovorans	24	15	8
Paracoccus dentrificans	23	16	7
Porphyrobacter sanguineus	17	11	6
Proteus mirabillis	27	17	8
Pseudomonas aeruginosae	22	15	6
Pseudomonas oleovorans	19	11	6
Rhizobium leguminosarum	24	16	8
Rhodococcus rhodochrous	22	14	7
Salmonella typhimurium	25	17	8
Simonsiella muelleri	19	12	6
Sphingomonas sp. (Alcaligenes sp)	22	14	7
Staphylococcus aureus	27	19	8
Streptococcus pneumoniae	29	20	8

Streptomyces scabiei	25	17	8
Tatlockia maceachernii	25	16	8
Vibrio parahaemolyticus	24	17	8
Xanthomonas translucens	24	16	8

TABLE 310. Porcine panel backgrounds screening in **multiplexed** format for the four BHV signatures against the below listed **soils**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. None of the signatures cross-reacted with any of the samples listed below.

Description	SVD-1 (50)	SVD-2 (51)	SVD-3 (52)
D 000107-49	20	16	8
D 000109 # 50	20	16	8
D 000402 # 53	23	17	9
D 000500 - 26 - 1	20	16	9
D 000501-14-1	19	15	9
D 000505 - 11 - 4	20	16	9
D 000521 - 23	23	17	8
D 000527 - 3	21	17	9
D 000531 - 21	21	17	9
D 000533 - 17 -1	17	15	8
D 000542 - 6	20	15	9
D 000550 - 20	20	16	9
D 000551 - 5	22	18	9
D 000561 - 8 - 6	21	16	8
D 000562 - 30 - 5	22	16	9
S 251	19	15	8
S 252	18	14	9
S 253	18	14	8
S 254	18	14	9
S 255	16	14	8
S 256	19	15	9
S 257	17	13	7
S 259	16	13	8
S 260	17	14	8
S 271	17	14	8
S 272	16	14	8
S 273	19	14	8
S 274	19	15	8
S 275	17	15	8
S 276	18	14	9
S 277	20	15	9
S 279	17	12	8
S 280	17	14	8
S 282	18	14	9
S 283	17	14	9

S 284	18	14	8
S 286	16	13	8
S 287	18	14	9
S 288	19	14	9
S 289	17	12	8
S 290	17	14	9
S 291	16	13	8
S 292	16	13	9
S 295	17	13	8
S 296	17	14	9
S 297	17	14	8
S 298	17	13	8
S 299	15	12	8
S 300	10	9	6
S 301	11	10	7
S 303	9	9	7
S 304	11	10	7
S 305	11	11	7
S 307	11	11	7

TABLE 311. Porcine Panel **Near-Neighbor** screening (Data from 20070601) against the 2 BHV signatures. Values shown are Median Fluorescence Intensity (MFI) units. “Blanks” are buffer-only samples (no nucleic acid added to reaction), shown below in red. Extracted nucleic acid was added to each PCR reaction in triplicate totaling 200pg/rxn. None of the signatures cross-reacted with any of the samples listed below.

Description	SVD_1 (50)	SVD_2 (51)	SVD_3 (52)
Blank	29	22	9
Blank	21	15	6
BDV Coos Bay	18	13	7
BHV (BFK) A03250006 DN-599	26	19	8
BHV A040150085	23	18	7
BHV-1 (IBR) Texas A030020072 CAHFS	24	20	8
BHV-1 A033640072	24	18	8
BHV-1 A040130066	28	21	9
BHV-1 ATCC VR 793	24	17	7
BHV-1 NVSL 10720	22	17	7
BHV-1 NVSL 200032	22	16	8
BHV-1 NVSL 231221	23	17	8
BHV-1 NVSL 51619	21	16	7
BHV-1 NVSL 86741	23	17	8
BHV-1 or IBR LA ATCC VR188	25	19	8
BHV-1 RA309	20	14	6
BHV-5 A032540006 CAHFS	18	13	7
BHV-5 A040150085 CAHFS	17	13	7
BHV-5 D9402133 CAHFS	17	12	6
BHV-5 D9403153 CAHFS	19	14	8

Caprine Herpes D0201157 CAHFS	18	14	7
Caprine Herpes-2 ATCC VR 462	22	15	7
Caprine Herpes-2 S0201998 CAHFS	18	12	7
EHD-1 A9904309	20	15	7
EHD-1 Georgia	19	14	7
EHD-1 New Jersey	14	10	7
EHD-1 Santa Barbara	16	10	7
EHD-2 Alberta	17	12	7
EHV-1 A011120004 CAHFS	26	19	8
EHV-1 A99043047	22	15	7
EHV-1 ATCC VR2003	21	16	7
EHV-2 ATCC VR701	21	16	7
EHV-2 D990 CAFHS	17	12	6
EHV-2 NVSL 0002	21	15	7
Feline Herpes ATCC VR 636	19	14	7
Fowl Pox	20	15	8
IBR CA 111903	26	19	8
IBR MN 111903	22	16	8
Parainfluenza Type 3	21	15	8
Porcine Herpesvirus or Pseudorabies Shope	19	14	7
Pseudorabies NVSL 92-12013	19	13	6
Pseudorabies NVSL 93-11745	24	17	8
Pseudorabies RA 180 CAHFS	19	13	7
Pseudorabies Titered	21	15	8
Respiratory Syncytial	20	14	8

RESULTS: These three SVD signatures have been added to the Porcine panel and tested against various background confounders. Target and near-neighbor is in process at PIADC and reporting will be amended when that data becomes available.

10. VESICULAR EXANTHEMA OF SWINE VIRUS (PORCINE PANEL)

OBJECTIVE: In 2005 we were tasked by the Department of Homeland Security to develop specific and reliable Real-time RT- PCR signatures for Vesicular Exanthema of Swine Virus [VESV], among other major agriculturally-impacting viruses for the development of a Foot-and-Mouth Disease rule-out panel. In 2006 the goal of these assays (collectively with other virus-specific signatures developed in parallel) is to develop a species specific (i.e. bovine, porcine) multiplexed detection assay for Foot-and-Mouth disease rule-out for use in veterinary diagnostic laboratories. The four signatures that were developed and incorporated into the Version 1.0 FMDV Rule-out panel have been re-evaluated for use in a porcine-specific FMDV rule-out panel. This document describes the historic data and information as well as preliminary 2006 data screening the 3 VESV signatures for inclusion into the Porcine panel. Screening of the

VESV signatures against target in the porcine multiplex was occurring at the writing of this report.

10.1. BACKGROUND AND ETIOLOGY OF VESV

Vesicular exanthema of swine (VESV) is an acute, highly infectious disease characterized by fever and formation of blisters on the snout, oral mucosa, soles of the feet, the coronary band, and between the toes.

Since 1972, a virus indistinguishable from VES virus (VESV), designated as San Miguel seal lion virus (SMSV), has been isolated from throat and rectal swabs from premature and 4-mo-old California sea lion pups, dead and weanling northern fur seal pups, and nursing northern elephant seal pups. It has also been isolated from vesicular lesions on marine mammals, commercial seal meat produced in Alaska, and perch-like fish collected from tidal pools off the southern California coast. SMSV isolated from both fish and marine mammals is capable of producing VES in pigs. In addition, caliciviruses isolated from throat and rectal swabs from dairy calves cause clinical vesicular exanthema in exposed pigs. One calicivirus serotype, SMSV-5, has been recovered from vesicular lesions on the palms and soles of a researcher working with the virus.

VESV, SMSV, and related viruses are members of the genus Vesivirus in the family Caliciviridae. Many immunologically distinct serotypes have been demonstrated (13 types of VESV from pigs and 16 types of SMSV from marine sources). Additionally, a number of serotypes have been named after the host species from which they were isolated: bovine, primate, cetacean, walrus, skunk, mink, and reptile caliciviruses. In some cases, serotypes initially isolated in terrestrial animals (e.g., reptile calicivirus) have subsequently been found in marine mammals. All of these viruses (except for SMSV-8, SMSV-12, and mink calicivirus) form a single species, vesicular exanthema of swine virus.

In pigs, the clinical disease is indistinguishable from foot-and-mouth disease (*Foot-and-mouth Disease: Introduction*), vesicular stomatitis (*Vesicular Stomatitis: Introduction*), and swine vesicular disease (*Swine Vesicular Disease: Introduction*). Originally confined to California, the disease became widespread in the USA during the 1950s, but a vigorous campaign to eradicate the disease was successful. In 1959, the USA was declared free of VES, and the disease was designated a foreign animal disease; it has never been reported as a natural infection of pigs in any other part of the world.

Presumptive diagnosis in pigs is based on fever and the presence of typical vesicles, which break within 24-48 hr to form erosions. Diagnosis can be confirmed by complement-fixation tests, ELISA, and electron microscopy on epithelial tissue, or after passage in swine tissue cultures. Serum neutralization tests and immunoelectron microscopy are also used. Various reverse transcriptase-PCR have been developed for the identification of vesiviruses, but none have been evaluated for diagnostic use[10].

[10] *Source: The Merck Veterinary Manual*

10.2. VESV COMPREHENSIVE ASSAY SUMMARY

Bioinformatics

Virus name: Vesicular Exanthema of Swine Virus
Signature generation reference: Single whole genome for VESV (Note: limited sequence avail. In Genbank)
Level of discrimination: species, VESV only
Number of Candidate Signatures: 44.
Number of Signatures forwarded to Gel PCR bench-screening: 20.
Number of Signatures forwarded to real-time PCR bench-screening: 6.

Real-time PCR Screening Summary

TABLE 312. Number of Final Signatures forwarded to multiplexed screening (6).

LLNL Signature Designation	Sequence
vesv_95653.F	GCCTTCTCCCTTCCCAAAA
vesv_95654.R	TGAAGGAATGGTTCCGTCAGT
vesv_95655.P	CCAAATTGCACATCTAAGGTTATCAACGATGATG
vesv_95677.F	TTTGATGTCCGCTCTTGACAA
vesv_95678.R	CGCTTTGCAAGGGCAAAT
vesv_95679.P	TCATTTTGACCGGACCTCCGGG
vesv_95680.F	GGGAATGAGGTGTGCATCATT
vesv_95681.R	CACGTCTTGATGTTGGCTTGAC
vesv_95682.P	CGACTCATCTGACAAGGTTGATTATGCCAATTT
vesv_95686.F	GGTCGCTCTCACTGATGATGAGTA
vesv_95687.R	GGTGTTATCAGCACCCATTGC
vesv_95688.P	CTTCCTCCAACTCAGGCACCGAGC
vesv_95692.F	ACCACCTCTGGAAACATCTATGG
vesv_95693.R	TTTGTGCACGTGTCACGAAT
vesv_95694.P	TGGTGACAAATGCCCGTCCCG
vesv_95701.F	AGGCTGTCGCAGCCTACAA
vesv_95702.R	GTTTTCCGTAGAGGTCGGTTAGGT
vesv_95703.P	ACCAAATCGCTCACGCGAGTGGT

TABLE 313. Summary of wet-bench screening in signature down-selection. Cross reactions were seen in gel screening . Additional real-time Target screening reported from Plum Island Animal Disease Center [PIADC] for 11 additional strains

	Soils	Eukaryotes	Prokaryotes	Aerosols[1]	Near Neighbors	Target
Gel Screening	2	6	none	none	none	12

http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/54600.htm&word=Veiscular%2cexanthema%2cof%2cswine

Real-time PCR Screening	15	16	14	1 Aerosol block	none	11

[1]There are 752 pooled samples in each Aerosol Block.

Multiplexed PCR Screening Summary

TABLE 314. Historic data from Version 1.0 panel. VESV signatures from multiplexed down-selection and their relative sensitivity ranges when challenged with extracted nucleic acid from target RNA. "N" indicates that the signatures did not react with the target or that they reacted very little, not enough to cross the positive detection threshold.

			Sensitivity Data for final signatures ($TCID_{50}$/mL)				
Agent	Serotype	Strain	VESV-1	VESV-3	VESV-4	VESV-5	Original titer (Log10 TCID50)
VESV	N/A	E54	1.78E+04	N	2.64E+01	N	5.99 ± 0.1
VESV	N/A	1934B	N	N	N	N	3.18 ± 0.13
VESV	N/A	J56	N	N	N	N	2.99 ± 0.1
VESV	N/A	C52	N	N	3.16E-02	N	5.11 ± 0.15
VESV	N/A	I55	N	N	1.00E+03	N	2.18 ± 0.14
VESV	N/A	D53	N	2.51E+02	1.52E-01	1.49E+01	5.86 ± 0.16
VESV	N/A	H54	1.53E+02	N	1.60E+00	N	3.43 ± 0.14
VESV	N/A	A48	3.22E-01	4.61E-02	4.18E-02	6.59E-01	5.43 ± 0.16
VESV	N/A	G55	N	N	6.09E+01	N	3.8 ± 0.12
VESV	N/A	B51	1.25E+01	1.56E+00	7.07E-01	3.74E+01	3.11 ± 0.15
VESV	N/A	K54	N	N	3.36E+02	N	3.99 ± 0.15

TABLE 315. Backgrounds screening in multiplexed format for VESV at LLNL and PIADC.

	Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors[2]	Targets
Mux PCR Screening	54	135	16	0	pending	pending

[1]There are 752 pooled samples in each Aerosol Block. [2]Near-neighbors indicated (43) apply to the near-neighbor panel that is not uniquely specific for BHV, but for the other panel constituents that were screened concurrently.

TABLE 316. Summary of multiplexed VESV signatures for Porcine panel.

LLNL Signature Designation	MUX Group ID	Gene ID	Relative Limit of detection[1]	Targets Screened	Near Neighbors Screened
vesv_95653	VESV-1	VESVgp1/911834	pending	pending	pending
vesv_95680	VESV-3	VESVgp1/911834	pending	pending	pending
vesv_95686	VESV-4	VESVgp1/911834	pending	pending	pending

[1] The relative "Limit of detection" is described as a range of lowest and highest sensitivity determined by multiple target strains screened and is described further in the Multiplex results summary report section.

10.3. VESV SIGNATURE BIOINFORMATICS AND GENERATION INFORMATION

Bioinformatics process:
The LLNL Bioinformatics team have developed a computational process that uses available DNA sequence information for a given target organism to generate candidate DNA signature sequences that are conserved and unique. Signatures are primarily used to underlie reliable and specific pathogen detection assays. Evidence of the success of the process is that several of the Real-time PCR signatures that are used in the national BioWatch monitoring system were generated by this process, and to date, has resulted in no false positives.

The general approach is the following: All available complete genomes of different strains of the target species are compared using a multiple genome alignment programs. A *consensus gestalt* is formed from the alignments that contain the sequence conserved amongst all target inputs. (This step is bypassed if only one target sequence is available).

To determine that organism-conserved sequence which does not occur in any other sequenced microbial organism, the consensus gestalt is compared against our immense, continuously updated database of microbial organism. A customized algorithm accomplishes this electronic subtraction, and the result is a *uniqueness gestalt* that is mined for potential signature candidates. A final computational screening is done to verify that cross-reactions are not detected.

Target Organism Information.

Virus name: Vesicular Exanthema of Swine virus.
Genome size: 8284 bp.

Primer/Probe Set Generation Information

Alignment and All Microbe Database subtraction.

TABLE 317. K-path run id: 13753. Genome Sequences used for alignment (1).

	Genome Description	GI Number	Sequence Length (bp)	K-path ID
1	Vesicular exanthema of swine virus, complete genome	10314005/NC_002551.1	8284	13753

TABLE 318. Primer/Probe Design from Candidates list using Primer3.

Primer3 Main Parameters	
Parameters	**Standard Settings**
PRIMER_OPT_SIZE	**20**
PRIMER_MIN_SIZE	**18**
PRIMER_MAX_SIZE	**27**
PRIMER_PRODUCT_OPT_SIZE	**100**
PRIMER_PRODUCT_SIZE_RANGE	**71-600**
PRIMER_OPT_TM	**62**
PRIMER_MIN_TM	**61**
PRIMER_MAX_TM	**63**

PRIMER_MIN_GC	**20**
PRIMER_MAX_GC	**80**
PRIMER_PICK_INTERNAL_OLIGO	**1**
PRIMER_INTERNAL_OLIGO_OPT_SIZE	**31**
PRIMER_INTERNAL_OLIGO_MIN_SIZE	**18**
PRIMER_INTERNAL_OLIGO_MAX_SIZE	**36**
PRIMER_INTERNAL_OLIGO_OPT_TM	**72**
PRIMER_INTERNAL_OLIGO_MIN_TM	**71**
PRIMER_INTERNAL_OLIGO_MAX_TM	**73**
Number of Primers/Probe Set generated:	**44**

Signature Information

Source: LLNL
Project name: ves_vesicular_exanthema_of_swine_virus.
Level of discrimination: species.

Number of Candidate Signatures: 44.
Number of Signatures forwarded to Gel PCR bench-screening: 20.
Number of Signatures forwarded to real-time PCR bench-screening: 6.

Signature list.

Note:For a listing of computationally predicted product sequences, please see attached document " VESV Taqsim Run Data"

Taqsim description

We used a computational TaqMan simulator program, "Taqsim", to identify all potential targets for each signature from all sequences available in genbank. Taqsim is a BLAST-based comparison of each signature as a triplet against all sequences in genbank to identify the targets that are predicted to produce a TaqMan reaction at 57 degrees primer annealing and 67 degrees for probe annealing (these temperatures are according to Primer 3 oligo Tm calculations). The first column of the excel spreadsheets list the signatures by the name, and the following columns report the predicted genbank targets, coordinates of signature region, amplicon size, and accession #'s for each target.

TABLE 319a-f. Signature bioinformatics. All sequences are listed in the 5'→ 3' direction.

(a) vesv_95653

Target Virus	vesivirus vesicular exanthema of swine virus A48
Forward Primer	vesv_95653.F
FWD Primer Length (bp)	19
FWD Primer TM (^{o}C)	61
FWD Primer GC Content (%)	52
Forward Sequence	GCCTTCTCCCTTCCCAAAA
Reverse Primer	vesv_95654.R

Rev Primer Length (bp)	21
Rev Primer TM (°C)	61
Rev Primer GC Content (%)	47
Reverse Sequence	TGAAGGAATGGTTCCGTCAGT
Probe Name	vesv_95655.P
Probe Length (bp)	34
Probe TM (°C)	71
Probe GC Content (%)	38
Probe Sequence	CCAAATTGCACATCTAAGGTTATCAACGATGATG
Probe strand	plus
Predicted Product Size	153

(b)vesv_95677

Target Virus	vesivirus vesicular exanthema of swine virus A48
Forward Primer	vesv_95677.F
FWD Primer Length (bp)	21
FWD Primer TM (°C)	61
FWD Primer GC Content (%)	42
Forward Sequence	TTTGATGTCCGCTCTTGACAA
Reverse Primer	vesv_95678.R
Rev Primer Length (bp)	18
Rev Primer TM (°C)	62
Rev Primer GC Content (%)	50
Reverse Sequence	CGCTTTGCAAGGGCAAAT
Probe Name	vesv_95679.P
Probe Length (bp)	22
Probe TM (°C)	71
Probe GC Content (%)	59
Probe Sequence	TCATTTTGACCGGACCTCCGGG
Probe strand	plus
Predicted Product Size	144

(c) vesv_95680

Target Virus	vesivirus vesicular exanthema of swine virus A48
Forward Primer	vesv_95680.F
FWD Primer Length (bp)	21
FWD Primer TM (°C)	62
FWD Primer GC Content (%)	47
Forward Sequence	GGGAATGAGGTGTGCATCATT
Reverse Primer	vesv_95681.R

Rev Primer Length (bp)	22
Rev Primer TM (°C)	62
Rev Primer GC Content (%)	50
Reverse Sequence	CACGTCTTGATGTTGGCTTGAC
Probe Name	vesv_95682.P
Probe Length (bp)	33
Probe TM (°C)	71
Probe GC Content (%)	39
Probe Sequence	CGACTCATCTGACAAGGTTGATTATGCCAATTT
Probe strand	plus
Predicted Product Size	199

(d) vesv_95686

Target Virus	vesivirus vesicular exanthema of swine virus A48
Forward Primer	vesv_95686.F
FWD Primer Length (bp)	24
FWD Primer TM (°C)	62
FWD Primer GC Content (%)	50
Forward Sequence	GGTCGCTCTCACTGATGATGAGTA
Reverse Primer	vesv_95687.R
Rev Primer Length (bp)	21
Rev Primer TM (°C)	62
Rev Primer GC Content (%)	52
Reverse Sequence	GGTGTTATCAGCACCCATTGC
Probe Name	vesv_95688.P
Probe Length (bp)	24
Probe TM (°C)	71
Probe GC Content (%)	62
Probe Sequence	CTTCCTCCAACTCAGGCACCGAGC
Probe strand	plus
Predicted Product Size	124

(e) VESV_95692

Target Virus	vesivirus vesicular exanthema of swine virus A48
Forward Primer	vesv_95692.F
FWD Primer Length (bp)	23
FWD Primer TM (°C)	61
FWD Primer GC Content (%)	47
Forward Sequence	ACCACCTCTGGAAACATCTATGG
Reverse Primer	vesv_95693.R

Rev Primer Length (bp)	20
Rev Primer TM (°C)	61
Rev Primer GC Content (%)	45
Reverse Sequence	TTTGTGCACGTGTCACGAAT
Probe Name	vesv_95694.P
Probe Length (bp)	21
Probe TM (°C)	72
Probe GC Content (%)	61
Probe Sequence	TGGTGACAAATGCCCGTCCCG
Probe strand	plus
Predicted Product Size	200

(f) VESV_95701

Target Virus	vesivirus vesicular exanthema of swine virus A48
Forward Primer	vesv_95701.F
FWD Primer Length (bp)	19
FWD Primer TM (°C)	62
FWD Primer GC Content (%)	57
Forward Sequence	AGGCTGTCGCAGCCTACAA
Reverse Primer	vesv_95702.R
Rev Primer Length (bp)	24
Rev Primer TM (°C)	62
Rev Primer GC Content (%)	50
Reverse Sequence	GTTTTCCGTAGAGGTCGGTTAGGT
Probe Name	vesv_95703.P
Probe Length (bp)	23
Probe TM (°C)	71
Probe GC Content (%)	56
Probe Sequence	ACCAAATCGCTCACGCGAGTGGT
Probe strand	plus
Predicted Product Size	200

Target Region Gene Information

TABLE 320a-b. (a) Reference genomes used for gene information. (b) Gene information for each signature

(a)

	Genome Description	GI Number	Sequence Length (bp)
1	Vesicular exanthema of swine virus, complete genome	10314005/NC_002551.1	8284

(b)

			Peptide Location		Target Region Location	
Primer	**Gene**	**Description**	**Start**	**End**	**Start**	**End**
vesv_95653	VESVgp1/911834	mature peptide, putative N-terminal leader protein	20	463	404	556

	VESVgp1/911834	mature peptide, putative counterpart of the picornavirus membrane modification protein 2B	464	1324	404	556
vesv_95677	VESVgp1/911834	mature peptide, putative Ntpase protein; probable ortholog of the 2C protein of picornavirus	1325	2392	1693	1836
vesv_95680	VESVgp1/911834	mature peptide, putative Ntpase protein; probable ortholog of the 2C protein of picornavirus	1325	2392	1898	2096
vesv_95686	VESVgp1/911834	mature peptide, putative VPg	3230	3568	3274	3397
vesv_95692	VESVgp1/911834	mature peptide, Putative cysteine proteinase	3569	4129	3869	4068
vesv_95701	VESVgp1/911834	mature peptide, putative RNA-dependent RNA polymerase	4130	5662	5556	5755
	VESVgp1/911832	leader of the capsid (LC)	5671	6126	5556	5755

10.4. VESV GEL AND REAL-TIME PCR SCREENING REPORT

Wet-lab screening process. To ensure extremely high selectivity and sensitivity, we have established a screening protocol that rigorously screens candidate DNA signatures to select the optimal sets for eventual field use. Candidate signature primer pairs that survive computational screening proceed to wet chemistry screening utilizing standard PCR with agarose gel electrophoresis. This step ensures that the primers will react across strains representative of the diversity of the pathogen (avoid false negative), but will not react with the nucleic acids of other organisms that could be present in a sample (avoid false positives). The collection of purified RNA templates used in this screening process for VESV genomes are listed below and include:

1) Strain panels representative of the diversity of the target organism in nature. We have a panel of 12 VESV strains
2) Eukaryotic DNA that may carry over from sample collection procedures
3) Fifteen soil samples from around the country representing diverse climates and contain a complex mixture of organisms from diverse environmental collections
4) Aerosol samples: 752 samples collected from aerosol collectors and pooled for background testing purposes.

Based on data from primer pair screening, a set of eight specific and reliable signatures were then further tested for suitability for real-time TaqMan fluorogenic PCR detection protocols. The selected signatures showed a robust signal in all target reactions.

List of Near-neighbors screened

Note: There were no near neighbors screened for VESV.

TABLE 321. List of Targets screened

Virus	Strain/ID[1]	Source	PIADC V#	Original Titer	Passage History	Extraction date/ID	Extraction Method	Use stock titer (Log10 TCID50 / ml)	Titer Method

VESV	E54	PIADC	V02033	**Unknown**	3 ST, 2 Vero	**Unknown**	Ambion MagMax 96	5.99 ± 0.1	**Spearmann-Karber**
VESV	B51	PIADC	V00758	**Unknown**	4 Vero	**Unknown**	Ambion MagMax 96	3.11 ± 0.15	**Spearmann-Karber**
VESV	J56	PIADC	V02037	**Unknown**	17 PPK, 3 ST, 2 Vero	**Unknown**	Ambion MagMax 96	2.99 ± 0.1	**Spearmann-Karber**
VESV	I55	PIADC	V02036	**Unknown**	12 PPK, 2 MVPK, 6 Vero	**Unknown**	Ambion MagMax 96	2.18 ± 0.14	**Spearmann-Karber**
VESV	G55	PIADC	V02034	**Unknown**	21 PPK, 2 MVPK, 7 Vero	**Unknown**	Ambion MagMax 96	3.8 ± 0.12	**Spearmann-Karber**
VESV	D53	PIADC	V02032	**Unknown**	12 PPK, 2 MVPK, 7 Vero	**Unknown**	Ambion MagMax 96	5.86 ± 0.16	**Spearmann-Karber**
VESV	A48	PIADC	V02028	**Unknown**	12 PPK, 2 MVPK, 6 Vero	**Unknown**	Ambion MagMax 96	5.43 ± 0.16	**Spearmann-Karber**
VESV	K54	PIADC	V02038	**Unknown**	12PPK, 2MVPK, 5 Vero	**Unknown**	Ambion MagMax 96	3.99 ± 0.15	**Spearmann-Karber**
VESV	C52	PIADC	V02031	**Unknown**	12PPK, 2MVPK, 6 Vero	**Unknown**	Ambion MagMax 96	5.11 ± 0.15	**Spearmann-Karber**
VESV	H54	PIADC	V02035	**Unknown**	12PPK, 2MVPK, 5 Vero	**Unknown**	Ambion MagMax 96	3.43 ± 0.14	**Spearmann-Karber**
VESV	1934B	PIADC	V02029	**Unknown**	10PPK, 2ST, 1 Vero	**Unknown**	Ambion MagMax 96	3.18 ± 0.13	**Spearmann-Karber**
VESV	F55	PIADC	V02027	**Unknown**	2 ST, 3PK, 1SW, 3PK, 2ST, 1 Vero	**Unknown**	Ambion MagMax 96	ND	**Spearmann-Karber**

10.4.1. Vesicular Exanthema of swine virus (VESV) - Gel Screening Report

List of signatures screened in gel format:
20 signatures were developed for VESV. For specific information on signature sequences please refer to CBNP database Plate Name 107_1 or LLNL computations group.

TABLE 322. Nucleic acid extracts used to challenge the initial set of 20 candidate signatures. These select backgrounds are used to determine which signatures will be down-selected against in the screening process. No prokaryotes were screened. All samples were screened in duplicate. From the 20 signatures generated, 10 were completely without any cross reactions. The cross reactions for the 6 signatures in the final assay are listed in TABLE 10 below.

Nucleic Acid Extract Type[1]	Description/ID
Soil Extract	D000505

Soil Extract	D000526
Eukaryotic DNA Extract	Mosquito
Eukaryotic DNA Extract	Flea
Eukaryotic DNA Extract	Tick
Eukaryotic DNA Extract	Porcine
Eukaryotic DNA Extract	Rabbit
Eukaryotic DNA Extract	Bovine

[1] No prokaryotes were screened at this stage.

TABLE 323. Summary of results and noted cross-reactions from background panel screening in gel format. "N" indicates that no reaction (no bands) was observed.

	vesv_95653.F vesv_95654.R	vesv_95677.F vesv_95678.R	vesv_95680.F vesv_95681.R	vesv_95686.F vesv_95687.R	vesv_95692.F vesv_95693.R	vesv_95701.F vesv_95702.R
Bovine	N	N	N	N	N	N
Flea	N	N	N	N	N	N
Mosquito	N	N	N	N	N	N
Rabbit	Not screened	Not screened	Not screened	N	Not screened	Not screened
Tick	N	N	N	N	N	N
Pig	N	N	N	N	N	1000bp multiple bands
Soil D000505	N	N	N	375bp multiple bands	N	N
Soil D000526	N	N	175bp multiple bands	N	N	N

***Signatures screened in gel format against available targets and near neighbors:**
All 20 signatures developed were screened against the 12 available targets. No near neighbors were screened. From the 20 signatures, the results of the 6 most favorable signatures are indicated below.

TABLE 324. Results of gel screening against available targets. None of the signatures were shown to be reactive with all targets and none of the signatures would react against the D53 strain. . "N" indicates that no reaction (no bands) was observed.

	vesv_95653.F vesv_95654.R	vesv_95677.F vesv_95678.R	vesv_95680.F vesv_95681.R	vesv_95686.F vesv_95687.R	vesv_95692.F vesv_95693.R	vesv_95701.F vesv_95702.R
Predicted Product Size	153	144	199	124	200	200
VESV (G55)	**N**	**140 bp**	**N**	**100 bp**	**200 bp**	**N**
VESV (K54)	**150 bp**	**125 bp**	**200 bp**	**110: 60,40 bp**	**180, 60 bp**	**200 bp**
VESV (A48)	**N**	**120 bp**	**N**	**100 bp**	**0**	**200 bp**
VESV (B51)	**50 bp**	**125 bp**	**200 bp**	**100 bp**	**150 bp**	**200 bp**
VESV (C52)	**N**	**N**	**200 bp**	**N**	**N**	**N**
VESV (D53)	**N**	**N**	**N**	**N**	**N**	**N**
VESV (H54)	**160 bp**	**110 bp**	**200 bp**	**100: 60 bp**	**180 bp**	**N**
VESV (I55)	**N**	**N**	**200 bp**	**100: 60 bp**	**N**	**N**
VESV	**150:**	**130 bp**	**190 bp**	**110 bp**	**180 bp**	**200 bp**

(1934B)	multiples					
VESV (E54)	150 bp	125 bp	190 bp	100 bp	200 bp	200 bp
VESV (F55)	150, 40 bp	N	200, 40 bp	100 bp	170 bp	N
VESV (J56)	N	N	200 bp	100 bp	180 bp	200 bp

Vesicular Exanthema of swine virus (VESV) - Real-time PCR Screening Report

TABLE 325. Signatures screened against Backgrounds in Real-time PCR Format.

vesv_95653.F	GCCTTCTCCCTTCCCAAAA
vesv_95654.R	TGAAGGAATGGTTCCGTCAGT
vesv_95655.P	CCAAATTGCACATCTAAGGTTATCAACGATGATG
vesv_95677.F	TTTGATGTCCGCTCTTGACAA
vesv_95678.R	CGCTTTGCAAGGGCAAAT
vesv_95679.P	TCATTTTGACCGGACCTCCGGG
vesv_95680.F	GGGAATGAGGTGTGCATCATT
vesv_95681.R	CACGTCTTGATGTTGGCTTGAC
vesv_95682.P	CGACTCATCTGACAAGGTTGATTATGCCAATTT
vesv_95686.F	GGTCGCTCTCACTGATGATGAGTA
vesv_95687.R	GGTGTTATCAGCACCCATTGC
vesv_95688.P	CTTCCTCCAACTCAGGCACCGAGC
vesv_95692.F	ACCACCTCTGGAAACATCTATGG
vesv_95693.R	TTTGTGCACGTGTCACGAAT
vesv_95694.P	TGGTGACAAATGCCCGTCCCG
vesv_95701.F	AGGCTGTCGCAGCCTACAA
vesv_95702.R	GTTTTCCGTAGAGGTCGGTTAGGT
vesv_95703.P	ACCAAATCGCTCACGCGAGTGGT

TABLE 326a-d. Nucleic acid extracts used to challenge the initial set of candidate signatures. These select backgrounds are used to determine which signatures will be down-selected against in the screening process. All reactions were done in duplicate, 200 pg per reaction. No cross-reactions were observed.

(a) Total of 15 soils screened

D000028	D000107
D000036	D000108
D000051	D000109
D000052	D000117
D000054	D000402
D000086	D000403
D000101	D000426
D000106	

(b) Total of 16 zoos screened

Monkey	Sheep	Rat	Drosophila
Human	Dog	Pig	Bovine
Tick	Chicken	Rabbit	Mosquito
Mouse	Cat	Equine	Flea

(c) Total of 14 microbes screened

E. coli	B. cereus	E. herbicola
B. globigii	S. pneumonia	B. thuringiensis
P. aeruginosae	C. burnetti	H. influenza
S. typhimurium	B. subtilis	S. aureus
L. monocytogenes	B. burgdorferi	

(d) Aerosol screening

Aerosol Block	Signatures Screened	Number of Samples in Block
Block 18 062804-061204	6	752
Total:	**6**	**752 samples**

TABLE 327. List of signatures screened in Real-time PCR format against available targets:

vesv_95653.F	GCCTTCTCCCTTCCCAAAA
vesv_95654.R	TGAAGGAATGGTTCCGTCAGT
vesv_95655.P	CCAAATTGCACATCTAAGGTTATCAACGATGATG
vesv_95677.F	TTTGATGTCCGCTCTTGACAA
vesv_95678.R	CGCTTTGCAAGGGCAAAT
vesv_95679.P	TCATTTTGACCGGACCTCCGGG
vesv_95680.F	GGGAATGAGGTGTGCATCATT
vesv_95681.R	CACGTCTTGATGTTGGCTTGAC
vesv_95682.P	CGACTCATCTGACAAGGTTGATTATGCCAATTT
vesv_95686.F	GGTCGCTCTCACTGATGATGAGTA
vesv_95687.R	GGTGTTATCAGCACCCATTGC
vesv_95688.P	CTTCCTCCAACTCAGGCACCGAGC
vesv_95692.F	ACCACCTCTGGAAACATCTATGG
vesv_95693.R	TTTGTGCACGTGTCACGAAT
vesv_95694.P	TGGTGACAAATGCCCGTCCCG
vesv_95701.F	AGGCTGTCGCAGCCTACAA
vesv_95702.R	GTTTTCCGTAGAGGTCGGTTAGGT
vesv_95703.P	ACCAAATCGCTCACGCGAGTGGT

TABLE 328. PIADC data, reference date 20050929. Results of real-time PCR target screening of VESV when spiked with 200 pg/ reaction of extracted nucleic acid. VESV_95686/87 reacted with all but 3 target strains, the others performed much more poorly. Near-neighbors were not tested. "N" indicates that no reaction was observed after 35 cycles of PCR.

	vesv_95653.F vesv_95654.R	vesv_95677.F vesv_95678.R	vesv_95680.F vesv_95681.R	vesv_95686.F vesv_95687.R	vesv_95692.F vesv_95693.R	vesv_95701.F vesv_95702.R
VESV (G55)	**N**	**N**	**N**	**N**	**N**	**N**
VESV (K54)	**N**	**N, N, 30.87**	**N**	**17.25, 21.11, 19.98**	**N**	**N**
VESV (A48)	**N**	**27.57, 17.52**	**N/A, 27.03**	**14.16, 14.22**	**15.68, 20.39**	**N**
VESV (B51)	**N**	**16.96, 28.73**	**N**	**16.86, 20.75**	**N**	**N**
VESV (C52)	**N**	**N**	**N**	**26.01, 29.60**	**N**	**N**
VESV (H54)	**N**	**N/A, 28.76**	**N**	**22.60, 21.17**	**N**	**N**

VESV (I55)	**N**	**N**	**N**	**22.15, 21.69**	**N**	**N**
VESV (1934B)	**N**	**N**	**N**	**N**	**N**	**N**
VESV (E54)	**N**	**18.23, N/A**	**15.71, N/A**	**18.64, 38.34**	**N**	**N**
VESV (F55)	**N**	**N/A, 33.73**	**N/A, 16.32**	**27.61, 28.08**	**N**	**N/A, 17.39**
VESV (J56)	**N**	**N**	**N**	**N**	**N**	**N**

Notes: (1) the number of Ct values denotes the number of replicate screenings against the particular template, the minimum standard replicates is 2, in some cases a repeat was necessary for validity of the results.

Vesicular Exanthema of Swine Virus (VESV) - LOD Report

TABLE 329a-k. Summary of real-time PCR titrations for VESV signatures when screened against available targets. A dilution series over 5-logs was prepared from extracted nucleic acid from untitered virus cell culture media. Values presented are in CT, cycle threshold. "N/A" indicates that no PCR product was detected for the 35 cycles of PCR. The tentative limit of detection is denoted by green highlighted cells. In some cases the LOD was not reached, thus no values are highlighted.

(a)

	Signature Average Ct Value[1]			
F55 Target Dilution Factor	**VESV 95654**	**VESV 95678**	**VESV 95687**	**VESV 95693**
1:10	36.2	**N/A**	27.9	**N/A**
1:100	**39.6**	**N/A**	28.2	**N/A**
1:1K	**N/A**	**N/A**	30.7	**N/A**
1:10K	**N/A**	**N/A**	35.1	**N/A**
1:100K	**N/A**	**N/A**	**37.1**	**N/A**
NTC	**N/A**	**N/A**	**N/A**	**N/A**

(b)

	Signature Average Ct Value			
1934B Dilution Factor	**VESV 95654**	**VESV 95678**	**VESV 95687**	**VESV 95693**
1:10	**N/A**	**N/A**	**N/A**	**38.9**
1:100	**N/A**	**N/A**	**N/A**	**N/A**
1:1K	**N/A**	**N/A**	**N/A**	**N/A**
1:10K	**N/A**	**N/A**	**N/A**	**N/A**
1:100K	**N/A**	**N/A**	**N/A**	**N/A**
NTC	**N/A**	**N/A**	**N/A**	**N/A**

(c)

	Signature Average Ct Value			
E54 Target Dilution Factor	**VESV 95654**	**VESV 95678**	**VESV 95687**	**VESV 95693**
1:10	28.7	**N/A**	28.8	**N/A**
1:100	29.1	**N/A**	28.7	**N/A**

1:1K	32.3	**N/A**	31.9	**N/A**
1:10K	34.6	**N/A**	34.8	**N/A**
1:100K	**38**	**N/A**	**38.9**	**N/A**
NTC	**N/A**	**N/A**	**N/A**	**N/A**

(d)

	Signature Average Ct Value			
K54 Target Dilution Factor	**VESV 95654**	**VESV 95678**	**VESV 95687**	**VESV 95693**
1:10	**N/A**	**N/A**	36.2	**N/A**
1:100	**N/A**	**N/A**	32.8	**N/A**
1:1K	**N/A**	**N/A**	**38.2**	**N/A**
1:10K	**N/A**	**N/A**	**N/A**	**N/A**
1:100K	**N/A**	**N/A**	**N/A**	**N/A**
NTC	**N/A**	**N/A**	**N/A**	**N/A**

(e)

	Signature Average Ct Value			
B51 Target Dilution Factor	**VESV 95654**	**VESV 95678**	**VESV 95687**	**VESV 95693**
1:10	N/A	**N/A**	34.3	**N/A**
1:100	**39.8**	**N/A**	33.8	**N/A**
1:1K	**N/A**	**N/A**	**38.9**	**N/A**
1:10K	**N/A**	**N/A**	**N/A**	**N/A**
1:100K	**N/A**	**N/A**	**N/A**	**N/A**
NTC	**N/A**	**N/A**	**N/A**	**N/A**

(f)

	Signature Average Ct Value			
G55 Target Dilution Factor	**VESV 95654**	**VESV 95678**	**VESV 95687**	**VESV 95693**
1:10	37.8	**N/A**	34.8	**N/A**
1:100	**39**	**N/A**	34.1	**N/A**
1:1K	**N/A**	**N/A**	36.3	**N/A**
1:10K	**N/A**	**N/A**	**39.9**	**N/A**
1:100K	**N/A**	**N/A**	**N/A**	**N/A**
NTC	**N/A**	**N/A**	**N/A**	**N/A**

(g)

	Signature Average Ct Value

I55 Target Dilution Factor	VESV 95654	VESV 95678	VESV 95687	VESV 95693
1:10	36	**N/A**	34.8	**N/A**
1:100	36.7	**N/A**	34.1	**N/A**
1:1K	**38.4**	**N/A**	36.3	**N/A**
1:10K	**N/A**	**N/A**	**39.9**	**N/A**
1:100K	**N/A**	**N/A**	**N/A**	**N/A**
NTC	**N/A**	**N/A**	**N/A**	**N/A**

(h)

	Signature Average Ct Value			
H54 Target Dilution Factor	**VESV 95654**	**VESV 95678**	**VESV 95687**	**VESV 95693**
1:10	34.3	**N/A**	31.8	**N/A**
1:100	35.9	**N/A**	32.4	**N/A**
1:1K	**38.6**	**N/A**	34.8	**N/A**
1:10K	**N/A**	**N/A**	**38.4**	**N/A**
1:100K	**N/A**	**N/A**	**N/A**	**N/A**
NTC	**N/A**	**N/A**	**N/A**	**N/A**

(i)

	Signature Average Ct Value			
C52 Target Dilution Factor	**VESV 95654**	**VESV 95678**	**VESV 95687**	**VESV 95693**
1:10	**37.8**	**N/A**	34.8	**N/A**
1:100	**N/A**	**N/A**	35.2	**N/A**
1:1K	**N/A**	**N/A**	36	**N/A**
1:10K	**N/A**	**N/A**	**39.2**	**N/A**
1:100K	**N/A**	**N/A**	**N/A**	**N/A**
NTC	**N/A**	**N/A**	**N/A**	**N/A**

(j)

	Signature Average Ct Value			
A48 Target Dilution Factor	**VESV 95654**	**VESV 95678**	**VESV 95687**	**VESV 95693**
1:10	25.7	31.9	28.1	28.8
1:100	26.1	32.6	28.5	29.1
1:1K	28.7	34.6	31.2	32.2
1:10K	31.7	**39.1**	34.2	35.1
1:100K	**35**	**N/A**	**36.8**	**38.3**
NTC	**N/A**	**N/A**	**N/A**	**N/A**

(k)

	Signature Average Ct Value			
D53 Target Dilution Factor	**VESV 95654**	**VESV 95678**	**VESV 95687**	**VESV 95693**
1:10	**N/A**	**N/A**	30	31.7
1:100	**N/A**	**N/A**	30.6	31.9
1:1K	**N/A**	**N/A**	32.9	34.1
1:10K	**N/A**	**N/A**	36.7	**36.9**
1:100K	**N/A**	**N/A**	**38.9**	**N/A**
NTC	**N/A**	**N/A**	**N/A**	**N/A**

[1]Dilution assays for VESV 95681 and VESV 95702 signatures were performed against all available targets but they are not listed below because they did not give a positive Ct value at any of the tested dilutions. All signatures were screened against dilutions of VESV J56 virus, however none of our signatures gave a positive Ct value with any of the tested dilutions, therefore that screening data has been omitted.

TABLE 330. Summary Table of Signature LODs. N/A indicates that no reaction was observed after 35 cycles.

TARGET	SIGNATURE			
	VESV 95654	**VESV 95678**	**VESV 95687**	**VESV 95693**
F55	**1:100**	N/A	**>1:100K**	N/A
1934B	N/A	N/A	N/A	**1:10**
E54	**>1:100K**	N/A	**>1:100K**	N/A
K54	N/A	N/A	**1:1K**	N/A
B51	**1:100**	N/A	**1:1K**	N/A
G55	**1:100**	N/A	**1:10K**	N/A
I55	**1:1K**	N/A	**1:10K**	N/A
H54	**1:1K**	N/A	**1:10K**	N/A
C52	**1:10**	N/A	**1:10K**	N/A
A48	**>1:100K**	**1:10K**	**>1:100K**	**>1:100K**
D53	N/A	N/A	**>1:100K**	**1:10K**

10.5. VESV MULTIPLEXED PCR SCREENING REPORT

SCREENING AND DOWN-SELECTION TECHNICAL APPROACH:

Signatures that pass gel and real-time PCR screening are candidates for multiplexed assays. If the number of candidate signatures is large than a subset of the available signatures are selected based on measured sensitivity and specificity observed in real-time screening. In preliminary screening the background of individual signatures is measured by running "blank" (no sample added) controls and observing signature response. In some cases a primer-to-primer non-specific interference will eliminate candidate signatures. Signatures are then added iteratively to each multiplexed panel, titrated, and with each signature addition, collectively, all signatures are re-titrated to determine if the presence of one signature will interfere with the performance of

another. During this process signatures are added or subtracted from the multiplexed panel depending on individual outcomes until all desirable signatures have been added.

Criterion for down-selection of multiplexed signatures

Signature specificity. In initial real-time PCR screening signatures undergo target and near neighbor screening and screening against environmental confounders which assess individual signature specificity. In multiplex format this is repeated to verify that a more complex reaction system gives the same level of specificity.

Signature sensitivity. In initial real-time PCR screening signatures undergo limit of detection testing where a dilution curve is generated for each signature. This determines individual signature sensitivity. In multiplex format this is repeated to verify that a more complex reaction system gives acceptable signature sensitivity.

Optimal signal to noise ratio. In the multiplex assay system the potential for cross reactivity is larger than in single reaction testing, this may cause unwanted PCR reaction products or non-specific cross-reactivity. Signatures are preferred to have a high signal to noise ratio, where desired signal is significantly greater than the background noise of the signatures when there is no target nucleic acid in the reaction.

Compatibility with other signatures in multiplex. In some cases a primer set may interfere with other primers or probes in the multiplex and there by cause limit of detection shifting for one or more signature in the multiplex. For each scenario it must be evaluated whether the shift in detection level is significant enough to remove (what could be a very desirable signature) from the panel.

TABLE 331. Order details for VESV signatures ordered for multiplexed assay screening and development.

ID	Modification details	Vendor
vesv_95653.BF	5'-/5Bio/GCCT/iBiodT/CTCCCT/iBiodT/CCCAAAA-3'	IDT DNA
vesv_95653.FCP	5'- /5AmMC6//iSp18/CATCATCGTTGATAACCTTAGATGTGCAATTTGG-3'	IDT DNA
vesv_95653.R	5'-TGAAGGAATGGTTCCGTCAGT-3'	IDT DNA
vesv_95680.BF	5'-/5Bio/GGGAA/iBiodT/GAGGTGTGCA/iBiodT/CATT-3'	IDT DNA
vesv_95680.FCP	5'- /5AmMC6//iSp18/AAATTGGCATAATCAACCTTGTCAGATGAGTCG-3'	IDT DNA
vesv_95680.R	5'-CACGTCTTGATGTTGGCTTGAC-3'	IDT DNA
vesv_95686.BF	5'-/5Bio/GGTCGC/iBiodT/CTCACTGATGA/iBiodT/GAGTA-3'	IDT DNA
vesv_95686.FCP	5'- /5AmMC6//iSp18/GCTCGGTGCCTGAGTTGGAGGAAG-3'	IDT DNA
vesv_95686.R	5'-GGTGTTATCAGCACCCATTGC-3'	IDT DNA
vesv_95692.BF	5'-/5Bio/ACCACC/iBiodT/CTGGAAACATC/iBiodT/ATGG-3'	IDT DNA
vesv_95692.FCP	5'- /5AmMC6//iSp18/CGGGACGGGCATTTGTCACCA-3'	IDT DNA
vesv_95692.R	5'-TTTGTGCACGTGTCACGAAT-3'	IDT DNA

Multiplex Data Analysis -Technical approach

Disease signature thresholds

Thresholds were initially determined using all available data from known negative samples (blanks) by plotting a histogram which showed the probability of a given sample generating a

particular MFI. In the absence of cross reactivity, each signature in the multiplex assay could be treated as an individual signature result. For example, for a sample spiked with VESV, data for other disease signatures could contribute towards negative data, providing that cross reaction between disease signature sets was not observed. This is a way to increase the number of samples that can be used to establish thresholds.

TABLE 332. Individual signature thresholds and ranges for VESV signatures. For FY06, threshold determinations have not yet been made and require a significant number of tests (>500) to generate this information. This information will be updated when it is available.

Signature Name	Mux Assay name	Panel Membership	Signature background in Tris NaCl Buffer (MFI +/-)	Signature background in Clinical matrices (MFI +/-)	Threshold
vesv_95653	VESV-1	Porcine	TBD	TBD	TBD
vesv_95680	VESV-3	Porcine	TBD	TBD	TBD
vesv_95686	VESV-4	Porcine	TBD	TBD	TBD
vesv_95692	VESV-5	Porcine	TBD	TBD	TBD

TABLE 333. List of targets screened in multiplex at PIADC(pending).

Virus	Strain/ID[1]	Source	PIADC V#	Original Titer	Passage History	Extraction date/ID	Extraction Method	Use stock titer Log10 $TCID_{50}$ / ml	Titer Method
VESV	E54	PIADC	V02033	**Unknown**	3 ST, 2 Vero	**Unknown**	Ambion MagMax 96	5.99 ± 0.1	**Spearmann-Karber**
VESV	B51	PIADC	V00758	**Unknown**	4 Vero	**Unknown**	Ambion MagMax 96	3.11 ± 0.15	**Spearmann-Karber**
VESV	J56	PIADC	V02037	**Unknown**	17 PPK, 3 ST, 2 Vero	**Unknown**	Ambion MagMax 96	2.99 ± 0.1	**Spearmann-Karber**
VESV	I55	PIADC	V02036	**Unknown**	12 PPK, 2 MVPK, 6 Vero	**Unknown**	Ambion MagMax 96	2.18 ± 0.14	**Spearmann-Karber**
VESV	G55	PIADC	V02034	**Unknown**	21 PPK, 2 MVPK, 7 Vero	**Unknown**	Ambion MagMax 96	3.8 ± 0.12	**Spearmann-Karber**
VESV	D53	PIADC	V02032	**Unknown**	12 PPK, 2 MVPK, 7 Vero	**Unknown**	Ambion MagMax 96	5.86 ± 0.16	**Spearmann-Karber**
VESV	A48	PIADC	V02028	**Unknown**	12 PPK, 2 MVPK, 6 Vero	**Unknown**	Ambion MagMax 96	5.43 ± 0.16	**Spearmann-Karber**
VESV	K54	PIADC	V02038	**Unknown**	12PPK, 2MVPK, 5 Vero	**Unknown**	Ambion MagMax 96	9.77 x 10^3	**Spearmann-Karber**
VESV	C52	PIADC	V02031	**Unknown**	12PPK, 2MVPK, 6 Vero	**Unknown**	Ambion MagMax 96	1.29 x 10^5	**Spearmann-Karber**
VESV	H54	PIADC	V02035	**Unknown**	12PPK,	**Unknown**	Ambion	2.69 x 10^3	**Spearmann-Karber**

					2MVPK, 5 Vero		MagMax 96		
VESV	1934B	PIADC	V02029	**Unknown**	10PPK, 2ST, 1 Vero	**Unknown**	Ambion MagMax 96	1.51 x 10^3	**Spearmann-Karber**
VESV	F55	PIADC	V02027	**Unknown**	2 ST, 3PK, 1SW, 3PK, 2ST, 1 Vero	**Unknown**	Ambion MagMax 96		**Spearmann-Karber**

TABLE 334. List of additional targets and near-neighbors screened against the porcine Panel. All near-neighbors samples were extracted nucleic acids with unknown titers.

Virus	Strain[1]	Unique ID	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	NA conc. used or stock titer	Titer Method
Border Disease virus	Coos Bay	4-6-92	NADC, Julia Ridpath	1.5 x 10^8 TCID50/mL	unknown	12/14/06	Trizol	1.18 x 10^6 TCID50/mL	Reed & Muench
Bovine Herpes Virus-1	California	NVSL 20032 111903	CAHFS	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Cooper	NVSL 86741 111903	CAHFS	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Los Angeles	Los Angeles ATCC VR188	ATCC (CAHFS)	1 x 10^5 TCID50/mL	(CAHFS) MDBK2(LLNL)MDBK1	5/17/07	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	RLB-106	ATCC VR793	ATCC (CAHFS)	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	A033640072	CAHFS, R. Mock, #5 Swab, 1BDFK1, 1/28/2004, A030020072	unknown	1BDFK1,	4/23/04	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	A040130066	CAHFS, R. Mock, #4 Lung, 1BFK1, 1/28/2004, A040130066	unknown	1BFK1	4/23/04	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	Texas A030020072	CAHFS, R. Mock, #80 Swab, 1BDFK1, 1/28/2004, A030020072	unknown	1BDFK1	4/23/2004	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	California	NVSL 200032	NVSL, CAHFS	unknown	MDBK CAHFS	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Virginia	NVSL 231221	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	California	NVSL 51619	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Minnesota	NVSL 86741	NVSL, CAHFS	unknown	MDBK CAHFS	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Minnesota	NVSL 97-10720	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Cooper	NVSL-Cooper RA 309	NVSL, CAHFS	unknown	MDBK CAHFS	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes-5	DN-599	A032540006	R. Mock, from lung tissue, 2BFK2, 2/3/2004	Unknown	CAHFS Lab 2BFK2 4/30/2004	6/3/2004	Phenol/Chloroform	40 pg/uL	N/A

Bovine Herpes-5	DN-599	A040150085	R. Mock, #2 Lung, 5BFK5, 2/3/04	Unknown	CAHFS Lab 5BFK5 4/23/2004	7/7/2004	Phenol/Chloroform	40 pg/uL	N/A
Bovine Herpes-5	D9402133	D9402133	Sonoma Co. 3/94 Severe necrotizing encephalitis, 1 week old calf	Unknown	CAHFS Lab (MDBK), (1 flasks) 11/19/2003	11/19/2003	Phenol/Chloroform	40 pg/uL	N/A
Bovine Herpes Virus	BHV 5	D9403153	CAHFS	unknown	unknown	unknown	unknown	40 pg/uL	unknown
Caprine Herpes Virus		VR462	ATCC	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Caprine Herpes Virus	D0201157	D0201157	CAHFS, Isolate D0201157 History: San Joaquin Co. 2/02 Aborted fetus-third trimester	unknown	CAHFS Lab (MDBK)	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Caprine Herpes Virus	S0201998	S0201998	CAHFS, Isolate S0201998 History: San Diego Co. 2/02 Aborted fetus-third trimester	unknown	CAHFS Lab (MDBK)	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-1	Santa Barbara	N/A	Bridget McLaughlin CAHFS	unknown	unknown	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-1	Georgia	N/A	Bridget McLaughlin CAHFS	unknown	unknown	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-2	Alberta	041 ODV 0401	NVSL	unknown	BHK	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-1 (SV123)	New Jersey (3TD) Deer, NJ 1955	041 ODV 0401	NVSL	1 x $10^{5.125}$ $TCID_{50}$/0.1 ml	+BHK-3 (12/7/83); (12/11/86); (12/18/86)	Unknown	Trizol	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A011120004	CAHFS, R. Mock, from brain tissue, 2BFK2, 2/3/2004, A011120004	unknown	CAHFS, 2BFK2	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A99043047	CAHFS, R. Mock, Spleen, 1BFK1, 2/3/2004, A99043047	unknown	CAHFS, 1BFK1	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A9904309	CAHFS	unknown	CAHFS, unknown	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Bovine 1247	Bovine 1247 ATCC VR2003	ATCC	unknown	CAHFS, unknown	unkown	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	NVSL 002	NVSL 002 1/28/04	NVSL	unknown	CAHFS, unknown	1/29/03	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	LK	LK ATCC VR701	ATCC	unknown	CAHFS, unknown	unkown	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	D990	D990	CAHFS	unknown	CAHFS, unknown	1/29/03	Phenol/ Chloroform	40 pg/uL	N/A

Feline Herpes	C-27	ATCC VR636	ATCC	unknown	CAHFS, unknown	unknown	unknown	40 pg/uL	N/A
Fowl Pox	Vaccine strain	Poxine™	Jeffers Livestock	unknown	ECE	5/17/07	Phenol/ Chloroform	40 pg/uL	N/A
Parainfluenza Virus type 3	C-243	TC-81335	LLNL	1 x 10^5 TCID50/0.1 mL	(VL)MK1 4(LLNL)H 292-1	5/17/07	Trizol	40 pg/uL	Reed and Muench
Porcine Coronavirus	AR310	ATCC VR-2384	LLNL	1 x $10^{4.25}$ $TCID_{50}$/0.1 ml	ST2				Reed and Muench
Porcine Herpes Pseudorabies	Shope	RA 180	CAHFS	1 x 10^5 TCID50/mL		5/17/07	Trizol	40 pg/uL	N/A
Pseudorabies		92-12013	NVSL 92-12013 bovine isolate Iowa December, 1991	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies		93-11745	NVSL 93-11745 Illinois December, 1992	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies	Shope	RA180	NVSL Shope (PRV) RA 180	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies Titered	Shope	N/A	NVSL	unknown		unknown	Phenol/ Chloroform		Reed & Muench
Respiratory Syncytial virus	Long	N/A		unknown	(VL)KB6 L2KB2HF DL2A549-1(LLNL)H eLa3	5/17/07	Trizol	40 pg/uL	N/A
Transmissible Gastroenteritis virus of hogs	Perdue	NVSL 9801	LLNL	1 x $10^{4.75}$ $TCID_{50}$/0.1 ml	ST 1		Trizol	40 pg/ul	Reed and Muench

TABLE 335. Panel membership for signature. The 3 of the 4 VESV signatures from the Version 1.0 panel were included in the **Porcine-specific multiplexed assay panel** for FMDV rule-out diagnostics. The below table describes the constituents of that multiplexed assay panel in which these signatures were tested.

Bead ID	Signature/ Target Name	Mux Group ID	Virus	Source	Forward Primer	Reverse Primer	Luminex Probe (-strand, FCP)
132	FMDV.TC	FMDV.TC	Foot and Mouth Virus	Tetracore	ACTGGGTTTTACAAACCTGTGA	GCGAGTCCTGCCACGGA	GTCCCACGGCGTGCAAAGGA
133	FMDV.Pir	FMDV.Pir	Foot and Mouth Virus	Pirbright	CACYTYAAGRTGACAYTGRTACTGGTAC	CAGATYCCRAGTGWCICITGTTA	CCTCGGGGTACCTGAAGGGCATCC
142	PRRS_1807709	PRRS__1807709	Porcine Reproductive and Respiratory Syndrome	LLNL	GAGCGGCAATTGTGTCTGTC	GCTGAGGGTGATGCTGTGAC	CGCACAGTATGATGCGTAGGCAAACTAAACTC
144	PRRS_1810351	PRRS_1810351	Porcine Reproductive and Respiratory Syndrome	LLNL	TTCTTGTGACCACGATTCGC	GACCCACCGAGTAACTTGCC	GCTCAAGAGCCAAAAGCTCAGCATGACA
145	PRRS_1807706	PRRS_1807706	Porcine Reproductive and Respiratory Syndrome	LLNL	ATTGGTTTGCTCCGCGATAC	AAATGAGCCACCACATCCAA	CGGTACATTCGACGCGACACCATTTC
148	PRRS_1810383	PRRS_1810383	Porcine Reproductive and Respiratory Syndrome	LLNL	CAGTGTGCACGCTTCCATTT	CTCGAATGATGTGTTGCCGT	AAACATAGCGTAGAGCTGGAATTCGAAGCCA
149	PRRS_1810386	PRRS_1810386	Porcine Reproductive and Respiratory Syndrome	LLNL	GCTTTCTGCGTGCCTTTTCT	ACAACGCCAGAGACATTCCC	TGACTTTGAAGCCTTTCTCGCTCATTTCTGA
150	SVD_1727049	SVD_1	Swine Vesicular Disease	LLNL	CAGGATAATTTCTTCCAAGGGC	ACGTGAACATTTCGAGCTTCC	TGCATTGTGTCTGATGGTACAACTTGTGACG

151	SVD_1727050	SVD_2	Swine Vesicular Disease	LLNL	GACTTGTTGTGGCTGGAGGA	CAGCGCCATGGTGAGGTAG	TGACCGTAATGAGGTCATCGTGATTTCTCAC
152	SVD_1727051	SVD_3	Swine Vesicular Disease	LLNL	GACAAAGTGGCCAAGGGAAA	CACGTAAACCACACTGGGCT	CTGGCGTCATAGCCTGAATAGTCAAACGCTA
154	VESV_95653.F, VESV_95654.R, VESV_95655.P	VESV_1	Vesicular Exanthema of Swine Virus	LLNL	GCCTTCTCCCTTCCCAAAA	TGAAGGAATGGTTCCGTCAGT	CATCATCGTTGATAACCTTAGATGTGCAATTTGG
157	VESV_95686.F, VESV_95687.R, VESV_95688.P	VESV_4	Vesicular Exanthema of Swine Virus	LLNL	GGTCGCTCTCACTGATGATGAGTA	GGTGTTATCAGCACCCATTGC	GCTCGGTGCCTGAGTTGGAGGAAG
158	VESV_95692.F, VESV_95693.R, VESV_95694.P	VESV_5	Vesicular Exanthema of Swine Virus	LLNL	ACCACCTCTGGAAACATCTATGG	TTTGTGCACGTGTCACGAAT	CGGGACGGGCATTTGTCACCA
163	VSV_1798943	VSV_1798943	Vesicular Stomatitis Virus -Indiana	LLNL	CGCCACAAGGCAGAGATGT	TGTCAAATTCTGACTTAGCATACTTGC	GCATACTGCATCATATCAGGAGTCGGTTTTCTG
164	VSV_1811409	VSV_1811409	Vesicular Stomatitis Virus –New Jersey	PIADC	CTCACAACATGGGTCCTGAA	TTCTTGCCCCGGATACATCAT	GGCACAGCTCATCTGCGACTTCCCT
166	VSV_1798947	VSV_1798947	Vesicular Stomatitis Virus -Indiana	LLNL	CCCAATCAATGCCATGATACA	CTCCAATGGAAGGGTCCAAA	TTTGAAAGTAGAACTGTGCAAGCCCGGTATC
167	VSV_1798949	VSV_1798949	Vesicular Stomatitis Virus -Indiana	LLNL	GGCGCTCATTATAAAATTCGGA	ACATTTTCTCGTAGTAATGCAGCAG	GAAGTCCCTGTAATGGATTCCCATTCCATGT
168	VSV_1811405	VSV_1811405	Vesicular Stomatitis Virus -Indiana	PIADC	AAGAGATGGTCACGAGTGAC	GAGCATTTGTGGAAACCGAGC	TGGGTATTTGGTCATTGGTGACACA
169	VSV_1811408	VSV_1811408	Vesicular Stomatitis Virus –New Jersey	PIADC	CTCACAACATGGGTCCTGAA	TTCTTGACCTGGATACATCAT	GGCATAGYTCGTCTGCRACTTCCCT
170	N/A	NC (MT-7)	Maritima	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
171	N/A	b-MT7 (FC)	N/A	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
172	N/A	MT7/Cy3 (IC)	N/A	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
173	Alien RNA	arRNA Control	None	Ambion	GACATCAAGGCTCAAACTAATTTTACC	CAAAGGCTGCCAACATAAAATG	CAAGCGTAAATGCAGCGTCCA

[1]FCP indicates that the probe is on the negative strand (Forward Compliment to the Probe).

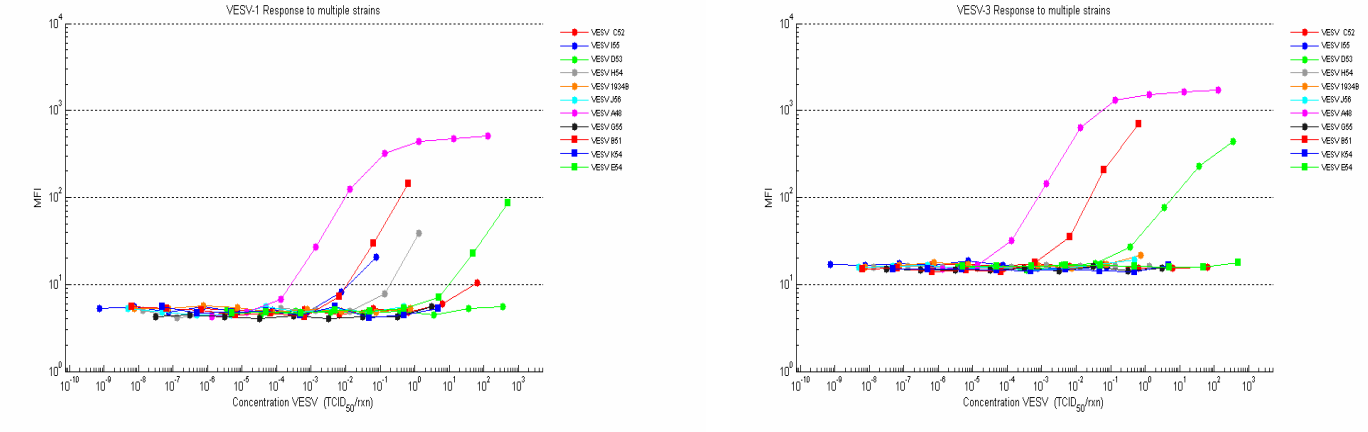
10.5.1. PORCINE PANEL MULTIPLEXED PCR DATA

Preliminary screening: Preliminary screening can be broken down into several steps. The signatures are preliminarily screened by first assessing how the signatures will perform under multiplex PCR conditions and whether or not the addition of the new signatures will adversely effect other signatures, these preliminary screening methods are described as “signature baseline screening” and “multiplex addition screening” respectively. First signature baseline screening tests the performance of the signature in a no-target reaction (blank), a “passing” score is indicated by a low (typically less than 100) MFI. All VESV signatures passed the signature baseline screening. Next in multiplex addition screening the new signature primers are added one-by –one to the other panel signatures to determine if there would be any cross-reactions between primer pairs in the panel. Further assessment of the signatures is done through target and near-neighbor screening and the determination of relative sensitivities. The signature down-selection for the VESV signatures was described in detail in the 2005 Agricultural Assay Report, here only a historical overview is provided.

Historic Data Overview: Version 1.0 Panel LLNL 2006

<u>Plotted by each signature against all strains</u>

(a) (b)



(c) (d)

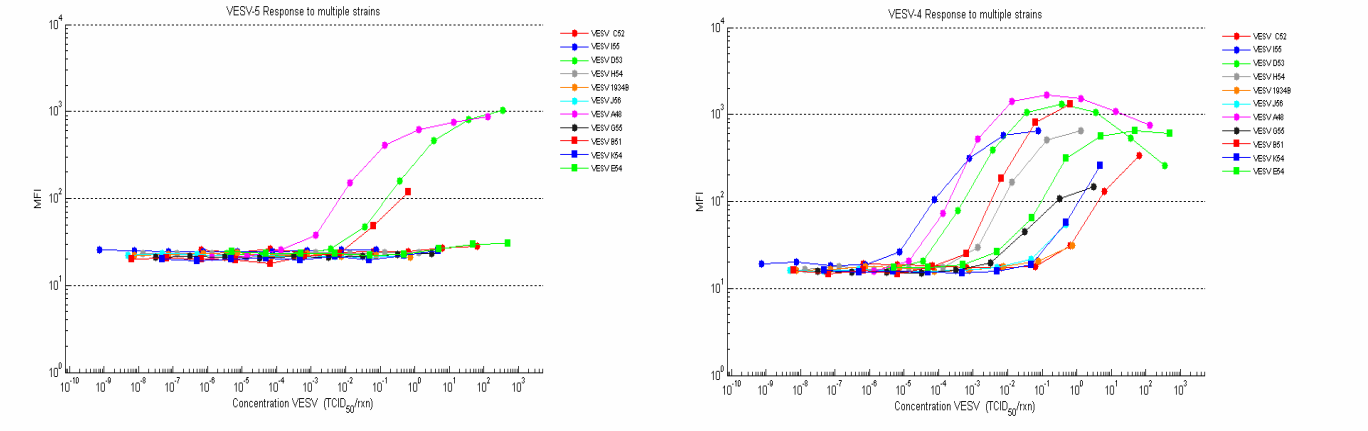


FIG. 50a-d. Version 1.0 panel summary plots for VESV. Each VESV signature plotted against all target strains of VESV at PIADC. (a) VESV-1 (b) VESV-3 (c) VESV-4 (d) VESV-5.

TABLE 336. Summary of limits of detection of each signature for all strains tested. "N" indicates that a limit of detection was not reached (or unable to assess) for those target strains. In most cases the reason for this was that the signatures were not able to detect those strains due to a lack of signature sensitivity to those strains, and in some less frequent instances, the concentration of the virus was not enough to detect. Additional limit of detection screening will be conducted at PIADC in Aug 2007.

Strain	VESV-1	VESV-3	VESV-4	VESV-5	units
E54	1.78E+04	N	2.64E+01	N	TCID50/ml
1934B	N	N	N	N	TCID50/ml
J56	N	N	N	N	TCID50/ml
C52	N	N	3.16E-02	N	TCID50/ml
I55	N	N	1.00E+03	N	TCID50/ml

D53	N	2.51E+02	1.52E-01	1.49E+01	TCID50/ml
H54	1.53E+02	N	1.60E+00	N	TCID50/ml
A48	3.22E-01	4.61E-02	4.18E-02	6.59E-01	TCID50/ml
G55	N	N	6.09E+01	N	TCID50/ml
B51	1.25E+01	1.56E+00	7.07E-01	3.74E+01	TCID50/ml
K54	N	N	3.36E+02	N	TCID50/ml

Near-neighbor and Target screening: The three Version 1.0 panel SVD signatures were added to the Porcine panel. The signatures exhibited a reasonably low background response (<30 MFI) in the Porcine panel.

TABLE 337. Backgrounds screening in multiplexed format for VESV at LLNL and PIADC. Target and near-neighbor testing is in process at PIADC.

	Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors	Targets
Mux PCR Screening	54	135	16	0	43 (pending)	pending

[1]-There are 752 pooled samples in each Aerosol Block.

TABLE 338. Porcine panel backgrounds screening in **multiplexed** format for down-selected VESV signatures against the below listed **eukaryotes**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. None of the signatures cross-reacted with any of the samples listed below.

Description	VESV-1 (54)	VESV-4 (57)	VESV-5 (58)
BOVINE	3	32	24
CAT	3	28	22
CHICKEN	3	22	19
DOG	3	23	21
DROSOPHILA MELANOGASTER	3	28	24
EQUINE	3	31	25
FLEA	4	26	25
HUMAN	3	24	21
MONKEY	3	38	25
MOSQUITO	3	22	20
MOUSE	3	28	23
PIG / PORCINE	4	29	27
RABBIT	4	28	26
RAT	4	34	28
SHEEP	4	32	27
TICK	4	29	26

TABLE 339. Porcine panel backgrounds screening in **multiplexed** format for the four BHV signatures against the below listed **prokaryotes**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in

Median Fluorescence Intensity (MFI) units. None of the signatures cross-reacted with any of the samples listed below.

Description	VESV_1 (54)	VESV_4 (57)	VESV_5 (58)
Erwinia amylovora	4	28	26
Actinobacillus suis	3	21	19
Aneurinbacillus migulanus	3	20	20
Bacillus cereus	4	31	27
Bacillus globigii	4	23	21
Bacillus subtilis	4	28	26
Bacillus thuringiensis	4	31	28
Bifidobacterium denticum	3	24	22
Borrelia burgdorferi	5	35	29
Burkholderia capacia	4	26	23
Caulobacter vibriodes	3	21	20
Clavibacter michganensis	4	26	26
Clostridium butyricum	4	30	29
Corynebacterium pseudodipthericum	4	28	26
Cytophaga marinoflava	4	31	27
Erwinia herbicola	4	30	27
Escherichia coli	4	34	30
Geobacillus caldoxylosilyticus	4	30	28
Halomonas halmophila	3	25	22
Haemophilus influenza	4	26	25
Herbaspirillium seropedicae	3	22	20
Lactobacillus garvieae	3	17	15
Lactobacillus gasseri	3	19	18
Listeria monocytogenes	4	27	23
Listeria seeligeri	5	30	30
Micrococcus luteus	3	21	20
Moraxella lacunatica	3	26	23
Oceanospirillium ssp. Maris	4	25	24
Paenibacillus napthalaenovorans	4	25	24
Paracoccus dentrificans	5	25	24
Porphyrobacter sanguineus	3	18	18
Proteus mirabillis	4	29	28
Pseudomonas aeruginosae	3	23	21
Pseudomonas oleovorans	3	19	18
Rhizobium leguminosarum	4	28	26
Rhodococcus rhodochrous	3	23	21
Salmonella typhimurium	4	26	23
Simonsiella muelleri	3	20	19
Sphingomonas sp. (Alcaligenes sp)	4	22	22
Staphylococcus aureus	4	29	26
Streptococcus pneumoniae	4	32	29

Streptomyces scabiei	4	28	27
Tatlockia maceachernii	4	26	26
Vibrio paraheamolyticus	3	27	26
Xanthomonas translucens	4	26	25

TABLE 340. Porcine panel backgrounds screening in **multiplexed** format for the four BHV signatures against the below listed **soils**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. None of the signatures cross-reacted with any of the samples listed below.

Description	VESV-1 (54)	VESV-4 (57)	VESV-5 (58)
D 000107-49	4	22	23
D 000109 # 50	5	23	22
D 000402 # 53	5	26	25
D 000500 - 26 - 1	5	23	24
D 000501-14-1	5	24	24
D 000505 - 11 - 4	5	24	23
D 000521 - 23	5	26	25
D 000527 - 3	5	25	24
D 000531 - 21	5	25	25
D 000533 - 17 -1	4	21	22
D 000542 - 6	5	24	24
D 000550 - 20	5	25	25
D 000551 - 5	5	26	25
D 000561 - 8 - 6	5	24	23
D 000562 - 30 - 5	5	24	25
S 251	5	21	23
S 252	4	22	22
S 253	5	23	23
S 254	5	24	22
S 255	4	22	22
S 256	5	23	23
S 257	4	20	21
S 259	5	20	21
S 260	5	22	21
S 271	5	25	22
S 272	5	21	22
S 273	4	24	23
S 274	5	24	24
S 275	4	22	24
S 276	4	27	23
S 277	5	29	25
S 279	4	21	21
S 280	5	22	23
S 282	5	23	23
S 283	5	23	23

S 284	5	22	23
S 286	4	22	23
S 287	5	23	23
S 288	4	24	24
S 289	5	21	22
S 290	5	23	23
S 291	5	21	22
S 292	4	22	23
S 295	5	23	23
S 296	4	23	24
S 297	4	24	24
S 298	4	22	22
S 299	4	20	20
S 300	3	17	19
S 301	3	20	20
S 303	2	21	20
S 304	3	20	21
S 305	3	21	21
S 307	3	21	21

TABLE 341. Porcine Panel **Near-Neighbor** screening (Data from 20070608) against the three VESV signatures. Values shown are Median Fluorescence Intensity (MFI) units. “Blanks” are buffer-only samples (no nucleic acid added to reaction), shown below in red. Extracted nucleic acid was added to each PCR reaction in triplicate totaling 200pg/rxn. None of the signatures cross-reacted with any of the samples listed below.

Description	VESV_1 (54)	VESV_4 (57)	VESV_5 (58)
Blank	4	29	29
Blank	2	22	20
BDV Coos Bay	4	21	22
BHV (BFK) A03250006 DN-599	4	27	27
BHV A040150085	5	27	25
BHV-1 (IBR) Texas A030020072 CAHFS	5	28	26
BHV-1 A033640072	4	24	25
BHV-1 A040130066	4	29	28
BHV-1 ATCC VR 793	5	25	23
BHV-1 NVSL 10720	4	27	24
BHV-1 NVSL 200032	4	27	23
BHV-1 NVSL 231221	5	26	24
BHV-1 NVSL 51619	4	25	22
BHV-1 NVSL 86741	4	28	24
BHV-1 or IBR LA ATCC VR188	5	28	26
BHV-1 RA309	4	21	20
BHV-5 A032540006 CAHFS	3	20	19
BHV-5 A040150085 CAHFS	4	27	21
BHV-5 D9402133 CAHFS	3	21	19

BHV-5 D9403153 CAHFS	4	24	22
Caprine Herpes D0201157 CAHFS	4	23	23
Caprine Herpes-2 ATCC VR 462	4	26	23
Caprine Herpes-2 S0201998 CAHFS	4	20	17
EHD-1 A9904309	4	24	22
EHD-1 Georgia	4	23	22
EHD-1 New Jersey	3	16	15
EHD-1 Santa Barbara	3	19	20
EHD-2 Alberta	4	20	20
EHV-1 A011120004 CAHFS	5	29	28
EHV-1 A99043047	4	24	23
EHV-1 ATCC VR2003	4	24	22
EHV-2 ATCC VR701	4	27	23
EHV-2 D990 CAFHS	4	21	18
EHV-2 NVSL 0002	4	25	23
Feline Herpes ATCC VR 636	4	23	23
Fowl Pox	4	23	23
IBR CA 111903	5	28	27
IBR MN 111903	4	25	23
Parainfluenza Type 3	4	24	24
Porcine Herpesvirus or Pseudorabies Shope	4	23	22
Pseudorabies NVSL 92-12013	4	23	20
Pseudorabies NVSL 93-11745	5	26	25
Pseudorabies RA 180 CAHFS	4	22	22
Pseudorabies Titered	4	29	25
Respiratory Syncytial	4	24	23

RESULTS: These four VESV signatures were added to the Porcine panel and tested against various background confounders. When the new constituents were added to the panel a cross-reaction with VESV-3 was and one of the VSV signatures was observed. As a result of the cross-reaction, the VESV-3 signature was dropped from the Porcine panel and the other two VESV signatures were retained. Target and near-neighbor is in process at PIADC this section of the report will be amended when that data becomes available.

11. PORCINE REPRODUCTIVE AND RESPIRATRY SYNDROME (PORCINE PANEL)

OBJECTIVE: We developed for the Department of Homeland Security specific and reliable Real-time RT- PCR signatures for Porcine Reproductive and Respiratory Syndrome [PRRS], among other major agriculturally-impacting viruses. The purpose of these assays (collectively with other virus-specific signatures developed in parallel) is to develop a species specific (i.e. porcine) multiplexed detection assay for Foot-and-Mouth disease rule-out for use in veterinary diagnostic laboratories. The signatures were designed to be uniquely capable of detecting all subtypes of PRRS. We believe the use of PRRS rather than pseudorabies virus PRV as a domestic porcine virus will allow the porcine panel to be more readily used for domestic porcine

surveillance in the NAHLN and thereby provide simultaneous surveillance for FMDV as well as SVD and VESV. Similar reasoning could be used as a partial explanation for the incorporation of BVDV (it is a domestic virus that would be routinely tested for in samples at NAHLN labs) however BVDV is a vesicular/ulcerative disease. This document describes the development of twenty seven optimal Real-time RT-PCR and multiplexed [MUX] PCR signatures to detect strains of Porcine Reproductive and Respiratory Syndrome [PRRS].

11.1. BACKGROUND AND ETIOLOGY OF PRRS

Porcine reproductive and respiratory syndrome (PRRS) was first reported in the USA in 1987. Since then, outbreaks of PRRS and successful isolation of the virus have been confirmed throughout North America and Europe. The etiologic agent is a virus in the group Arteriviridae. The virus is enveloped and ranges in size from 45 to 80 mm. Inactivation is possible after treatment with ether or chloroform; however, the virus is very stable under freezing conditions, retaining its infectivity for 4 mo at -70°C. As the temperature rises, infectivity is reduced (15-20 min at 56°C). Following infection of a naive herd, exposure of all members of the breeding population is inconsistent, leading to the development of naive, exposed, and persistently infected subpopulations of sows. This situation is exacerbated over time through the addition of improperly acclimated replacement gilts and leads to shedding of the virus from carrier animals to those that have not been previously exposed.

The primary vector for transmission of the virus is the infected pig. Contact transmission has been demonstrated experimentally, and the spread of virus from infected seedstock originating from a single source has been described. Introduction of infected seedstock can lead to the introduction and coexistence of genetically diverse isolates of PRRS virus on the same farm. Controlled studies have indicated that infected swine may be longterm carriers, with adults able to shed PRRS virus for up to 86 days after infection, while weaned pigs may harbor virus for 157 days. Experimentally infected boars can shed virus in the semen up to 93 days after infection.

Aerosol transmission of the virus has been considered to be a potential route of transmission, particularly under conditions of high humidity, low temperatures, and low wind speeds; however, this has been difficult to consistently reproduce under controlled field conditions and in the laboratory. PRRS virus can also be transmitted by fomites, such as contaminated needles, boots, coveralls, transport vehicles, and shipping containers. Farm personnel are not a risk, unless hands are contaminated with blood from viremic pigs. Finally, transmission via certain species of insects (mosquitos [*Aedes vexans*] and house flies [*Musca domestica*]) has been reported. The role of migratory waterfowl has not been determined. While biologic transmission of PRRS virus has been documented in immature Mallard ducks, results have not been reproducible experimentally using adult Mallards, nor have infected pigs been able to transmit virus to adult Mallards housed under field conditions[11].

[11] *Source: The Merck Veterinary Manual*

11.2. PRRS VIRUS COMPREHENSIVE ASSAY SUMMARY

Bioinformatics

Virus name: Porcine Reproductive and Respiratory Syndrome (PRRS) Virus

Project name: *PRRS Domestic and European*

Level of discrimination: *species*

Total number of Genome Sequences available for alignment: 25
Number of Initial Signatures: 62
Number of Signatures forwarded to PCR gel screening: 62
Number of Signatures forwarded to Real-time RT-PCR screening: 30

Real-time RT- PCR Screening Summary

TABLE 342. Final signatures down-selected in real-time RT-PCR screening (27).

#	LLNL Signature Designation	Sequence	#	LLNL Signature Designation	Sequence
1	PRRS_1807661_F	CCAGGACATCAGCTGCCTTA	15	PRRS_1810365_F	TTGGCCATATTGGTAAGGCG
	PRRS_1807661_R	TGACATGTTGGACGTAGCTGG		PRRS_1810365_R	CACAGTTTGCCAATTCTCCTTG
	PRRS_1807661_P	TTTCACTCATCTCAGAAGCATAGAAAAGGCAAGA		PRRS_1810365_P	TTTCAGGTATGCTCTGAACGTCCTTTGTTGG
2	PRRS_1807662_F	GGTCGCGCTCACTATGGG	16	PRRS_1810367_F	CCCGCCATTGTAAGATGGTT
	PRRS_1807662_R	GCTTTTCTGCCACCCAACAC		PRRS_1810367_R	GAAGGCACCATCCTGTGTTG
	PRRS_1807662_P	CCGGACGACAAATGCGTGGTTATCATTT		PRRS_1810367_P	TGGCAGCAATTAAGCACATAGCTAGGCAAGT
3	PRRS_1807703_F	CTAACCCGTTTGCCGTCC	17	PRRS_1810368_F	CGTCACCAGTGTGTCCAACA
	PRRS_1807703_R	GCAACCAGCAAGGAAACACA		PRRS_1810368_R	GGGTCTTTCAGCGTACAAGACAA
	PRRS_1807703_P	TACCCAAACATAGCTGGCAATTGCAAGC		PRRS_1810368_P	TGTCGGCCTTGAAAATGGGTCATGAAAT
4	PRRS_1807706_F	ATTGGTTTGCTCCGCGATAC	18	PRRS_1810369_F	GGTTTCAGAACGGACCCAAA
	PRRS_1807706_R	AAATGAGCCACCACATCCAA		PRRS_1810369_R	CGGCAGCAGACGCATAATAC

http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/54100.htm&word=porcine%2creproductive%2cand%2crespiratory%2cand

	PRRS_1807706_P	CGGTACATTCGACGCGACACCATTTC		PRRS_1810369_P	TCATAACTGATAAACCCAGCTTCCTCGGCTG
5	PRRS_1807709_F	GAGCGGCAATTGTGTCTGTC	19	PRRS_1810374_F	TTGCATCTACCATCGCCAAA
	PRRS_1807709_R	GCTGAGGGTGATGCTGTGAC		PRRS_1810374_R	CCACGGCTGAACACAAGGT
	PRRS_1807709_P	CGCACAGTATGATGCGTAGGCAAACTAAACTC		PRRS_1810374_P	CAAGACACGGGTTGTTCATTTATGACCCTCA
6	PRRS_1810342_F	CCAAGTCTTTTGCACACGGT	20	PRRS_1810375_F	TGCGGATAATGCAGTCACAA
	PRRS_1810342_R	GCACCCGGATGGAGTACATT		PRRS_1810375_R	CCACTTGCGGTAGTGGCATA
	PRRS_1810342_P	CTTCTCTCTCCAGAGCTTCAGGACACTGACC		PRRS_1810375_P	TCAGACCCGAGGTGCAAGTCTCTCTTAGC
7	PRRS_1810344_F	ACGTCTCATTCTTGCGGTCA	21	PRRS_1810382_F	GTGGTTGGTGAGGCCACTCT
	PRRS_1810344_R	TTGGTTTGGTAACCGAAGGC		PRRS_1810382_R	ACATTGCCAACGGCAAGATT
	PRRS_1810344_P	ACGTGTTTGATGGCAAGTGCTGGCTC		PRRS_1810382_P	CATCACGAGTCGTGAGCTGAGAAAGCG
8	PRRS_1810346_F	TGACTTCACGTCCCCTCTGA	22	PRRS_1810383_F	CAGTGTGCACGCTTCCATTT
	PRRS_1810346_R	ATTCCGAACCACGGTAGCAG		PRRS_1810383_R	CTCGAATGATGTGTTGCCGT
	PRRS_1810346_P	CAGTACAACAGACCAGAGGATGATTGGGCTT		PRRS_1810383_P	AAACATAGCGTAGAGCTGGAATTCGAAGCCA
9	PRRS_1810347_F	TCCTCGCTCCCTTTCTCGT	23	PRRS_1810384_F	GTTAATGTCCATCCCGTCCG
	PRRS_1810347_R	CAAGAAAGTCAGCAATACCAGAGC		PRRS_1810384_R	ATCATGCTCGGCACAAATGA
	PRRS_1810347_P	ACACAATCGGAGGGTAGTCTGTAAGCAGACG		PRRS_1810384_P	CAAACTTGAGGGTTATTATGCTTGGCTGGCT
10	PRRS_1810348_F	ATCCTTTCGAATTTGCCGAA	24	PRRS_1810386_F	GCTTTCTGCGTGCCTTTTCT
	PRRS_1810348_R	CATGTCCACCCTATCCCACA		PRRS_1810386_R	ACAACGCCAGAGACATTCCC
	PRRS_1810348_P	CGTTTCTCCGCACAAGCCTTAATTGACC		PRRS_1810386_P	TGACTTTGAAGCCTTTCTCGCTCATTTCTGA
11	PRRS_1810351_F	TTCTTGTGACCACGATTCGC	25	PRRS_1810387_F	GAGACCTTTGTGCTTTACCCG
	PRRS_1810351_R	GACCCACCGAGTAACTTGCC		PRRS_1810387_R	TTTTAGCAGCACGGATGACAA
	PRRS_1810351_P	TGTCATGCTGAGCTTTTGGCTCTTGAGC		PRRS_1810387_P	CATACGAACGCTGCGAAAGCACAAGC
12	PRRS_1810355_F	GAAGGCACTTATATGGCCGC	26	PRRS_1810388_F	CCGTACCCGGTTTACCAACT
	PRRS_1810355_R	CACGGTGTTAAGGCAGGGTT		PRRS_1810388_R	CTAGGCCTCCCATTGCTCAG
	PRRS_1810355_P	ACTTTAATCTTCACCCCGTCTGCAGTTGGAT		PRRS_1810388_P	CTTTAACCCCTTCGAGGACGACATGTTTGAT
13	PRRS_1810360_F	GCGGCTCCAAATTCAGTGTT	27	PRRS_1810391_F	CGTGACTTCTACATCCGCCA
	PRRS_1810360_R	GATGACGCGGACCATTCTC		PRRS_1810391_R	GTCACATGGTTCCTGCCTGA
	PRRS_1810360_P	ATCCCACTCCAGACACCAACCCCTCTTT		PRRS_1810391_P	CCATGTGATCGCCCTAATTGAATAGGTGACT

14	PRRS_1810362_F	CACGCTGTTGTGGCAAACTT
	PRRS_1810362_R	CCGGGTTTCAGAAGAACGTC
	PRRS_1810362_P	ACGGTGGGTGAGGTCTCATCAAGATGAC

TABLE 343. Summary of wet-bench screening in signature down-selection. No cross reactions were seen in real-time RT- PCR screening against backgrounds.

	Soils	Prokaryotes	Eukaryotes	Aerosols[1]	Near Neighbors	Target
Gel Screening	5	5	5	none	0	9
Real-time RT- PCR Screening	45	54	16	3 Aerosol Blocks	0	9

TABLE 344a-b. Limit of Detection for PRRS signatures (a) Domestic (b) European against available PRRS isolates recorded using the amount of total extracted target RNA added to each 25 ul reaction that was detectable. The diluted targets were then tested with each signature using the standard Real-time RT- PCR protocol in triplicate and average Ct values are reported for each dilution. "N" indicates no detectable PCR product after 35 cycles of PCR.

(a)

North American (Domestic)	DOM 112	DOM 124	DOM 134	DOM 184	DOM 251	DOM 262
1807661	N	1 pg	1 pg	N	200 pg	N
1807662	200 pg	200 pg	100 pg	100 pg	10 pg	100 fg
1807703	N	100 fg	N	1 pg	100 pg	100 pg
1807706	100 pg	100 pg	100 pg	100 pg	100 pg	100 pg
1807709	N	10 pg	100 pg	100 pg	100 pg	100 pg

(b)

European	EUR 8	EUR 13
1810342	100 pg	100 pg
1810344	100 pg	N
1810346	N	1 pg
1810347	100 pg	100 pg
1810348	100 pg	100 pg
1810351	100 fg	100 fg
1810355	100 fg	N
1810360	100 pg	100 pg
1810362	1 pg	N
1810365	N	100 pg
1810367	N	1 fg
1810368	1 pg	1 pg
1810369	100 pg	100 pg
1810374	1 pg	10 fg
1810375	10 pg	10 pg

1810382	100 pg	10 pg
1810383	1 pg	100 fg
1810384	1 pg	100 pg
1810386	1 pg	10 fg
1810387	100 pg	100 pg
1810388	100 pg	10 pg
1810391	100 pg	10 pg

Multiplexed PCR Screening Summary

TABLE 345. Backgrounds screening in multiplexed format for PRRS.

	Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors[2]	Targets
Mux PCR Screening	54	135	16	0	43* (0)	9

[1]-There are 752 pooled samples in each Aerosol Block.
[2]Near-neighbors indicated (43) apply to the near-neighbor panel that is not uniquely specific for PRRS, but for the other panel constituents that were screened concurrently.

TABLE 346. Signature summary for PRRS multiplexed assays in the porcine panel.

LLNL Signature Designation	MUX Group ID	Gene ID	Limit of detection[1]	Targets Screened	Near Neighbors Screened[2]
PRRS_1807706 (Domestic)	PRRS_1807706	PRRSVgp3(ORF2)/1494886	$1x10^{-1}$ $TCID_{50}$/rxn	9	0
PRRS_1807709 (Domestic)	PRRS_1807709	PRRSVgp8(ORF7)149488	$1x10^{-2}$ $TCID_{50}$/rxn	9	0
PRRS_1810351 (European)	PRRS_1810351	PRRSVgp2(ORF1a)/ 1494889	N/A (pending)	9	0
PRRS_1810383 (European)	PRRS_1810383	PRRSVgp3(ORF2a)/1494886; PRRSVgp4 (ORF3)/1494891.	N/A (pending)	9	0
PRRS_1810386 (European)	PRRS_1810386	PRRSVgp5(ORF4)/1494887	N/A (pending)	9	0

[1] The relative "Limit of detection"was evaluated using one strain of domestic PRRS (North American strain). [2]Near-neighbors indicated (43) apply to the near-neighbor panel that is not uniquely specific for PRRS, but for the other panel constituents that were screened concurrently.

11.3. PRRS SIGNATURE BIOINFORMATICS AND GENERATION INFORMATION

Bioinformatics process:
The LLNL Bioinformatics team have developed a computational process that uses available DNA sequence information for a given target organism to generate candidate DNA signature sequences that are conserved and unique. Signatures are primarily used to underlie reliable and specific pathogen detection assays. Evidence of the success of the process is that several of the Real-time RT- PCR signatures that are used in the national BioWatch monitoring system were

generated by this process, and to date, has resulted in no false positives from a total of over 5 million assays performed.

The general approach is the following: All available complete genomes of different strains of the target species are compared using a multiple genome alignment programs. A *consensus gestalt* is formed from the alignments that contain the sequence conserved amongst all target inputs. (This step is bypassed if only one target sequence is available).

To determine that organism-conserved sequence which does not occur in any other sequenced microbial organism, the consensus gestalt is compared against our immense, continuously updated database of microbial organism. A customized algorithm accomplishes this electronic subtraction, and the result is a *uniqueness gestalt* that is mined for potential signature candidates. A final computational screening is done to verify that cross-reactions are not detected.

Target Virus Information:
Virus name: *Porcine Reproductive and Respiratory Syndrome* Virus
Type: ssRNA positive-strand virus
Genome size: 15428 bp.

Primer/Probe Set Generation Information

Method: Alignment and All Microbe Database subtraction.

Note: The twenty seven PRRS signatures were generated from three separate kpath runs and they are listed below with their corresponding genome sequences information used for the alignment.

TABLE 347. K-path run id: Domestic: 99484. List of reference genomes used for alignment.

	Genome Description	GI Number	Sequence Length (bp)
1	Porcine reproductive and respiratory syndrome virus strain CH-1a, complete genome	14250956	15432
2	Porcine reproductive and respiratory syndrome virus isolate NVSL 97-7985 IA 1-4-2, complete genome	12744849	15393
3	Porcine reproductive and respiratory syndrome virus strain SP, complete genome	7650192	15520
4	Porcine reproductive and respiratory syndrome virus isolate P129, complete genome	20271246	15450
5	Porcine reproductive and respiratory syndrome virus strain JA142, complete genome	40646796	15413
6	Porcine reproductive and respiratory syndrome virus strain NVSL 97-7895, complete genome	45360239	15414
7	Porcine reproductive and respiratory syndrome virus isolate HB-2(sh)/2002, complete genome	31747018	15398
8	Porcine reproductive and respiratory syndrome virus isolate HB-1(sh)/2002, complete genome	25361009	15447

TABLE 348. K-path run id: Domestic: 99485. List of reference genomes used for alignment.

	Genome Description	GI Number	Sequence Length (bp)
1	Porcine reproductive and respiratory syndrome virus BJ-4, complete genome	12240324	15504
2	Porcine reproductive and respiratory syndrome virus strain VR-2332, complete genome	11192298	15411
3	Porcine reproductive and respiratory syndrome virus isolate MLV RespPRRS/Repro, complete genome	9931316	15390
4	Porcine reproductive and respiratory syndrome virus isolate VR-2332, complete genome	27549163	15451
5	Porcine reproductive and respiratory syndrome virus isolate PA8 complete genome	22658020	15483
6	Porcine reproductive and respiratory syndrome virus strain PL97-1, complete genome	46519708	15465
7	Porcine reproductive and respiratory syndrome virus, complete genome	9630807	15428
8	Porcine respiratory and reproductive syndrome virus strain 01NP1.2, complete genome	66735372	15412
9	Porcine reproductive and respiratory syndrome virus RespPRRS MLV, complete genome	66735498	15412
10	Porcine reproductive and respiratory syndrome virus strain PL97-1/LP1, complete genome	51980219	15465
11	Porcine respiratory and reproductive syndrome virus isolate S1, complete genome	92090664	15411
12	Porcine reproductive and respiratory syndrome virus HN1, complete genome	38385769	15423
13	Porcine reproductive and respiratory syndrome virus PRRS MN184A from UMN on May 30 2006 2:25PM	No information	15073
14	Porcine reproductive and respiratory syndrome virus PRRS MN184B from UMN on May 30 2006 2:36PM	No information	15072

TABLE 349. K-path run id: European: 99872. List of reference genomes used for alignment.

	Genome Description	GI Number	Sequence Length (bp)
1	Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome	38146324	15047
2	Lelystad virus, complete genome	11125727	15111
3	PRRSV LV4.2.1, complete genome	51094057	15146

TABLE 350. List of main parameters and settings used in generating candidate signatures using Primer 3 signature generation tool.

Primer3 Main Parameters	
Parameters	**Standard Settings**

PRIMER_OPT_SIZE	20
PRIMER_MIN_SIZE	18
PRIMER_MAX_SIZE	27
PRIMER_PRODUCT_OPT_SIZE	100
PRIMER_PRODUCT_SIZE_RANGE	71-600
PRIMER_OPT_TM	62
PRIMER_MIN_TM	61
PRIMER_MAX_TM	63
PRIMER_MIN_GC	20
PRIMER_MAX_GC	80
PRIMER_PICK_INTERNAL_OLIGO	1
PRIMER_INTERNAL_OLIGO_OPT_SIZE	31
PRIMER_INTERNAL_OLIGO_MIN_SIZE	18
PRIMER_INTERNAL_OLIGO_MAX_SIZE	36
PRIMER_INTERNAL_OLIGO_OPT_TM	72
PRIMER_INTERNAL_OLIGO_MIN_TM	71
PRIMER_INTERNAL_OLIGO_MAX_TM	73
Number of Primers/Probe Set generated:	**62**

Signature Information
Source: LLNL
Project name: PRRS Domestic and European
Level of discrimination: Species.

Number of Initial Signatures: 62
Number of Signatures forwarded to gel bench-screening: 62
Number of Signatures forwarded to real-time RT_PCR TaqMan screening: 30
Number of Final real-time RT-PCR Signatures: 27

Taqsim description
We used a computational Real-time RT- PCR simulator program, "Taqsim", to identify all potential targets for each signature from all sequences available in genbank. Taqsim is a BLAST-based comparison of each signature as a triplet against all sequences in genbank to identify the targets that are predicted to produce a Real-time RT- PCR reaction at 57 degrees primer annealing and 67 degrees for probe annealing (these temperatures are according to Primer 3 oligo Tm calculations). The first column of the excel spreadsheets list the signatures by the name, and the following columns report the predicted genbank targets, coordinates of signature region, amplicon size, and accession #'s for each target.

Note: For a listing of computationally predicted product sequences and potential cross targets for each signature, please see Appendix II, Taqsim Run Data.

Signature bioinformatics

TABLE 351. Signature bioinformatics (a) PRRS_1807661, (b) PRRS_1807662 (c) PRRS_18077703, (d) PRRS_1807706, (e) PRRS_1807709, (f) PRRS_1810342, (g) PRRS_1810344, (h) PRRS_1810346, (i) PRRS_1810347, (j) PRRS_1810348, (k)

PRRS_1810351, (l) PRRS_1810355, (m) PRRS_1810360, (n) PRRS_1810362, (0) PRRS_1810365, (p) PRRS_1810367, (q) PRRS_1810368, (r), PRRS_1810369, (s) PRRS_1810374, (t) PRRS_1810375, (u) PRRS_1810382, (v) PRRS_1810383, (w) PRRS_1810384, (x) PRRS_1810386, (y) PRRS_1810387, (z) PRRS_1810388, (aa) PRRS_1810391.

	(a)	(b)	(c)
Forward Primer	PRRS_1807661_F	PRRS_1807662_F	PRRS_1807703_F
Primer Length (bp)	20	18	18
FWD Primer TM (^{o}C)	54	55	53
FWD Primer GC Content (%)	55	67	61
Forward Location	13490	14693	5043
Reverse Primer	PRRS_1807661_R	PRRS_1807662_R	PRRS_1807703_R
Rev Primer Length (bp)	21	20	20
Rev Primer TM (^{o}C)	55	55	54
Rev Primer GC Content (%)	52	55	50
Reverse location	13744	14946	5296
Probe Name	PRRS_1807661_P	PRRS_1807662_P	PRRS_1807703_P
Probe Length (bp)	34	28	28
Probe TM (^{o}C)	61	61	61
Probe GC Content (%)	38	46	46
Probe location	13650	14864	5248
Probe strand	minus	minus	minus
Predicted Product Size	275	273	273

	(d)	(e)
Forward Primer	PRRS_1807706_F	PRRS_1807709_F
FWD Primer Length (bp)	20	20
FWD Primer TM (^{o}C)	54	55
FWD Primer GC Content (%)	50	55
Forward Location	12228	15097
Reverse Primer	PRRS_1807706_R	PRRS_1807709_R
Rev Primer Length (bp)	20	20
Rev Primer TM (^{o}C)	53	56
Rev Primer GC Content (%)	45	60
Reverse location	12494	15235
Probe Name	PRRS_1807706_P	PRRS_1807709_P
Probe Length (bp)	26	32
Probe TM (^{o}C)	61	62
Probe GC Content (%)	54	47
Probe location	12395	15193
Probe strand	minus	minus
Predicted Product Size	286	158

	(f)	(g)	(h)
Forward Primer	PRRS_1810342_F	PRRS_1810344_F	PRRS_1810346_F
FWD Primer Length (bp)	20	20	20
FWD Primer TM (^{o}C)	54	54	55
FWD Primer GC Content (%)	50	50	55
Forward Location	284	995	1553
Reverse Primer	PRRS_1810342_R	PRRS_1810344_R	PRRS_1810346_R
Rev Primer Length (bp)	20	20	20
Rev Primer TM (^{o}C)	55	53	55
Rev Primer GC Content (%)	55	50	55
Reverse location	427	1113	1648
Probe Name	PRRS_1810342_P	PRRS_1810344_P	PRRS_1810346_P
Probe Length (bp)	31	26	31
Probe TM (^{o}C)	64	63	63
Probe GC Content (%)	55	54	48
Probe location	321	1030	1575
Probe strand	plus	plus	plus
Predicted Product Size	163	140	115

	(i)	(j)	(k)
Forward Primer	PRRS_1810347_F	PRRS_1810348_F	PRRS_1810351_F
FWD Primer Length (bp)	19	20	20
FWD Primer TM (^{o}C)	55	51	53
FWD Primer GC Content (%)	58	40	50
Forward Location	1745	2935	3773
Reverse Primer	PRRS_1810347_R	PRRS_1810348_R	PRRS_1810351_R
Rev Primer Length (bp)	24	20	20
Rev Primer TM (^{o}C)	55	54	55
Rev Primer GC Content (%)	46	55	60
Reverse location	1885	3115	3894
Probe Name	PRRS_1810347_P	PRRS_1810348_P	PRRS_1810351_P
Probe Length (bp)	31	28	28
Probe TM (^{o}C)	64	61	63
Probe GC Content (%)	52	50	50
Probe location	1852	2967	3798
Probe strand	minus	plus	plus
Predicted Product Size	164	200	141

	(l)	(m)	(n)
Forward Primer	PRRS_1810355_F	PRRS_1810360_F	PRRS_1810362_F
FWD Primer Length (bp)	20	20	20
FWD Primer TM (^{o}C)	54	54	55
FWD Primer GC Content (%)	55	50	50
Forward Location	5160	6769	7517

Reverse Primer	PRRS_1810355_R	PRRS_1810360_R	PRRS_1810362_R
Rev Primer Length (bp)	20	19	20
Rev Primer TM (^{o}C)	55	53	53
Rev Primer GC Content (%)	55	58	55
Reverse location	5269	6861	7583
Probe Name	PRRS_1810355_P	PRRS_1810360_P	PRRS_1810362_P
Probe Length (bp)	31	28	28
Probe TM (^{o}C)	63	64	63
Probe GC Content (%)	45	54	54
Probe location	5208	6831	7547
Probe strand	plus	plus	Minus
Predicted Product Size	129	111	86

	(o)	(p)	(q)
Forward Primer	PRRS_1810365_F	PRRS_1810367_F	PRRS_1810368_F
FWD Primer Length (bp)	20	20	20
FWD Primer TM (^{o}C)	53	53	55
FWD Primer GC Content (%)	50	50	55
Forward Location	8154	8558	8713
Reverse Primer	PRRS_1810365_R	PRRS_1810367_R	PRRS_1810368_R
Rev Primer Length (bp)	22	20	23
Rev Primer TM (^{o}C)	53	54	56
Rev Primer GC Content (%)	46	55	48
Reverse location	8299	8661	8880
Probe Name	PRRS_1810365_P	PRRS_1810367_P	PRRS_1810368_P
Probe Length (bp)	31	31	28
Probe TM (^{o}C)	62	63	61
Probe GC Content (%)	45	45	43
Probe location	8243	8619	8772
Probe strand	minus	minus	plus
Predicted Product Size	167	123	190

	(r)	(s)	(t)
Forward Primer	PRRS_1810369_F	PRRS_1810374_F	PRRS_1810375_F
FWD Primer Length (bp)	20	20	20
FWD Primer TM (^{o}C)	53	53	53
FWD Primer GC Content (%)	50	45	45
Forward Location	8951	10307	10489
Reverse Primer	PRRS_1810369_R	PRRS_1810374_R	PRRS_1810375_R
Rev Primer Length (bp)	20	19	20
Rev Primer TM (^{o}C)	54	55	55
Rev Primer GC Content (%)	55	58	55
Reverse location	9100	10449	10612

Probe Name	PRRS_1810369_P	PRRS_1810374_P	PRRS_1810375_P
Probe Length (bp)	31	31	29
Probe TM (^{o}C)	63	62	64
Probe GC Content (%)	48	45	55
Probe location	8979	10368	10553
Probe strand	plus	plus	plus
Predicted Product Size	169	161	143

	(u)	(v)	(w)
Forward Primer	PRRS_1810382_F	PRRS_1810383_F	PRRS_1810384_F
FWD Primer Length (bp)	20	20	20
FWD Primer TM (^{o}C)	57	54	53
FWD Primer GC Content (%)	60	50	55
Forward Location	12123	12362	12622
Reverse Primer	PRRS_1810382_R	PRRS_1810383_R	PRRS_1810384_R
Rev Primer Length (bp)	20	20	20
Rev Primer TM (^{o}C)	54	53	53
Rev Primer GC Content (%)	45	50	45
Reverse location	12246	12471	12777
Probe Name	PRRS_1810382_P	PRRS_1810383_P	PRRS_1810384_P
Probe Length (bp)	27	31	31
Probe TM (^{o}C)	62	63	62
Probe GC Content (%)	56	45	45
Probe location	12213	12423	12655
Probe strand	minus	minus	plus
Predicted Product Size	143	129	175

	(x)	(y)	(z)
Forward Primer	PRRS_1810386_F	PRRS_1810387_F	PRRS_1810388_F
FWD Primer Length (bp)	20	21	20
FWD Primer TM (^{o}C)	54	54	55
FWD Primer GC Content (%)	50	52	55
Forward Location	13200	13641	13859
Reverse Primer	PRRS_1810386_R	PRRS_1810387_R	PRRS_1810388_R
Rev Primer Length (bp)	20	21	20
Rev Primer TM (^{o}C)	55	53	55
Rev Primer GC Content (%)	55	43	60
Reverse location	13261	13816	14029
Probe Name	PRRS_1810386_P	PRRS_1810387_P	PRRS_1810388_P
Probe Length (bp)	31	26	31
Probe TM (^{o}C)	62	62	62
Probe GC Content (%)	42	54	45
Probe location	13225	13788	13974

Probe strand	minus	minus	minus
Predicted Product Size	81	196	190

(aa)

Forward Primer	PRRS_1810391_F
FWD Primer Length (bp)	20
FWD Primer TM (^{o}C)	54
FWD Primer GC Content (%)	55
Forward Location	14897
Reverse Primer	PRRS_1810391_R
Rev Primer Length (bp)	20
Rev Primer TM (^{o}C)	55
Rev Primer GC Content (%)	55
Reverse location	14977
Probe Name	PRRS_1810391_P
Probe Length (bp)	31
Probe TM (^{o}C)	61
Probe GC Content (%)	45
Probe location	15021
Probe strand	Minus
Predicted Product Size	144

Target Region Gene Information

TABLE 352a-b. (a) Reference Genomes used for Gene Information. (b) Gene information for each signature.

(a)

Genome Description	GI Number	Sequence Length (bp)
Porcine reproductive and respiratory syndrome virus strain SP, complete genome	gi|7650192|gb|AF184212.1|	15520
Porcine reproductive and respiratory syndrome virus BJ-4, complete genome	gi|12240324|gb|AF331831.1	15504
Porcine reproductive and respiratory syndrome virus strain EuroPRRSV	gi|38146324|gb|AY366525.1	15047

(b)

			Gene Location		Target Region Location	
Kpath Signature ID	**Gene/ID**	**Description**	**Start**	**End**	**Start**	**End**

1807661	PRRSVgp4(ORF3) /1494891; PRRSVgp5(ORF4)/ 1494887	GP3 envelope protein; GP4.	12805;13350	13569;13886	13490	13764
1807662	PRRSVgp7(ORF6)/1494890	Membrane protein M	14484	15008	14693	14965
1807703	PRRSVgp2(ORF1a)/ 1494889	RNA polymerase	191	7699	5043	5315
1807706	PRRSVgp3(ORF2)/1494886	envelope protein GP2	12071	12841	12228	12513
1807709	PRRSVgp8(ORF7)149488	nucleocapsid protein N	14887	15258	15097	15254
1810342	PRRSVgp2(ORF1a)/ 1494889	replicase polyprotein 1A	222	7361	284	446
1810344	PRRSVgp2(ORF1a)/ 1494889	replicase polyprotein 1A	222	7361	995	1132
1810346	PRRSVgp2(ORF1a)/ 1494889	replicase polyprotein 1A	222	7361	1553	1667
1810347	PRRSVgp2(ORF1a)/ 1494889	replicase polyprotein 1A	222	7361	1745	1908
1810348	PRRSVgp2(ORF1a)/ 1494889	replicase polyprotein 1A	222	7361	2935	3134
1810351	PRRSVgp2(ORF1a)/ 1494889	replicase polyprotein 1A; encodes papain-like cysteine protease, cysteine protease and 3C-like serine protease.	222	7361	3773	3913
1810355	PRRSVgp2(ORF1a)/ 1494889	replicase polyprotein 1A	222	7361	5160	5288
1810360	PRRSVgp2(ORF1a)/ 1494889	replicase polyprotein 1A	222	7361	6769	6879
1810362	PRRSVgp1(ORF1B)/1494884	replicase polyprotein 1B	7358	11734	7517	7602
1810365	PRRSVgp1(ORF1B)/1494884	replicase polyprotein 1B	7358	11734	8154	8320
1810367	PRRSVgp1(ORF1B)/1494884	replicase polyprotein 1B	7358	11734	8558	8680
1810368	PRRSVgp1(ORF1B)/1494884	replicase polyprotein 1B; encodes polymerase, helicase, zinc finger and nidovirus motifs;	7358	11734	8713	8902
1810369	PRRSVgp1(ORF1B)/1494884	replicase polyprotein 1B	7358	11734	8951	9119
1810374	PRRSVgp1(ORF1B)/1494884	replicase polyprotein 1B; encodes polymerase, helicase, zinc finger and nidovirus motifs;	7358	11734	10307	10467
1810375	PRRSVgp1(ORF1B)/1494884	replicase polyprotein 1B	7358	11734	10489	10631
1810382	PRRSVgp3(ORF2a)/1494886	Envelope glycoprotein (GP2)	11745	12494	12123	12265
1810383	PRRSVgp3(ORF2a)/1494886; PRRSVgp4 (ORF3)/1494891.	Glycosylated envelope protein (GP2); envelope	11745; 12353	12494;13150	12362	12490

		glycoprotein (GP3).				
1810384	PRRSVgp4 (ORF3)/1494891	envelope glycoprotein (GP3)	12353	13150	12622	12796
1810386	PRRSVgp5(ORF4)/1494887	envelope glycoprotein (GP4).	12895	13446	13200	13280
1810387	PRRSVgp6(ORF5)/1494885	major envelope glycoprotein (GP5)	13443	14048	13641	13836
1810388	PRRSVgp6(ORF5)/1494885; PRRSVgp7/1494890	major envelope glycoprotein; Membrane protein M	13443; 14036	14048; 14557	13859	14048
1810391	PRRSVgp8/(ORF7/1494888	Nucleocapsid protein N	14547	14933	14897	15040

11.4. PRRS GEL AND REAL-TIME PCR SCREENING REPORT

Wet-lab screening process. To ensure extremely high selectivity and sensitivity, we have established a screening protocol that rigorously screens candidate DNA signatures to select the optimal sets for eventual field use. Candidate signature primer pairs that survive computational screening proceed to wet chemistry screening utilizing standard PCR with agarose gel electrophoresis. This step ensures that the primers will react across strains representative of the diversity of the pathogen (avoid false negative), but will not react with the nucleic acids of other organisms that could be present in a sample (avoid false positives). The collection of purified DNA templates used in this screening process is listed below:

1) Strain panels representative of the diversity of the target organism in nature.
2) Near neighbors-organisms that are closely related at the genetic level and therefore have the greatest likelihood of cross-reaction.
3) Eukaryotic DNA that may carry over from sample collection procedures.
4) Prokaryotic DNA that would be expected to be present in environmental samples and may also be present in Bovine and Porcine oral cavities.
5) Forty five soil samples from around the country representing diverse climates and contain a complex mixture of organisms from diverse environmental collections
6) Aerosol samples: 752 samples collected from aerosol collectors and pooled for background testing purposes.

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TABLE 353. List of targets screened at LLNL. The PRRS virus known as the North American strain or NVSL strain is derived from a field isolate we made from tissues collected and submitted to us in 1989 during the "mystery pig disease" days. When a non-proprietary cell line became available for PRRS isolation and propagation, we went back to some of the original

submissions and reisolated the virus on the non-proprietary cells. The "official" designation is the NVSL strain; it is genetically and antigenically typical of the PRRS viruses that evolved in the Western Hemisphere. The cell culture passage history is unknown.

Virus	Strain[1]	Isolate	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	Use stock titer	Titer Method
PPRS	North American	field isolate	NVSL	10^6 $TCID_{50}$/1.0mL	Unknown	9/5/06	TRIZOL	$9.06x10^4$ $TCID_{50}$/mL	Reed & Muench

TABLE 354. List of targets screened at University of Minnesota. Nucleic acids isolated via TRIZOL extraction at University of Minnesota from PRRS isolates grown at University of Minnesota.

Virus	Strain[1]	Isolate	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	Use stock titer	Titer Method
PPRS	Domestic 112	unknown	NVSL	10^6 $TCID_{50}$/1.0mL	Marc-145	2006	TRIZOL	No titer	N/A
PPRS	Domestic 112	Texas	Univ of Minnesota	unknown	PAM	2006	TRIZOL	No titer	N/A
PPRS	Domestic 124	Oklahoma	Univ of Minnesota	unknown	PAM	2006	TRIZOL	No titer	N/A
PPRS	Domestic 134	Nebraska	Univ of Minnesota	unknown	PAM	2006	TRIZOL	No titer	N/A
PPRS	Domestic 184	Minnesota	Univ of Minnesota	unknown	PAM	2006	TRIZOL	No titer	N/A
PPRS	Domestic 251	Missouri	Univ of Minnesota	unknown	Marc-145	2006	TRIZOL	No titer	N/A
PPRS	Domestic 262	Missouri	Univ of Minnesota	unknown	PAM	2006	TRIZOL	No titer	N/A
PPRS	European 8	Missouri	Univ of Minnesota	unknown	PAM	2006	TRIZOL	No titer	N/A
PPRS	European 13	North Carolina	Univ of Minnesota	unknown	PAM	2006	TRIZOL	No titer	N/A

TABLE 355. Screening summary for PRRS at LLNL and University of Minnesota.

	Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors	Targets
Gel Screening	5	5	5	none	0	9
Real-time_PCR Screening	45	54	16	3_Aerosol Blocks[1]	0	9

[1]-There are 752 pooled samples in each Aerosol Block.

PRRS - Gel Screening Report

Background gel screening was carried out in duplicate as 25ul reactions in 96 well PCR plates on MJ thermal cyclers. Each reaction mix consisted of 1X PCR Buffer [200mM Tris-HCL (pH 8.4), 500mM KCl], 1.5mM $MgCl_2$, 0.8mM each dNTP, 80ng BSA, 0.4uM each forward and reverse primers, 0.75U Platinum *Taq* DNA polymerase, PCR water and a variable amount of extracted DNA template. A total of 5ng of soil DNA or 1ng of Eukaryotic DNA or 200pg of

Prokaryotic DNA was added to each 25ul reaction mix. Background template data and extraction protocols are available upon request.

Because the target is RNA, we used the Clontech Powerscript RT-PCR kit. Each reaction was performed in triplicate as per the manufacturer's suggested protocol, replacing probe with PCR water, in a 25ul volume with a total of 200pg TRIZOL extracted RNA.

Reaction products were visualized by gel electrophoresis on 4% agarose gels. PCR product sizes are listed as visual estimates based on a 20bp ladder that was run on each gel for reference. I f a signature screened against a background produced a PCR product size that fell below 100 base pairs greater in size than the predicted product size for a signature screened against its target, the signature was dropped from further screening. The theory behind this selection process is that a much larger than target PCR product would not cause inhibitory PCR competition. However, a PCR product of correct size or smaller would inhibit PCR through competition.

TABLE 356a-b. List of signatures screened in gel format: 62 signatures were gel screened. Signatures that did not pass the initial gel screening did not have probes ordered. (a) Signatures designed to identify domestic isolates of PRRS. (b) Signatures designed to identify European isolates of PRRS.

(a) 12 signatures designed to identify domestic isolates of PRRS

PRRS_1807659_F	TTCTGGGTCTTCTCGGCG
PRRS_1807659_R	AAAGCACATAAGCTCCAGCCA
PRRS_1807659_P	Probe not ordered
PRRS_1807660_F	TTTTGTGGATGCTTTCACGG
PRRS_1807660_R	TCACCACCTGTTTCCAGGC
PRRS_1807660_P	ACACCATTTCATCAATCAGGGTTGACACCTT
PRRS_1807661_F	CCAGGACATCAGCTGCCTTA
PRRS_1807661_R	TGACATGTTGGACGTAGCTGG
PRRS_1807661_P	TTTCACTCATCTCAGAAGCATAGAAAAGGCAAGA
PRRS_1807662_F	GGTCGCGCTCACTATGGG
PRRS_1807662_R	GCTTTTCTGCCACCCAACAC
PRRS_1807662_P	CCGGACGACAAATGCGTGGTTATCATTT
PRRS_1807702_F	GCTGCACAGAAACACCCTTCT
PRRS_1807702_R	TTTCCACTGGTCATTCGTGC
PRRS_1807702_P	ACTGCTTTACGGTCTCTCCACCCCTTTAACC
PRRS_1807703_F	CTAACCCGTTTGCCGTCC
PRRS_1807703_R	GCAACCAGCAAGGAAACACA
PRRS_1807703_P	TACCCAAACATAGCTGGCAATTGCAAGC
PRRS_1807704_F	GACAAACTCCAGGGCCTGAC
PRRS_1807704_R	GGACTCAAAATCCCAGAGCG
PRRS_1807704_P	Probe not ordered
PRRS_1807705_F	TGATTATGCTAGCACCGCCTT
PRRS_1807705_R	GAATTGCAGCGTTGTGCC

PRRS_1807705_P	Probe not ordered
PRRS_1807706_F	ATTGGTTTGCTCCGCGATAC
PRRS_1807706_R	AAATGAGCCACCACATCCAA
PRRS_1807706_P	CGGTACATTCGACGCGACACCATTTC
PRRS_1807707_F	TCTCCAGCGAAGGCCACT
PRRS_1807707_R	CGAACGCCTGAGAAACCAA
PRRS_1807707_P	Probe not ordered
PRRS_1807708_F	AGTTTCAGCGGAACAATGGG
PRRS_1807708_R	GGGCCAGAATGTACTTGCG
PRRS_1807708_P	CCTAGCAAGCACAAACGGCATCTGGA
PRRS_1807709_F	GAGCGGCAATTGTGTCTGTC
PRRS_1807709_R	GCTGAGGGTGATGCTGTGAC
PRRS_1807709_P	CGCACAGTATGATGCGTAGGCAAACTAAACTC

(b) 50 signatures designed to identify European isolates of PRRS

PRRS_1810342_F	CCAAGTCTTTTGCACACGGT
PRRS_1810342_R	GCACCCGGATGGAGTACATT
PRRS_1810342_P	CTTCTCTCTCCAGAGCTTCAGGACACTGACC
PRRS_1810343_F	ATGCACGTATCCGACCAGC
PRRS_1810343_R	TCCTCAAATGGACAGAACGG
PRRS_1810343_P	Probe not ordered
PRRS_1810344_F	ACGTCTCATTCTTGCGGTCA
PRRS_1810344_R	TTGGTTTGGTAACCGAAGGC
PRRS_1810344_P	ACGTGTTTGATGGCAAGTGCTGGCTC
PRRS_1810345_F	GCATAAGTGGTATGGCGCTG
PRRS_1810345_R	CATCCGGTTCATTATGGCG
PRRS_1810345_P	Probe not ordered
PRRS_1810346_F	TGACTTCACGTCCCCTCTGA
PRRS_1810346_R	ATTCCGAACCACGGTAGCAG
PRRS_1810346_P	CAGTACAACAGACCAGAGGATGATTGGGCTT
PRRS_1810347_F	TCCTCGCTCCCTTTCTCGT
PRRS_1810347_R	CAAGAAAGTCAGCAATACCAGAGC
PRRS_1810347_P	ACACAATCGGAGGGTAGTCTGTAAGCAGACG
PRRS_1810348_F	ATCCTTTCGAATTTGCCGAA
PRRS_1810348_R	CATGTCCACCCTATCCCACA
PRRS_1810348_P	CGTTTCTCCGCACAAGCCTTAATTGACC
PRRS_1810349_F	AGTTCCAAGCTGGTCGCATT
PRRS_1810349_R	CACTGTGCCACCAGTTGCTT
PRRS_1810349_P	Probe not ordered
PRRS_1810350_F	GGCTCTATGGCTCCAGGTGA
PRRS_1810350_R	GCAAAAGCCATCCAAGAACC
PRRS_1810350_P	Probe not ordered
PRRS_1810351_F	TTCTTGTGACCACGATTCGC

PRRS_1810351_R	GACCCACCGAGTAACTTGCC
PRRS_1810351_P	TGTCATGCTGAGCTTTTGGCTCTTGAGC
PRRS_1810352_F	GGCGTTGTCACAAGTGTTGG
PRRS_1810352_R	AGTGCACAGGATCAACCCCT
PRRS_1810352_P	Probe not ordered
PRRS_1810353_F	ATCGTGGACCAGCCTACACC
PRRS_1810353_R	CAGCCACAAAAGTGTCCGAA
PRRS_1810353_P	Probe not ordered
PRRS_1810354_F	GGCCTCTTACACCCTTGCTG
PRRS_1810354_R	CGAGAGGAGCACACAACTCC
PRRS_1810354_P	Probe not ordered
PRRS_1810355_F	GAAGGCACTTATATGGCCGC
PRRS_1810355_R	CACGGTGTTAAGGCAGGGTT
PRRS_1810355_P	ACTTTAATCTTCACCCCGTCTGCAGTTGGAT
PRRS_1810356_F	ACTCCTACAACCGCATGCAC
PRRS_1810356_R	CTTCGCAACCTTGACCACAG
PRRS_1810356_P	Probe not ordered
PRRS_1810357_F	CAGCAGTTTTGGTCCGAGC
PRRS_1810357_R	TTGTTGCGGTTGAGAGATGC
PRRS_1810357_P	Probe not ordered
PRRS_1810358_F	AACATGCTGGTTGGTGATGG
PRRS_1810358_R	TGCCACAGGACTGTGAAACAC
PRRS_1810358_P	Probe not ordered
PRRS_1810359_F	TGTTCTGCTTGGGCAACATC
PRRS_1810359_R	TTGCACGGACACAGTTTTCC
PRRS_1810359_P	Probe not ordered
PRRS_1810360_F	GCGGCTCCAAATTCAGTGTT
PRRS_1810360_R	GATGACGCGGACCATTCTC
PRRS_1810360_P	ATCCCACTCCAGACACCAACCCCTCTTT
PRRS_1810361_F	CCAAGCCTGACAACTGCCTT
PRRS_1810361_R	TTTAGCTTTTCCACCTCGGC
PRRS_1810361_P	TTTCACTCATCTCAGAAGCATAGAAAAGGCAAGA
PRRS_1810362_F	CACGCTGTTGTGGCAAACTT
PRRS_1810362_R	CCGGGTTTCAGAAGAACGTC
PRRS_1810362_P	ACGGTGGGTGAGGTCTCATCAAGATGAC
PRRS_1810363_F	ACCCACAAAGGCAGAACTCG
PRRS_1810363_R	TGCGGACTTGGTGTCTTGAG
PRRS_1810363_P	Probe not ordered
PRRS_1810364_F	TGGTTTCGAGCTTTATGTCCC
PRRS_1810364_R	GTCCTCTGCAGCAGCCTTG
PRRS_1810364_P	Probe not ordered
PRRS_1810365_F	TTGGCCATATTGGTAAGGCG
PRRS_1810365_R	CACAGTTTGCCAATTCTCCTTG
PRRS_1810365_P	TTTCAGGTATGCTCTGAACGTCCTTTGTTGG

PRRS_1810366_F	AAGCCCAAAACCAGGACCAT
PRRS_1810366_R	TCTTCATGAATGCCTGGGTG
PRRS_1810366_P	Probe not ordered
PRRS_1810367_F	CCCGCCATTGTAAGATGGTT
PRRS_1810367_R	GAAGGCACCATCCTGTGTTG
PRRS_1810367_P	TGGCAGCAATTAAGCACATAGCTAGGCAAGT
PRRS_1810368_F	CGTCACCAGTGTGTCCAACA
PRRS_1810368_R	GGGTCTTTCAGCGTACAAGACAA
PRRS_1810368_P	TGTCGGCCTTGAAAATGGGTCATGAAAT
PRRS_1810369_F	GGTTTCAGAACGGACCCAAA
PRRS_1810369_R	CGGCAGCAGACGCATAATAC
PRRS_1810369_P	TCATAACTGATAAACCCAGCTTCCTCGGCTG
PRRS_1810370_F	ACCTCATCTGCGGTATTGCC
PRRS_1810370_R	CAAACACAAATCAAGCCCACA
PRRS_1810370_P	Probe not ordered
PRRS_1810371_F	CGAGGTCTCGTTGCAGTCAA
PRRS_1810371_R	AGGTGGTCTTTCCGGAACCT
PRRS_1810371_P	Probe not ordered
PRRS_1810372_F	ACGTCCCTGGCCGAGTATC
PRRS_1810372_R	CAAAGCCGACAGGGTGAAGT
PRRS_1810372_P	Probe not ordered
PRRS_1810373_F	ATTTGGCCCTAACATCTGCG
PRRS_1810373_R	ATGGTTATCGCAGAGCCGAT
PRRS_1810373_P	Probe not ordered
PRRS_1810374_F	TTGCATCTACCATCGCCAAA
PRRS_1810374_R	CCACGGCTGAACACAAGGT
PRRS_1810374_P	CAAGACACGGGTTGTTCATTTATGACCCTCA
PRRS_1810375_F	TGCGGATAATGCAGTCACAA
PRRS_1810375_R	CCACTTGCGGTAGTGGCATA
PRRS_1810375_P	TCAGACCCGAGGTGCAAGTCTCTCTTAGC
PRRS_1810376_F	ATGGTCGGTGCAGGGTATGT
PRRS_1810376_R	GGGAGTTCTTTTGCTGCCTC
PRRS_1810376_P	Probe not ordered
PRRS_1810377_F	CAACCTGAGACGGCATCAAA
PRRS_1810377_R	AAATAGGCGGTGGCTCCTTT
PRRS_1810377_P	Probe not ordered
PRRS_1810378_F	CCAGGTTTATTCAGCTGCCC
PRRS_1810378_R	TACGGTGTCACTGCCAGGTC
PRRS_1810378_P	Probe not ordered
PRRS_1810379_F	TTTGACAACAGCCTGGTTCG
PRRS_1810379_R	TGTAGTCCTTGCCGTCATTCA
PRRS_1810379_P	Probe not ordered
PRRS_1810380_F	GAGCTAGGTAAACCCCGGCT
PRRS_1810380_R	CGACGGTGAACCCAAACAG

PRRS_1810380_P	Probe not ordered
PRRS_1810381_F	CCTATGAAGGCTTGTTGCCC
PRRS_1810381_R	TCCATGGTCTGGTAAATGCG
PRRS_1810381_P	Probe not ordered
PRRS_1810382_F	GTGGTTGGTGAGGCCACTCT
PRRS_1810382_R	ACATTGCCAACGGCAAGATT
PRRS_1810382_P	CATCACGAGTCGTGAGCTGAGAAAGCG
PRRS_1810383_F	CAGTGTGCACGCTTCCATTT
PRRS_1810383_R	CTCGAATGATGTGTTGCCGT
PRRS_1810383_P	AAACATAGCGTAGAGCTGGAATTCGAAGCCA
PRRS_1810384_F	GTTAATGTCCATCCCGTCCG
PRRS_1810384_R	ATCATGCTCGGCACAAATGA
PRRS_1810384_P	CAAACTTGAGGGTTATTATGCTTGGCTGGCT
PRRS_1810385_F	TACCGGACACAACATCTCCG
PRRS_1810385_R	AAACAGGGCTTACAGGCGAA
PRRS_1810385_P	Probe not ordered
PRRS_1810386_F	GCTTTCTGCGTGCCTTTTCT
PRRS_1810386_R	ACAACGCCAGAGACATTCCC
PRRS_1810386_P	TGACTTTGAAGCCTTTCTCGCTCATTTCTGA
PRRS_1810387_F	GAGACCTTTGTGCTTTACCCG
PRRS_1810387_R	TTTTAGCAGCACGGATGACAA
PRRS_1810387_P	CATACGAACGCTGCGAAAGCACAAGC
PRRS_1810388_F	CCGTACCCGGTTTACCAACT
PRRS_1810388_R	CTAGGCCTCCCATTGCTCAG
PRRS_1810388_P	CTTTAACCCCTTCGAGGACGACATGTTTGAT
PRRS_1810389_F	AACCGTGTCGCACTTACCCT
PRRS_1810389_R	ATGCTCGGTTACCAGACGCT
PRRS_1810389_P	Probe not ordered
PRRS_1810390_F	AGGACTTCGGAGCCTCGTG
PRRS_1810390_R	ATCATTGCACCCAGCAACTG
PRRS_1810390_P	Probe not ordered
PRRS_1810391_F	CGTGACTTCTACATCCGCCA
PRRS_1810391_R	GTCACATGGTTCCTGCCTGA
PRRS_1810391_P	CCATGTGATCGCCCTAATTGAATAGGTGACT

TABLE 357. Nucleic acid extracts used to challenge the initial set of candidate signatures. These select backgrounds are used to determine which signatures will be down-selected against in the screening process.

Nucleic Acid Extract Type	Description/ID
Soil Extract	D000500
Soil Extract	D000501
Soil Extract	D000502
Soil Extract	D000526
Soil Extract	D000541

Prokaryotic DNA Extract	*R. leguminosarium*
Prokaryotic DNA Extract	*P. aeruginosae*
Prokaryotic DNA Extract	*L. gassi*
Prokaryotic DNA Extract	*S. aureus*
Prokaryotic DNA Extract	*B. cereus*
Eukaryotic DNA Extract	Mosquito
Eukaryotic DNA Extract	Chicken
Eukaryotic DNA Extract	Flea
Eukaryotic DNA Extract	Pig
Eukaryotic DNA Extract	Bovine

TABLE 358. Gel background screening results: The amplicon is the predicted target size for each signature when screened against target RNA. Signatures in red were dropped from further screening because they produced PCR product similar in size to the expected target or multiple sized products that could interfere with downstream multiplex assays. Signatures in green passed all criteria and were moved on to target and near-neighbor screening. Prokaryotic bacterial background screenings were left off the table because only one signature (1807356), produced PCR product when crossed with *S. aureus*. "N" indicates no detectable PCR product.

		Soils					Zoos				
Signature	**Amplicon**	**D000500**	**D000501**	**D000502**	**D000526**	**D000541**	**Bovine**	**Chicken**	**Flea**	**Mosquito**	**Pig**
1807659	311	2xmulti	N	2xmulti	2x500	2xmulti	N	N	N	N	N
1807660	348	N	N	N	N	2x350	N	N	N	N	N
1807661	275	N	N	1x400	1x100	2x400	N	N	N	N	2x500
1807662	273	N	N	N	2xmulti	2xmulti	N	N	N	N	1x200
1807702	384	N	N	N	N	N	N	N	N	N	N
1807703	273	N	N	N	N	N	N	N	N	N	N
1807704	368	N	N	N	2xmulti	1x600	N	N	N	N	2x500
1807705	244	N	N	N	2xmulti	2xmulti	N	N	N	N	N
1807706	286	N	N	N	N	N	N	N	N	N	N
1807707	317	2x500	2x400	2x500	2xmulti	2x400	2x350	2x700	N	N	2x300
1807708	349	N	1x350	2x400	N	2x500	N	N	N	N	N
1807709	158	N	N	N	N	N	N	N	N	N	N
1807342	163	N	N	N	N	N	N	N	N	N	N
1807343	101	2xmulti	2x400	N	2xmulti	2xmulti	N	N	N	N	N
1807344	140	N	N	N	N	N	N	N	N	N	N
1807345	184	2x500	1x600	1x500	2xmulti	2xmulti	N	N	N	N	N
1807346	115	N	N	N	N	N	N	N	N	N	N
1807347	164	N	N	N	N	N	N	N	N	N	N
1807348	200	N	N	N	N	N	N	N	N	N	N
1807349	130	2x600	2x600	2xmulti	2x400	2xmulti	N	N	2x100	N	N
1807350	164	2x500	N	N	N	N	N	N	N	N	1x300
1807351	141	2x600	N	1x400	2xmulti	2x600	N	N	N	1x200	N
1807352	150	N	N	N	2xmulti	2xmulti	N	N	1x140	N	2xmulti

1807353	127	N	N	N	2xmulti	2x600	N	2x600	2x500	N	N
1807354	171	N	N	N	2xmulti	2xmulti	2xmulti	2x600	N	N	2xmulti
1807355	129	N	N	N	2x600	2xmulti	N	N	N	N	N
1807356	101	N	2x100	2x500	2xmulti	2xmulti	N	2x300	N	N	2x500
1807357	118	2x500	N	2x400	2xmulti	2xmulti	N	N	N	N	1x500
1807358	97	1x500	N	N	2xmulti	2x500	2xmulti	2x500	N	N	2x500
1807359	79	N	N	N	2x400	N	N	N	N	N	N
1807360	111	N	N	N	N	N	N	N	N	N	N
1807361	106	N	2x150	1x500	2xmulti	2x500	N	N	N	N	N
1807362	86	N	N	N	2x600	N	N	N	N	N	N
1807363	196	2x400	1x500	2xmulti	2xmulti	2x400	N	2x500	N	N	N
1807364	118	2x180	N	N	2x600	1xmulti	N	2x600	N	N	N
1807365	167	N	N	N	2x600	N	N	N	N	N	N
1807366	94	N	N	1x200	2xmulti	2xmulti	N	N	1x60	N	2xmulti
1807367	123	N	N	1x500	2x500	2x500	2x500	2x500	N	1x400	2x500
1807368	190	N	2x500	N	1x600	N	1x190	N	N	N	N
1807369	169	N	N	N	N	N	N	N	N	N	N
1807370	182	N	N	N	N	2xmulti	N	N	N	N	N
1807371	178	2x600	2x600	N	2x500	2xmulti	N	N	N	N	N
1807372	129	2x600	N	2x500	2xmulti	N	N	N	N	N	N
1807373	159	N	N	N	2xmulti	2xmulti	N	N	N	N	N
1807374	161	N	2x600	N	N	N	N	N	N	N	2x600
1807375	143	N	N	N	N	N	N	N	N	N	N
1807376	194	N	N	2xmulti	N	2xmulti	2x200	2x40	2xmulti	N	2x300
1807377	89	2xmulti	N	+/+	2xmulti	2xmulti	N	2xmulti	2x50	N	N
1807378	124	2xmulti	N	2xmulti	2xmulti	2xmulti	2xmulti	2x600	N	N	2xmulti
1807379	140	N	2x400	N	2xmulti	2xmulti	N	N	N	N	N
1807380	172	N	N	N	2xmulti	2xmulti	N	N	N	N	N
1807381	139	2xmulti	N	N	2xmulti	2xmulti	N	2x600	N	N	2x600
1807382	143	N	N	N	N	N	N	N	N	N	N
1807383	129	N	N	N	N	N	N	N	N	N	N
1807384	175	N	N	N	N	N	N	N	N	N	N
1807385	158	2xmulti	N	2xmulti	2xmulti	2xmulti	N	N	N	N	N
1807386	81	N	N	N	N	N	N	2x600	1x40	N	N
1807387	196	N	N	N	N	N	N	N	N	N	N
1807388	190	N	N	N	N	N	N	N	N	N	N
1807389	193	N	N	N	2xmulti	2x500	N	N	N	N	N
1807390	169	N	N	N	2x500	N	N	N	N	N	N
1807391	144	N	N	N	N	N	N	N	N	N	N

TABLE 359. Eight domestic signatures and 22 European PRRS signatures passed background gel screening. Signatures that were not eliminated by producing products when screened against backgrounds were then screened against a North American PRRS isolate target at LLNL.

Signatures in green did produce an amplicon and signatures in red did not produce an amplicaon when screened against the domestic target template.It was not essential that signatures hit this particular isolate. All 30 signatures were sent to the Faaberg lab at the University of Minnesota for additional target screening. “N” indicates no detectable PCR product after 35 cycles of PCR.

Signature	Origin	Amplicon	200pg North American PRRS
1807660	Domestic	348	3 x N
1807661	Domestic	275	3 x 275
1807662	Domestic	273	3 x 273
1807702	Domestic	384	3 x N
1807703	Domestic	273	3 x 273
1807706	Domestic	286	3 x 286
1807708	Domestic	349	3 x N
1807709	Domestic	158	3 x 160
1810342	European	163	3 x N
1810344	European	140	3 x N
1810346	European	115	3 x N
1810347	European	164	3 x N
1810348	European	200	3 x N
1810351	European	141	3 x N
1810355	European	129	3 x N
1810360	European	111	3 x N
1810362	European	86	3 x N
1810365	European	167	3 x N
1810367	European	123	3 x N
1810368	European	190	3 x N
1810369	European	169	3 x N
1810374	European	161	3 x N
1810375	European	143	3 x N
1810382	European	143	3 x N
1810383	European	129	3 x N
1810384	European	175	3 x N
1810386	European	81	3 x N
1810387	European	196	3 x N
1810388	European	190	3 x N
1810391	European	144	2 x 144, 1 x N

TABLE 360. Gel target screening results using samples from the University of Minnesota. Real time PCR reactions with available targets that were performed at the University of Minnesota were frozen and sent back to LLNL for gel analysis of product size. Signatures in red were dropped from further screening because they failed to produce an amplicon with any of our target templates. Signatures in green were moved forward for real-time RT-PCR screening. Of the original 62 PRRS signatures, 30 signatures were chosen to move forward to real time screening. These signatures were chosen based on their ability to recognize some or all of the

targets and not produce PCR products against backgrounds that would inhibit real-time RT-PCR target detection. Eight of the signatures are designed to detect domestic isolates of PRRS and 22 of the signatures are designed to detect European isolates. "N" indicates no detectable PCR product after 35 cycles of PCR

Signature	Origin	Amplicon	Dom 112	Dom 124	Dom 134	Dom 184	Dom 251	Dom 162	Euro 8	Euro 13
1807660	Domestic	348	3 x N	3 x N	3 x N	3 x N	3 x N	3 x N	3 x N	3 x N
1807661	Domestic	275	3 x 275	3 x 275	3 x 275	3 x 275	3 x 275	3 x 275	3 x N	3 x N
1807662	Domestic	273	3 x 273	3 x 275	3 x 275	3 x 275	3 x 275	3 x 275	3 x N	3 x N
1807702	Domestic	384	3 x N	3 x N	3 x N	3 x N	3 x N	3 x N	3 x N	3 x N
1807703	Domestic	273	3 x N	3 x 275	3 x 275	3 x 275	3 x 275	3 x 275	3 x N	3 x N
1807706	Domestic	286	3 x 290	3 x 290	3 x 290	3 x 290	3 x 290	3 x 290	3 x N	3 x N
1807708	Domestic	349	3 x N	3 x N	3 x N	3 x N	3 x N	3 x N	3 x N	3 x N
1807709	Domestic	158	3 x 160	3 x 160	3 x 160	3 x 160	3 x 160	3 x 160	3 x N	3 x N
1810342	European	163	3 x N	3 x N	3 x N	3 x N	3 x N	3 x N	3 x 165	3 x 165
1810344	European	140	3 x N	3 x N	3 x N	3 x N	3 x N	3 x N	3 x 140	3 x 140
1810346	European	115	3 x N	3 x N	3 x N	3 x N	3 x N	3 x N	3 x N	3 x 115
1810347	European	164	3 x N	3 x N	3 x N	3 x N	3 x N	3 x N	3 x 165	3 x 165
1810348	European	200	3 x N	3 x N	3 x N	3 x N	3 x N	3 x N	3 x 200	3 x 200
1810351	European	141	3 x N	3 x N	3 x N	3 x N	3 x N	3 x N	3 x 140	3 x 140
1810355	European	129	3 x N	3 x N	3 x N	3 x N	3 x N	3 x N	3 x 90	3 x 90
1810360	European	111	3 x N	3 x N	3 x N	3 x N	3 x 110	3 x 110	3 x 110	3 x 110
1810362	European	86	3 x N	3 x N	3 x N	3 x N	3 x N	3 x N	3 x 90	3 x 90
1810365	European	167	3 x N	3 x N	3 x N	3 x N	3 x N	3 x N	3 x N	3 x N
1810367	European	123	3 x N	3 x N	3 x N	3 x N	3 x N	3 x N	3 x 125	3 x 125
1810368	European	190	3 x N	3 x N	3 x N	3 x N	3 x N	3 x N	3 x 200	3 x 200
1810369	European	169	3 x N	3 x N	3 x N	3 x N	3 x N	3 x N	3 x 170	3 x 170
1810374	European	161	3 x N	3 x N	3 x N	3 x N	3 x N	3 x N	3 x 160	3 x 160
1810375	European	143	3 x N	3 x N	3 x N	3 x N	3 x N	3 x N	3 x 140	3 x 140
1810382	European	143	3 x N	3 x N	3 x N	3 x N	3 x N	3 x N	3 x 140	3 x 140
1810383	European	129	3 x N	3 x N	3 x N	3 x N	3 x N	3 x N	3 x 130	3 x 130
1810384	European	175	3 x N	3 x N	3 x N	3 x N	3 x N	3 x N	3 x 180	3 x 180
1810386	European	81	3 x N	3 x N	3 x N	3 x N	3 x N	3 x N	3 x 80	3 x 80
1810387	European	196	3 x N	3 x N	3 x N	3 x N	3 x N	3 x N	3 x 200	3 x 200
1810388	European	190	3 x N	3 x N	3 x N	3 x N	3 x N	3 x N	3 x 200	3 x 200
1810391	European	144	3 x N	3 x N	3 x N	3 x N	3 x N	3 x N	3 x 145	3 x 145

PRRS – Real-time RT- PCR Screening Report

Background real-time RT-PCR assays were carried out in triplicate as 25ul reactions in 96 well PCR plates on BioRad's iCYCLERs. Each reaction mix consisted of 1X PCR Buffer [200mM Tris-HCL (pH 8.4), 500mM KCl], 6mM $MgCl_2$, 0.2mM each dNTP, 80ng BSA, 0.2uM each forward and reverse primers, 0.4uM Probe with a 5' Fam and a 3' BHQ modification, 1.25U Platinum *Taq* DNA polymerase, PCR water and a variable amount of extracted DNA template. A total of 5ng of soil DNA or 1ng of Eukaryotic DNA or 200pg of Prokaryotic DNA was added to each 25ul reaction mix.

Because the target is RNA, we used the Clontech Powerscript RT-PCR kit. Each reaction was performed in triplicate as per the manufacturer's suggested protocol, in a 25ul volume with a total of 200pg TRIZOL extracted RNA.

A real-time RT- PCR reaction was deemed positive if a Ct (cycle threshold) value of below 36 cycles was observed at least 2 of the 3 times the reaction was performed.

TABLE 361(a-b). List of signatures that were considered successful through gel screening and were brought forward to be screened in real-time RT- PCR format.

(a) 8 signatures designed to identify domestic isolates of PRRS

PRRS_1807661_F	CCAGGACATCAGCTGCCTTA
PRRS_1807661_R	TGACATGTTGGACGTAGCTGG
PRRS_1807661_P	TTTCACTCATCTCAGAAGCATAGAAAAGGCAAGA
PRRS_1807662_F	GGTCGCGCTCACTATGGG
PRRS_1807662_R	GCTTTTCTGCCACCCAACAC
PRRS_1807662_P	CCGGACGACAAATGCGTGGTTATCATTT
PRRS_1807702_F	GCTGCACAGAAACACCCTTCT
PRRS_1807702_R	TTTCCACTGGTCATTCGTGC
PRRS_1807702_P	ACTGCTTTACGGTCTCTCCACCCCTTTAACC
PRRS_1807703_F	CTAACCCGTTTGCCGTCC
PRRS_1807703_R	GCAACCAGCAAGGAAACACA
PRRS_1807703_P	TACCCAAACATAGCTGGCAATTGCAAGC
PRRS_1807706_F	ATTGGTTTGCTCCGCGATAC
PRRS_1807706_R	AAATGAGCCACCACATCCAA
PRRS_1807706_P	CGGTACATTCGACGCGACACCATTTC
PRRS_1807707_F	TCTCCAGCGAAGGCCACT
PRRS_1807707_R	CGAACGCCTGAGAAACCAA
PRRS_1807707_P	Probe not ordered
PRRS_1807708_F	AGTTTCAGCGGAACAATGGG
PRRS_1807708_R	GGGCCAGAATGTACTTGCG
PRRS_1807708_P	CCTAGCAAGCACAAACGGCATCTGGA
PRRS_1807709_F	GAGCGGCAATTGTGTCTGTC
PRRS_1807709_R	GCTGAGGGTGATGCTGTGAC
PRRS_1807709_P	CGCACAGTATGATGCGTAGGCAAACTAAACTC

(b) 22 signatures designed to identify European isolates of PRRS

PRRS_1810342_F	CCAAGTCTTTTGCACACGGT
PRRS_1810342_R	GCACCCGGATGGAGTACATT
PRRS_1810342_P	CTTCTCTCTCCAGAGCTTCAGGACACTGACC
PRRS_1810344_F	ACGTCTCATTCTTGCGGTCA
PRRS_1810344_R	TTGGTTTGGTAACCGAAGGC

PRRS_1810344_P	ACGTGTTTGATGGCAAGTGCTGGCTC
PRRS_1810346_F	TGACTTCACGTCCCCTCTGA
PRRS_1810346_R	ATTCCGAACCACGGTAGCAG
PRRS_1810346_P	CAGTACAACAGACCAGAGGATGATTGGGCTT
PRRS_1810347_F	TCCTCGCTCCCTTTCTCGT
PRRS_1810347_R	CAAGAAAGTCAGCAATACCAGAGC
PRRS_1810347_P	ACACAATCGGAGGGTAGTCTGTAAGCAGACG
PRRS_1810348_F	ATCCTTTCGAATTTGCCGAA
PRRS_1810348_R	CATGTCCACCCTATCCCACA
PRRS_1810348_P	CGTTTCTCCGCACAAGCCTTAATTGACC
PRRS_1810351_F	TTCTTGTGACCACGATTCGC
PRRS_1810351_R	GACCCACCGAGTAACTTGCC
PRRS_1810351_P	TGTCATGCTGAGCTTTTGGCTCTTGAGC
PRRS_1810355_F	GAAGGCACTTATATGGCCGC
PRRS_1810355_R	CACGGTGTTAAGGCAGGGTT
PRRS_1810355_P	ACTTTAATCTTCACCCCGTCTGCAGTTGGAT
PRRS_1810360_F	GCGGCTCCAAATTCAGTGTT
PRRS_1810360_R	GATGACGCGGACCATTCTC
PRRS_1810360_P	ATCCCACTCCAGACACCAACCCCTCTTT
PRRS_1810362_F	CACGCTGTTGTGGCAAACTT
PRRS_1810362_R	CCGGGTTTCAGAAGAACGTC
PRRS_1810362_P	ACGGTGGGTGAGGTCTCATCAAGATGAC
PRRS_1810365_F	TTGGCCATATTGGTAAGGCG
PRRS_1810365_R	CACAGTTTGCCAATTCTCCTTG
PRRS_1810365_P	TTTCAGGTATGCTCTGAACGTCCTTTGTTGG
PRRS_1810367_F	CCCGCCATTGTAAGATGGTT
PRRS_1810367_R	GAAGGCACCATCCTGTGTTG
PRRS_1810367_P	TGGCAGCAATTAAGCACATAGCTAGGCAAGT
PRRS_1810368_F	CGTCACCAGTGTGTCCAACA
PRRS_1810368_R	GGGTCTTTCAGCGTACAAGACAA
PRRS_1810368_P	TGTCGGCCTTGAAAATGGGTCATGAAAT
PRRS_1810369_F	GGTTTCAGAACGGACCCAAA
PRRS_1810369_R	CGGCAGCAGACGCATAATAC
PRRS_1810369_P	TCATAACTGATAAACCCAGCTTCCTCGGCTG
PRRS_1810374_F	TTGCATCTACCATCGCCAAA
PRRS_1810374_R	CCACGGCTGAACACAAGGT
PRRS_1810374_P	CAAGACACGGGTTGTTCATTTATGACCCTCA
PRRS_1810375_F	TGCGGATAATGCAGTCACAA
PRRS_1810375_R	CCACTTGCGGTAGTGGCATA
PRRS_1810375_P	TCAGACCCGAGGTGCAAGTCTCTCTTAGC
PRRS_1810382_F	GTGGTTGGTGAGGCCACTCT
PRRS_1810382_R	ACATTGCCAACGGCAAGATT

PRRS_1810382_P	CATCACGAGTCGTGAGCTGAGAAAGCG
PRRS_1810383_F	CAGTGTGCACGCTTCCATTT
PRRS_1810383_R	CTCGAATGATGTGTTGCCGT
PRRS_1810383_P	AAACATAGCGTAGAGCTGGAATTCGAAGCCA
PRRS_1810384_F	GTTAATGTCCATCCCGTCCG
PRRS_1810384_R	ATCATGCTCGGCACAAATGA
PRRS_1810384_P	CAAACTTGAGGGTTATTATGCTTGGCTGGCT
PRRS_1810386_F	GCTTTCTGCGTGCCTTTTCT
PRRS_1810386_R	ACAACGCCAGAGACATTCCC
PRRS_1810386_P	TGACTTTGAAGCCTTTCTCGCTCATTTCTGA
PRRS_1810387_F	GAGACCTTTGTGCTTTACCCG
PRRS_1810387_R	TTTTAGCAGCACGGATGACAA
PRRS_1810387_P	CATACGAACGCTGCGAAAGCACAAGC
PRRS_1810388_F	CCGTACCCGGTTTACCAACT
PRRS_1810388_R	CTAGGCCTCCCATTGCTCAG
PRRS_1810388_P	CTTTAACCCCTTCGAGGACGACATGTTTGAT
PRRS_1810391_F	CGTGACTTCTACATCCGCCA
PRRS_1810391_R	GTCACATGGTTCCTGCCTGA
PRRS_1810391_P	CCATGTGATCGCCCTAATTGAATAGGTGACT

TABLE 362(a-c). Real-time RT- PCR background screening consisted of an extensive list of soil, Prokaryotic and Eukaryotic extracted DNAs in addition to 3 Aerosol Blocks: Panel 1, 2 and 3, each consisting of 752 samples of extracted nucleic acids from filters under in aerosol monitoring. All signatures passed real-time RT- PCR background screening. None of the 8 domestic PRRS signatures or the 22 European signatures produced an amplicon when screened against the listed backgrounds. All signatures moved forward to be screened against the available PRRS target at LLNL. As stated previously, it was not necessary for a signature to produce PCR product with the single PRRS isolate available to LLNL for screening.

(a) Total of 45 soils screened

D000402	D000531	S252	S271	S280	S290	S300
D000109	D000542	S253	S272	S282	S291	S301
D000107	D000533	S254	S273	S283	S292	S303
D000500	D000561	S255	S274	S284	S295	S304
D000505	D000562	S256	S275	S286	S296	S305
D000521	D000501	S257	S276	S287	S297	S307
D000551	D000550	S259	S277	S288	S298	
D000527	S251	S260	S279	S289	S299	

(b) Total of 16 Eukaryotes screened

Bovine	Drosophila	Monkey	Rabbit
Cat	Equine	Mosquito	Rat
Chicken	Flea	Mouse	Sheep
Dog	Human	Porcine	Tick

(c) Total of 54 Prokaryotes screened.

A. suis	*C. butyricum*	*L. gasseri*	*P. oleovorans*
A migulanus	*C. pseudodipthericum*	*L. moncytogenes*	*R. leguminosarum*
B. cereus	*C. marinoflava*	*L. seeligeri*	*R. rhodochrous*
B. globigii	*E. amylovora*	*M. luteus*	*S. typhimurium*
B. subtilis	*E. herbicola*	*M. lacunatica*	*S. muelleri*
B thuringiensis	*E. coli*	*O. ssp. Maris*	*Alcaligenes sp.*
B. denticum	*G.caldoxylosilyticus*	*P. napthalaenovorans*	*S. aureus*
B. burgdorferi	*H. halmophila*	*P. dentrificans*	*S. pneumoniae*
B. capacia	*H. influenza*	*P sanguineus*	*S. scabiei*
C. vibriodes	*H. seropedicae*	*P. mirabillis*	*T. maceachernii*
C. michganensis	*L. garviease*	*P aeruginosae*	*V. paraheamolyticus*
			X. translucens

TABLE 363. Real-time RT-PCR target screening results from LLNL and the University of Minnesota. The listed Ct values are the average Ct value of each signature run in triplicate with 200pg total RNA in each 25ul volume reaction. Signatures in green passed all criterion and were considered successful. Signatures in red were dropped because they either produced a PCR product with a background template or they did not produce a PCR product with any of the target templates. "N" indicates no detectable PCR product after 35 cycles of PCR

Signature	Origin	Dom LLNL	Dom 112	Dom 124	Dom 134	Dom 184	Dom 251	Dom 262	Euro 8	Euro 13
1807660	Domestic	N	N	N	N	N	N	N	N	N
1807661	Domestic	32	N	32.8	32.4	N	N	N	N	N
1807662	Domestic	35.16	30.69	29.54	34.67	32.69	30.87	N	N	N
1807702	Domestic	N	N	N	N	N	N	N	N	N
1807703	Domestic	N	34.54	N	28.85	29.72	30.34	N	N	N
1807706	Domestic	36.39	23.79	25.18	26.09	28.46	27.45	N	N	N
1807708	Domestic	N	N	N	N	N	N	N	N	N
1807709	Domestic	31.9	N	29.8	33.6	27.2	32	30.3	N	N
1810342	European	N	N	N	N	N	N	N	35.42	30.54
1810344	European	N	N	N	N	N	N	N	35.89	N
1810346	European	N	N	N	N	N	N	N	N	22.60
1810347	European	N	N	N	N	N	N	N	26.75	26.74
1810348	European	N	N	N	N	N	N	N	30.91	37.95
1810351	European	N	N	N	N	N	N	N	24.17	21.90
1810355	European	N	N	N	N	N	N	N	27.17	N
1810360	European	N	N	N	N	N	N	N	23.65	35.74
1810362	European	N	N	N	N	N	N	N	23.90	N
1810365	European	N	N	N	N	N	N	N	N	35.83
1810367	European	N	N	N	N	N	N	N	N	22.80
1810368	European	N	N	N	N	N	N	N	27.77	26.11
1810369	European	N	N	N	N	N	N	N	33.10	28.00
1810374	European	N	N	N	N	N	N	N	27.00	23.47
1810375	European	N	N	N	N	N	N	N	26.95	26.47
1810382	European	N	N	N	N	N	N	N	31.11	32.92

1810383	European	N	N	N	N	N	N	N	32.63	23.33
1810384	European	N	N	N	N	N	N	N	27.32	33.15
1810386	European	N	N	N	N	N	N	N	30.47	23.64
1810387	European	N	N	N	N	N	N	N	30.44	33.32
1810388	European	N	N	N	N	N	N	N	27.76	26.99
1810391	European	N	N	N	N	N	N	N	29.90	26.90

PRRS LLNL TaqMan Conclusions: From the initial 62 signatures generated and 30 signatures screened, 27 signatures were deemed successful. These signatures did not produce positive Ct values when preformed with the vast number of background templates and did yield positive Ct values with some of the available PRRS isolates.

Porcine Reproductive and Respiratory Syndrome (PRRS) - LOD Report

The nucleic acids used in this target screening were not purified RNA, but rather total RNA extract from cell culture, thus LODs reported here are not absolute LODs. Rather, they are relative LODs comparing one signature with another. In order to determine relative limits of detection for each Real-time RT- PCR signature developed, a dilution series of available targets was made [6 logs, from 1 fg to 10^5 fg per reaction]. The diluted targets were tested against the 27 signatures that had previously produced PCR product with a target template.

TABLE 364. Limit of Detection for PRRS signatures (a) Domestic (b) European against available PRRS isolates recorded using the amount of total extracted target RNA added to each 25 ul reaction that was detectable. The diluted targets were then tested with each signature using the standard Real-time RT- PCR protocol in triplicate and average Ct values are reported for each dilution. "N" indicates no detectable PCR product after 35 cycles of PCR

(a)

North American (Domestic)	DOM 112	DOM 124	DOM 134	DOM 184	DOM 251	DOM 262
1807661	N	1 pg	1 pg	N	200 pg	N
1807662	200 pg	200 pg	100 pg	100 pg	10 pg	100 fg
1807703	N	100 fg	N	1 pg	100 pg	100 pg
1807706	100 pg	100 pg	100 pg	100 pg	100 pg	100 pg
1807709	N	10 pg	100 pg	100 pg	100 pg	100 pg

(b)

European	EUR 8	EUR 13
1810342	100 pg	100 pg
1810344	100 pg	N
1810346	N	1 pg
1810347	100 pg	100 pg
1810348	100 pg	100 pg
1810351	100 fg	100 fg
1810355	100 fg	N

1810360	100 pg	100 pg
1810362	1 pg	N
1810365	N	100 pg
1810367	N	1 fg
1810368	1 pg	1 pg
1810369	100 pg	100 pg
1810374	1 pg	10 fg
1810375	10 pg	10 pg
1810382	100 pg	10 pg
1810383	1 pg	100 fg
1810384	1 pg	100 pg
1810386	1 pg	10 fg
1810387	100 pg	100 pg
1810388	100 pg	10 pg
1810391	100 pg	10 pg

11.5. PRRS MULTIPLEXED PCR SCREENING REPORT

SCREENING AND DOWN-SELECTION TECHNICAL APPROACH:

Signatures that pass gel and real-time PCR screening are candidates for multiplexed assays. If the number of candidate signatures is large than a subset of the available signatures are selected based on measured sensitivity and specificity observed in real-time screening. In preliminary screening the background of individual signatures is measured by running "blank" (no sample added) controls and observing signature response. In some cases a primer-to-primer non-specific interference will eliminate candidate signatures. Signatures are then added iteratively to each multiplexed panel, titrated, and with each signature addition, collectively, all signatures are re-titrated to determine if the presence of one signature will interfere with the performance of another. During this process signatures are added or subtracted from the multiplexed panel depending on individual outcomes until all desirable signatures have been added.

Criterion for down-selection of multiplexed signatures

Signature specificity. In initial real-time PCR screening signatures undergo target and near neighbor screening and screening against environmental confounders which assess individual signature specificity. In multiplex format this is repeated to verify that a more complex reaction system gives the same level of specificity.

Signature sensitivity. In initial real-time PCR screening signatures undergo limit of detection testing where a dilution curve is generated for each signature. This determines individual signature sensitivity. In multiplex format this is repeated to verify that a more complex reaction system gives acceptable signature sensitivity.

Optimal signal to noise ratio. In the multiplex signature system the potential for cross reactivity is larger than in single reaction testing, this may cause unwanted PCR reaction products or non-specific cross-reactivity. Signatures are preferred to have a high signal to noise ratio, where desired signal is significantly greater than the background noise of the signatures when there is no target nucleic acid in the reaction.

Compatibility with other signatures in multiplex. In some cases a primer set may interfere with other primers or probes in the multiplex and there by cause limit of detection shifting for one or more signature in the multiplex. For each scenario it must be evaluated whether the shift in detection level is significant enough to remove (what could be a very desirable signature) from the panel.

TABLE 365. Order details for PRRS signatures ordered for multiplexed assay screening and development.

ID	Modification details	Vendor
PRRS_0351_BF	5'-/5Bio/TTCTTGTGACCACGATTCGC-3'	Biosearch
PRRS_0351_FCP	5'-/5AmMC6/iSp18/GCTCAAGAGCCAAAAGCTCAGCATGACA-3'	Biosearch
PRRS_0351_R	5'-GACCCACCGAGTAACTTGCC-3'	Biosearch
PRRS_0383_BF	5'-/5Bio/CAGTGTGCACGCTTCCATTT-3'	Biosearch
PRRS_0383_FCP	5'-/5AmMC6/iSp18/AAACATAGCGTAGAGCTGGAATTCGAAGCCA-3'	Biosearch
PRRS_0383_R	5'-CTCGAATGATGTGTTGCCGT-3'	Biosearch
PRRS_0386_BF	5'-/5Bio/GCTTTCTGCGTGCCTTTTCT-3'	Biosearch
PRRS_0386_FCP	5'-/5AmMC6/iSp18/TGACTTTGAAGCCTTTCTCGCTCATTTCTGA-3'	Biosearch
PRRS_0386_R	5'-ACAACGCCAGAGACATTCCC-3'	Biosearch
PRRS_7706_BF	5'-/5Bio/ATTGGTTTGCTCCGCGATAC-3'	Biosearch
PRRS_7706_FCP	5'-/5AmMC6/iSp18/CGGTACATTCGACGCGACACCATTTC-3'	Biosearch
PRRS_7706_R	5'-AAATGAGCCACCACATCCAA-3'	Biosearch
PRRS_DOM_7709.BF	5'-/5Bio/GAGCGGCAATTGTGTCTGTC-3'	Biosearch
PRRS_DOM_7709.FCP	5'-/5AmMC6/iSp18/CGCACAGTATGATGCGTAGGCAAACTAAACTC	Biosearch
PRRS_DOM_7709.R	5'-GCTGAGGGTGATGCTGTGAC-3'	Biosearch

Multiplex Data Analysis -Technical approach

Disease signature thresholds

Thresholds were initially determined using all available data from known negative samples (blanks) by plotting a histogram which showed the probability of a given sample generating a particular MFI. In the absence of cross reactivity, each signature in the multiplex assay could be treated as an individual signature result. For example, for a sample spiked with RPV, data for other disease signatures could contribute towards negative data, providing that cross reaction between disease signature sets was not observed. This is a way to increase the number of samples that can be used to establish thresholds.

TABLE 366. Individual signature thresholds and ranges for the final set of PRRS signatures. For FY06, threshold determinations have not yet been made as they require a significant number of tests (>500) to generate this information. This information will be updated when available.

Signature Name	Mux Assay name	Panel Membership	Signature background in Tris NaCl Buffer (MFI +/-)	Signature background in Clinical matrices (MFI +/-)	Threshold
PRRS_1807709	PRRS_1807709	Porcine	TBD	TBD	TBD
PRRS_1810351	PRRS_1810351	Porcine	TBD	TBD	TBD
PRRS_1807706	PRRS_1807706	Porcine	TBD	TBD	TBD
PRRS_1810383	PRRS_1810383	Porcine	TBD	TBD	TBD
PRRS_1810386	PRRS_1810386	Porcine	TBD	TBD	TBD

TABLE 367. List of targets screened at LLNL. The PRRS virus known as the North American strain or NVSL strain is derived from a field isolate we made from tissues collected and submitted to us in 1989 during the "mystery pig disease" days. When a non-proprietary cell line became available for PRRS isolation and propagation, we went back to some of the original submissions and reisolated the virus on the non-proprietary cells. The "official" designation is the NVSL strain; it is apparently genetically and antigenically typical of the PRRS viruses that evolved in the Western Hemisphere. The cell culture passage history is unknown.

Virus	Strain[1]	Unique ID	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	Use stock titer	Titer Method
PPRS	North American	field isolate	NVSL	10^6 $TCID_{50}$/1.0mL	Unknown	9/5/06	TRIZOL	$9.06x10^4$ $TCID_{50}$/mL	Reed & Muench

TABLE 368. List of targets screened at University of Minnesota. Nucleic acids isolated via TRIZOL extraction at University of Minnesota from PRRS isolates grown at University of Minnesota.

Virus	Strain[1]	Isolate	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	Use stock titer	Titer Method
PPRS	Domestic 112	unknown	NVSL	10^6 $TCID_{50}$/1.0mL	Marc-145	2006	TRIZOL	No titer	N/A
PPRS	Domestic 112	Texas	Univ of Minnesota	unknown	PAM	2006	TRIZOL	No titer	N/A
PPRS	Domestic 124	Oklahoma	Univ of Minnesota	unknown	PAM	2006	TRIZOL	No titer	N/A
PPRS	Domestic 134	Nebraska	Univ of Minnesota	unknown	PAM	2006	TRIZOL	No titer	N/A
PPRS	Domestic 184	Minnesota	Univ of Minnesota	unknown	PAM	2006	TRIZOL	No titer	N/A
PPRS	Domestic 251	Missouri	Univ of Minnesota	unknown	Marc-145	2006	TRIZOL	No titer	N/A
PPRS	Domestic 262	Missouri	Univ of Minnesota	unknown	PAM	2006	TRIZOL	No titer	N/A
PPRS	European 8	Missouri	Univ of Minnesota	unknown	PAM	2006	TRIZOL	No titer	N/A
PPRS	European 13	North Carolina	Univ of Minnesota	unknown	PAM	2006	TRIZOL	No titer	N/A

TABLE 369. List of additional near-neighbors screened against the porcine panel. All near-neighbors samples were extracted nucleic acids with unknown titers.

Virus	Strain[1]	Unique ID	Source	Original Titer	Passage History	Extraction date/ID	Extraction Method	NA conc. used or stock titer	Titer Method
Border Disease virus	Coos Bay	4-6-92	NADC, Julia Ridpath	1.5 x 10^8 TCID50/mL	unknown	12/14/06	Trizol	1.18 x 10^6 TCID50/mL	Reed & Muench
Bovine Herpes Virus-1	California	NVSL 20032 111903	CAHFS	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Cooper	NVSL 86741 111903	CAHFS	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Los Angeles	Los Angeles ATCC VR188	ATCC (CAHFS)	1 x 10^5 TCID50/mL	(CAHFS) MDBK2(LLNL)MDBK1	5/17/07	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	RLB-106	ATCC VR793	ATCC (CAHFS)	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	A033640072	CAHFS, R. Mock, #5 Swab, 1BDFK1, 1/28/2004, A030020072	unknown	1BDFK1,	4/23/04	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	A040130066	CAHFS, R. Mock, #4 Lung, 1BFK1, 1/28/2004, A040130066	unknown	1BFK1	4/23/04	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	IBR	Texas A030020072	CAHFS, R. Mock, #80 Swab, 1BDFK1, 1/28/2004, A030020072	unknown	1BDFK1	4/23/2004	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	California	NVSL 200032	NVSL, CAHFS	unknown	MDBK CAHFS	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Virginia	NVSL 231221	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	California	NVSL 51619	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Minnesota	NVSL 86741	NVSL, CAHFS	unknown	MDBK CAHFS	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Minnesota	NVSL 97-10720	NVSL, CAHFS	unknown	MDBK CAHFS	11/12/03	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes Virus-1	Cooper	NVSL-Cooper RA 309	NVSL, CAHFS	unknown	MDBK CAHFS	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Bovine Herpes-5	DN-599	A032540006	R. Mock, from lung tissue, 2BFK2, 2/3/2004	Unknown	CAHFS Lab 2BFK2 4/30/2004	6/3/2004	Phenol/Chloroform	40 pg/uL	N/A
Bovine Herpes-5	DN-599	A040150085	R. Mock, #2 Lung, 5BFK5, 2/3/04	Unknown	CAHFS Lab 5BFK5 4/23/2004	7/7/2004	Phenol/Chloroform	40 pg/uL	N/A
Bovine Herpes-5	D9402133	D9402133	Sonoma Co. 3/94 Severe necrotizing encephalitis, 1 week old calf	Unknown	CAHFS Lab (MDBK), (1 flasks) 11/19/2003	11/19/2003	Phenol/Chloroform	40 pg/uL	N/A
Bovine Herpes Virus	BHV 5	D9403153	CAHFS	unknown	unknown	unknown	unknown	40 pg/uL	unknown

Caprine Herpes Virus		VR462	ATCC	unknown	unknown	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Caprine Herpes Virus	D0201157	D0201157	CAHFS, Isolate D0201157 History: San Joaquin Co. 2/02 Aborted fetus-third trimester	unknown	CAHFS Lab (MDBK)	unknown	Phenol/ Chloroform	40 pg/uL	N/A
Caprine Herpes Virus	S0201998	S0201998	CAHFS, Isolate S0201998 History: San Diego Co. 2/02 Aborted fetus-third trimester	unknown	CAHFS Lab (MDBK)	11/19/03	Phenol/ Chloroform	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-1	Santa Barbara	N/A	Bridget McLaughlin CAHFS	unknown	unknown	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-1	Georgia	N/A	Bridget McLaughlin CAHFS	unknown	unknown	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-2	Alberta	041 ODV 0401	NVSL	unknown	BHK	unknown	Trizol	40 pg/uL	N/A
Epizootic Hemorrhagic Disease virus-1 (SV123)	New Jersey (3TD) Deer, NJ 1955	041 ODV 0401	NVSL	1 x $10^{5.125}$ $TCID_{50}$/0.1 ml	+BHK-3 (12/7/83); (12/11/86); (12/18/86)	Unknown	Trizol	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A011120004	CAHFS, R. Mock, from brain tissue, 2BFK2, 2/3/2004, A011120004	unknown	CAHFS, 2BFK2	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A99043047	CAHFS, R. Mock, Spleen, 1BFK1, 2/3/2004, A99043047	unknown	CAHFS, 1BFK1	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Equine Herpes 1	A9904309	CAHFS	unknown	CAHFS, unknown	6/3/04	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 1	Bovine 1247	Bovine 1247 ATCC VR2003	ATCC	unknown	CAHFS, unknown	unkown	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	NVSL 002	NVSL 002 1/28/04	NVSL	unknown	CAHFS, unknown	1/29/03	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	LK	LK ATCC VR701	ATCC	unknown	CAHFS, unknown	unkown	Phenol/ Chloroform	40 pg/uL	N/A
Equine Herpes 2	D990	D990	CAHFS	unknown	CAHFS, unknown	1/29/03	Phenol/ Chloroform	40 pg/uL	N/A
Feline Herpes	C-27	ATCC VR636	ATCC	unknown	CAHFS, unknown	unknown	unknown	40 pg/uL	N/A
Fowl Pox	Vaccine strain	Poxine™	Jeffers Livestock	unknown	ECE	5/17/07	Phenol/ Chloroform	40 pg/uL	N/A
Parainfluenza Virus type 3	C-243	TC-81335	LLNL	1 x 10^{5} TCID50/0.1 mL	(VL)MK1 4(LLNL)H 292-1	5/17/07	Trizol	40 pg/uL	Reed and Muench
Porcine Coronavirus	AR310	ATCC VR-2384	LLNL	1 x $10^{4.25}$ $TCID_{50}$/0.1 ml	ST2				Reed and Muench

Porcine Herpes Pseudorabies	Shope	RA 180	CAHFS	1 x 10^5 TCID50/mL		5/17/07	Trizol	40 pg/uL	N/A
Pseudorabies		92-12013	NVSL 92-12013 bovine isolate Iowa December, 1991	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies		93-11745	NVSL 93-11745 Illinois December, 1992	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies	Shope	RA180	NVSL Shope (PRV) RA 180	unknown	CAHFS, unknown	12/3/03	Phenol/ Chloroform	40 pg/uL	N/A
Pseudorabies Titered	Shope	N/A	NVSL	unknown		unknown	Phenol/ Chloroform		Reed & Muench
Respiratory Syncytial virus	Long	N/A		unknown	(VL)KB6 L2KB2HF DL2A549-1(LLNL)H eLa3	5/17/07	Trizol	40 pg/uL	N/A
Transmissible Gastroenteritis virus of hogs	Perdue	NVSL 9801	LLNL	1 x $10^{4.75}$ $TCID_{50}$/0.1 ml	ST 1		Trizol	40 pg/ul	Reed and Muench

TABLE 370. Panel membership for signature. Five PRRS signatures were included in the **Porcine-specific multiplexed assay panel** for FMDV rule-out diagnostics. The below table describes the constituents of that multiplexed assay panel in which these signatures were tested.

Bead ID	Signature/ Target Name	Mux Group ID	Virus	Source	Forward Primer	Reverse Primer	Luminex Probe (-strand, FCP)
132	FMDV.TC	FMDV.TC	Foot and Mouth Virus	Tetracore	ACTGGGTTTTACAAACCTGTGA	GCGAGTCCTGCCACGGA	GTCCCACGGCGTGCAAAGGA
133	FMDV.Pir	FMDV.Pir	Foot and Mouth Virus	Pirbright	CACYTYAAGRTGACAYTGRTACTGGTAC	CAGATYCCRAGTGWCICITGTTA	CCTCGGGGTACCTGAAGGGCATCC
142	PRRS_1807709	PRRS__1807709	Porcine Reproductive and Respiratory Syndrome	LLNL	GAGCGGCAATTGTGTCTGTC	GCTGAGGGTGATGCTGTGAC	CGCACAGTATGATGCGTAGGCAAACTAAACTC
144	PRRS_1810351	PRRS_1810351	Porcine Reproductive and Respiratory Syndrome	LLNL	TTCTTGTGACCACGATTCGC	GACCCACCGAGTAACTTGCC	GCTCAAGAGCCAAAAGCTCAGCATGACA
145	PRRS_1807706	PRRS_1807706	Porcine Reproductive and Respiratory Syndrome	LLNL	ATTGGTTTGCTCCGCGATAC	AAATGAGCCACCACATCCAA	CGGTACATTCGACGCGACACCATTTC
148	PRRS_1810383	PRRS_1810383	Porcine Reproductive and Respiratory Syndrome	LLNL	CAGTGTGCACGCTTCCATTT	CTCGAATGATGTGTTGCCGT	AAACATAGCGTAGAGCTGGAATTCGAAGCCA
149	PRRS_1810386	PRRS_1810386	Porcine Reproductive and Respiratory Syndrome	LLNL	GCTTTCTGCGTGCCTTTTCT	ACAACGCCAGAGACATTCCC	TGACTTTGAAGCCTTTCTCGCTCATTTCTGA
150	SVD_1727049	SVD_1	Swine Vesicular Disease	LLNL	CAGGATAATTTCTTCCAAGGGC	ACGTGAACATTTCGAGCTTCC	TGCATTGTGTCTGATGGTACAACTTGTGACG
151	SVD_1727050	SVD_2	Swine Vesicular Disease	LLNL	GACTTGTTGTGGCTGGAGGA	CAGCGCCATGGTGAGGTAG	TGACCGTAATGAGGTCATCGTGATTTCTCAC
152	SVD_1727051	SVD_3	Swine Vesicular Disease	LLNL	GACAAAGTGGCCAAGGGAAA	CACGTAAACCACACTGGGCT	CTGGCGTCATAGCCTGAATAGTCAAACGCTA
154	VESV_95653.F, VESV_95654.R, VESV_95655.P	VESV_1	Vesicular Exanthema of Swine Virus	LLNL	GCCTTCTCCCTTCCCAAAA	TGAAGGAATGGTTCCGTCAGT	CATCATCGTTGATAACCTTAGATGTGCAATTTGG
157	VESV_95686.F, VESV_95687.R, VESV_95688.P	VESV_4	Vesicular Exanthema of Swine Virus	LLNL	GGTCGCTCTCACTGATGATGAGTA	GGTGTTATCAGCACCCATTGC	GCTCGGTGCCTGAGTTGGAGGAAG
158	VESV_95692.F, VESV_95693.R, VESV_95694.P	VESV_5	Vesicular Exanthema of Swine Virus	LLNL	ACCACCTCTGGAAACATCTATGG	TTTGTGCACGTGTCACGAAT	CGGGACGGGCATTTGTCACCA
163	VSV_1798943	VSV_1798943	Vesicular Stomatitis Virus -Indiana	LLNL	CGCCACAAGGCAGAGATGT	TGTCAAATTCTGACTTAGCATACTTGC	GCATACTGCATCATATCAGGAGTCGGTTTTCTG

164	VSV_1811409	VSV_1811409	Vesicular Stomatitis Virus –New Jersey	PIADC	CTCACAACATGGGTCCTGAA	TTCTTGCCCCGGATACATCAT	GGCACAGCTCATCTGCGACTTCCCT
166	VSV_1798947	VSV_1798947	Vesicular Stomatitis Virus -Indiana	LLNL	CCCAATCAATGCCATGATACA	CTCCAATGGAAGGGTCCAAA	TTTGAAAGTAGAACTGTGCAAGCCCGGTATC
167	VSV_1798949	VSV_1798949	Vesicular Stomatitis Virus -Indiana	LLNL	GGCGCTCATTATAAAATTCGGA	ACATTTTCTCGTAGTAATGCAGCAG	GAAGTCCCTGTAATGGATTCCCATTCCATGT
168	VSV_1811405	VSV_1811405	Vesicular Stomatitis Virus -Indiana	PIADC	AAGAGATGGTCACGAGTGAC	GAGCATTTGTGGAAACCGAGC	TGGGTATTTGGTCATTGGTGACACA
169	VSV_1811408	VSV_1811408	Vesicular Stomatitis Virus –New Jersey	PIADC	CTCACAACATGGGTCCTGAA	TTCTTGACCTGGATACATCAT	GGCATAGYTCGTCTGCRACTTCCCT
170	N/A	NC (MT-7)	Maritima	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
171	N/A	b-MT7 (FC)	N/A	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
172	N/A	MT7/Cy3 (IC)	N/A	LLNL	NT	NT	CAAAGTGGGAGACGTCGTTG
173	Alien RNA	arRNA Control	None	Ambion	GACATCAAGGCTCAAACTAATTTTACC	CAAAGGCTGCCAACATAAAATG	CAAGCGTAAATGCAGCGTCCA

11.5.1. PORCINE PANEL MULTIPLEXED PCR DATA

Preliminary screening: Preliminary screening can be broken down into several steps. The signatures are preliminarily screened by first assessing how the signatures will perform under multiplex PCR conditions and whether or not the addition of the new signatures will adversely effect other signatures, these preliminary screening methods are described as “signature baseline screening” and “multiplex addition screening” respectively. First signature baseline screening tests the performance of the signature in a no-target reaction (blank), a “passing” score is indicated by a low (typically less than 100) MFI. All but one PRRS signature passed the signature baseline screening. Next in multiplex addition screening the new signature primers are added one-by –one to the other panel signatures to determine if there would be any cross-reactions between primer pairs in the panel. Further assessment of the signatures is done through target and near-neighbor screening and the determination of relative sensitivities. The signature down-selection for the PRRS signature is further described below.

TABLE 371. Preliminary screening of PRRS signatures by adding step-wise into the **Porcine panel**. PRRS-7706 and PRRS-0368 showed moderately elevated baselines in the multiplexed panel. When all signatures were titrated with PRRS NVSL strain 2 (shown in bold) of the 7 signatures reacted. PRRS-0368 was dropped from further screening.

Description	**PRRS-7658 (43)**	**PRRS-0351 (44)**	**PRRS-7706 (45)**	**PRRS-0368 (46)**	**PRRS-0374 (47)**	**PRRS-0383 (48)**	**PRRS-0386 (49)**
Porcine + PRRS Panel Blank	8	14	156	107	23	17	17
Blank	8	14	188	104	25	18	17
Blank	8	14	204	104	24	17	16
Blank	9	13	211	109	25	17	16
Blank	8	15	210.5	121	24	16	17
Blank	7	14	206	118.5	24	17	16.5

Blank	8	14	190	109	24	16	16
Blank	8	14	192	127.5	27	17	17
PRRS LLNL .00001 pg/rxn	**7**	14	**103**	71	24	17	17
0.0001	**7**	13	**150**	79.5	25	17.5	18
0.001	**8**	13	**151**	120	24	17	15
0.01	**7**	12	**106**	98	20	14	15
0.1	**10**	13.5	**132**	304	24	16	15.5
1	**57**	11	**176.5**	106	20	15	15
10	**268**	15	**405**	78	22	14	15.5
100	**401**	21	**754.5**	78	25	17	19

TABLE 372. Additional screening of the 2 domestic PRRS signatures in multiplex (PRRS-7658 and PRRS-7706. PRRS-7706 gives slightly better MFIs than 7658. PRRS- 7706 had elevated backgrounds seen in previous work.

Description	PRRS-765 (43)	PRRS-0351 (44)	PRRS-7706 (45)	PRRS-0374 (47)	PRRS-0383 (48)	PRRS-0386 (49)
Porcine Panel (Both Dom PRRS) Blank	9	15	166	29	19	17
Porcine Panel (Both Dom PRRS) Blank	10	14	174	27	17	17
Porcine Panel (Both Dom PRRS) Blank	9	13	175	26	17	17
Porcine Panel (Both Dom PRRS) Blank	11	15	181	25	17	16
Porcine Panel (Both Dom PRRS) Blank	11	14	171	28	16	16
Porcine Panel (Both Dom PRRS) Blank	11	14	189	26	17	16
Porcine Panel (Both Dom PRRS) Blank	11	14	171	27	17	17
Porcine Panel (Both Dom PRRS) Blank	9	15	171	30	18	18
PRRS-Domestic strain _.00001 pg/rxn	9	13	132	28	16	16
0.0001	9	14	140	26	18	16
0.001	9	14	167	28	17	16
0.01	9	15	164	31	18	17
0.1	15	15	186	29	18	16
1	53	15	189	29	18	18
10	237	18	353	30	18	19
100	368	21	607	29	18	20

TABLE 373. Multiplexed assay down-selection summary for PRRS in the **Porcine Panel**. Signature baseline screening indicates the performance of the signature in a no-sample reaction (blank), a "passing" score is indicated by a low MFI. All signatures passed the signature baseline screening. In the multiplex addition screening the primers are added one-by –one to the other panel constituents to determine if there would be any cross-reactions between primer pairs in the panel. The signatures in red were dropped from the panel as explained below.

	Mux Screening: Assay Down Selection					
Signature	Signature baseline screening	Multiplex addition screening	Target screening	Near-neighbor screening	Limit of detection screening	Noted cross-reactions

PRRS_1807658	N/A	Fail (3-1-07): Crossreacts with PRRS_1807706	No further testing	No further testing	No further testing	PRRS_1807658 primers crossreact with PRRS_1807706 probe
PRRS_1807706	N/A	Pass	Fail	Pass	TBD	None observed
PRRS_1807709	N/A	Pass (4-13-07): Some cross-reactions seen with other PRRS signatures	Pass	Pass	TBD	PRRS_1807709 probe cross-reacts with at least one of the other PRRS primers
PRRS_1810351	N/A	Pass	Pass	Pass	TBD	None observed
PRRS_1810368	N/A	Fail (2-13-07): High backgrounds in multiplex also appears to cross-react with SVD-1 based on subsequent tests	No further testing	No further testing	No further testing	PRRS_1810368 primers cross-react with SVD-1 probe
PRRS_1810374	N/A	Pass	Fail (2-13-07): Does not react with any templates	No further testing	No further testing	None observed
PRRS_1810383	N/A	Pass	Pass	Pass	TBD	None observed
PRRS_1810386	N/A	Pass	Pass	Pass	TBD	None observed

Near-neighbor and Target screening: Five PRRS signatures were added to the Porcine panel. The signatures exhibited a reasonably low background response (<60 MFI) in the Porcine panel. Target screening for PRRS was conducted at LLNL with isolates obtained from a collaboration with the University of Minnesota.

TABLE 374. Backgrounds screening in multiplexed format for PRRS at LLNL and PIADC.

	Soils	Prokaryotes	Eukaryotes	Aerosols	Near Neighbors	Targets
Mux PCR Screening	54	135	16	0	2	9

[1]-There are 752 pooled samples in each Aerosol Block.

TABLE 375. Porcine panel backgrounds screening in **multiplexed** format for down-selected PRRS signatures against the below listed **eukaryotes**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. Very low level cross-reactions observed with PRRS-7709.

Description	PRRS-7709 (42)	PRRS-0351 (44)	PRRS-7706 (45)	PRRS-03863 (48)	PRRS-0386 (49)
BOVINE	46	7	14	9	16
CAT	43	7	14	9	38
CHICKEN	40	7	12	8	16
DOG	44	7	11	8	14
DROSOPHILA MELANOGASTER	53	8	14	9	20
EQUINE	53	8	16	9	20
FLEA	53	8	13	9	15
HUMAN	41	7	11	8	14
MONKEY	71	9	18	9	23
MOSQUITO	63	7	12	8	16
MOUSE	68	8	14	9	18
PIG / PORCINE	85	9	20	10	19
RABBIT	78	9	15	10	19
RAT	104	10	18	11	21
SHEEP	84	9	18	10	29
TICK	69	9	20	10	20

TABLE 376. Porcine panel backgrounds screening in **multiplexed** format for the five PRRS signatures against the below listed **prokaryotes**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. Some minor cross-reactions were observed with VSV-7709. No other significant cross-reactions were noted.

Description	PRRS_1807709 (42)	PRRS_1810351 (44)	PRRS_1807706 (45)	PRRS_1810383 (48)	PRRS_1810386 (49)
Erwinia amylovora	91	9	16	10	18
Actinobacillus suis	46	6	11	8	14
Aneurinbacillus migulanus	53	6	10	8	13
Bacillus cereus	142	9	19	9	21
Bacillus globigii	95	7	14	8	17
Bacillus subtilis	122	9	16	9	20
Bacillus thuringiensis	138	10	19	10	21
Bifidobacterium denticum	69	8	13	8	14
Borrelia burgdorferi	148	11	21	10	22
Burkholderia capacia	73	8	14	10	17
Caulobacter vibriodes	42	7	11	8	13
Clavibacter michganensis	69	9	14	10	16
Clostridium butyricum	107	9	23	10	19

Corynebacterium pseudodiptericum	88	9	17	10	17
Cytophaga marinoflava	95	10	17	11	18
Erwinia herbicola	114	9	19	10	20
Escherichia coli	135	10	20	10	22
Geobacillus caldoxylosilyticus	90	9	17	11	18
Halomonas halmophila	69	7	14	9	15
Haemophilus influenza	99	8	16	9	18
Herbaspirillium seropedicae	50	7	12	9	13
Lactobacillus garvieae	38	5	9	6	11
Lactobacillus gasseri	47	6	10	7	12
Listeria monocytogenes	95	8	16	9	18
Listeria seeligeri	113	10	19	10	20
Micrococcus luteus	52	6	11	8	13
Moraxella lacunatica	71	8	15	9	15
Oceanospirillium ssp. Maris	62	8	14	9	16
Paenibacillus napthalaenovorans	70	9	15	9	16
Paracoccus dentrificans	68	8	14	9	15
Porphyrobacter sanguineus	40	6	11	8	17
Proteus mirabillis	77	9	16	10	17
Pseudomonas aeruginosae	82	7	13	9	15
Pseudomonas oleovorans	47	6	10	9	13
Rhizobium leguminosarum	64	8	14	10	16
Rhodococcus rhodochrous	57	7	12	9	13
Salmonella typhimurium	100	8	15	9	17
Simonsiella muelleri	53	7	12	8	14
Sphingomonas sp. (Alcaligenes sp)	60	7	13	8	16
Staphylococcus aureus	108	9	17	10	18
Streptococcus pneumoniae	115	10	19	10	19
Streptomyces scabiei	74	9	14	11	17
Tatlockia maceachernii	64	9	15	10	16
Vibrio parahaemolyticus	70	8	14	10	16
Xanthomonas translucens	67	9	14	10	16

TABLE 377. Porcine panel backgrounds screening in **multiplexed** format for the five PRRS signatures against the below listed **soils**. 200pg per reaction of extracted total nucleic acid is added to each PCR in triplicate. Results are indicated below as averages of 3 replicates in Median Fluorescence Intensity (MFI) units. None of the signatures cross-reacted with any of the samples listed below.

Description	PRRS-7709 (42)	PRRS-0351 (44)	PRRS-7706 (45)	PRRS-0383 (48)	PRRS-0386 (49)

D 000107-49	69	8	13	9	13
D 000109 # 50	68	8	13	9	14
D 000402 # 53	72	8	14	9	15
D 000500 - 26 - 1	71	9	14	9	15
D 000501-14-1	54	8	12	9	13
D 000505 - 11 - 4	63	7	14	10	16
D 000521 - 23	69	9	15	9	15
D 000527 - 3	62	8	14	10	14
D 000531 - 21	59	9	13	9	14
D 000533 - 17 -1	55	7	12	8	13
D 000542 - 6	53	8	13	10	14
D 000550 - 20	55	8	13	10	14
D 000551 - 5	68	9	14	10	15
D 000561 - 8 - 6	56	8	13	10	14
D 000562 - 30 - 5	59	8	12	10	15
S 251	46	8	12	9	12
S 252	45	7	10	8	12
S 253	43	8	12	9	12
S 254	40	8	11	9	12
S 255	41	7	11	8	12
S 256	41	8	12	9	13
S 257	39	7	10	8	12
S 259	40	7	11	8	12
S 260	39	7	11	8	12
S 271	37	7	11	9	12
S 272	36	7	11	9	12
S 273	38	8	12	9	12
S 274	43	8	12	10	12
S 275	41	7	11	9	12
S 276	40	8	11	10	12
S 277	39	8	12	9	13
S 279	34	7	11	9	11
S 280	34	8	11	9	11
S 282	36	7	12	10	12
S 283	33	8	11	9	12
S 284	32	8	12	10	13
S 286	33	8	13	9	12
S 287	34	8	11	9	12
S 288	33	7	11	10	12
S 289	31	7	10	9	11
S 290	32	8	11	9	12
S 291	30	7	10	8	11
S 292	29	7	12	8	12
S 295	33	7	11	8	11
S 296	33	7	11	9	11
S 297	32	8	11	9	12
S 298	31	7	11	8	11

S 299	29	7	10	8	10
S 300	13	6	7	7	9
S 301	13	6	8	8	10
S 303	10	6	8	8	10
S 304	13	7	8	9	10
S 305	13	7	9	8	12
S 307	12	7	9	8	12

TABLE 378. Porcine Panel **Near-Neighbor** screening (Data from 20070601) against the PRRS signatures. Values shown are Median Fluorescence Intensity (MFI) units. “Blanks” are buffer-only samples (no nucleic acid added to reaction), shown below in red. Extracted nucleic acid was added to each PCR reaction in triplicate totaling 200pg/rxn. The data shows that the PRRS signatures did not cross- reacted with any listed near-neighbors of the Porcine panel constituents.

Description	PRRS_180 7709 (42)	PRRS_181 0351 (44)	PRRS_180 7706 (45)	PRRS_181 0383 (48)	PRRS_181 0386 (49)
Blank Average	158	9	18	9	17
Blank St Dev	81	3	6	3	5
BDV Coos Bay	72	7	13	9	12
BHV (BFK) A03250006 DN-599	140	9	18	10	16
BHV A040150085	132	8	17	9	16
BHV-1 (IBR) Texas A030020072 CAHFS	117	9	17	10	18
BHV-1 A033640072	141	8	18	9	15
BHV-1 A040130066	159	10	19	9	17
BHV-1 ATCC VR 793	132	8	18	9	14
BHV-1 NVSL 10720	117	8	16	9	14
BHV-1 NVSL 200032	108	8	18	9	14
BHV-1 NVSL 231221	125	8	16	9	15
BHV-1 NVSL 51619	99	7	15	8	13
BHV-1 NVSL 86741	118	8	16	10	15
BHV-1 or IBR LA ATCC VR188	122	8	18	10	16
BHV-1 RA309	97	7	14	8	13
BHV-5 A032540006 CAHFS	71	6	12	8	11
BHV-5 A040150085 CAHFS	68	7	13	9	13
BHV-5 D9402133 CAHFS	70	7	12	7	12
BHV-5 D9403153 CAHFS	81	7	14	9	12
Caprine Herpes D0201157 CAHFS	72	7	13	9	13
Caprine Herpes-2 ATCC VR 462	89	7	14	9	13
Caprine Herpes-2 S0201998 CAHFS	71	6	12	8	11
EHD-1 A9904309	85	7	13	8	14
EHD-1 Georgia	75	7	13	9	13
EHD-1 New Jersey	52	5	9	7	10
EHD-1 Santa Barbara	64	6	10	8	11
EHD-2 Alberta	63	7	11	8	12

EHV-1 A011120004 CAHFS	113	10	17	11	16
EHV-1 A99043047	97	8	14	9	14
EHV-1 ATCC VR2003	99	7	14	9	14
EHV-2 ATCC VR701	97	8	15	9	14
EHV-2 D990 CAFHS	75	6	11	8	12
EHV-2 NVSL 0002	92	8	15	9	16
Feline Herpes ATCC VR 636	79	7	14	9	14
Fowl Pox	66	8	13	9	13
IBR CA 111903	135	9	25	10	17
IBR MN 111903	111	8	16	8	15
Parainfluenza Type 3	75	8	14	10	13
Porcine Herpesvirus or Pseudorabies Shope	77	7	18	9	13
Pseudorabies NVSL 92-12013	81	6	13	9	13
Pseudorabies NVSL 93-11745	100	8	19	10	15
Pseudorabies RA 180 CAHFS	84	7	13	9	13
Pseudorabies Titered	94	7	14	9	14
Respiratory Syncytial	69	8	13	10	13

TABLE 379. Porcine Panel **Near-Neighbor** screening (Data from 20070608) against the PRRS signatures. Values shown are Median Fluorescence Intensity (MFI) units. “Blanks” are buffer-only samples (no nucleic acid added to reaction), shown below in red. Extracted nucleic acid was added to each PCR reaction in triplicate totaling 200pg/rxn. The data shows that the PRRS signatures did not cross- reacted with any listed near-neighbors. The MFI values for the blanks on signature PRRS-7709 are elevated for an unknown reason.

Description	**PRRS-7709 (42)**	**PRRS-0351 (44)**	**PRRS-7706 (45)**	**PRRS-0383 (48)**	**PRRS-0386 (49)**
Blank	141	10	18	10	20
Blank	114	9	16	8	15
Porcine respiratory coronavirus	58	7	11	8	12
Porcine respiratory coronavirus	55	8	12	8	11
Porcine respiratory coronavirus	59	8	14	8	12
Transmissible gastroenteritis of hogs	68	7	14	9	12
Transmissible gastroenteritis of hogs	63	8	14	9	13
Transmissible gastroenteritis of hogs	64	8	14	11	13

Multiplexed PCR Assay Titrations

Upon finalization of the assay panel constituents each agent assay is characterized in multiplexed PCR format by running dose-response (titration) curves against titered virus extracted from virus infected cell culture for each available agent strain. Each sample is tested in duplicate and reported as a mean value of the median fluorescence intensity units (MFI) against agent

concentration (per reaction volume, 5uL) on a double log plot. Concentration units are reported in TCID50/rxn (assumes 5uL sample volume per reaction) or pfu/rxn depending on virus type and source. In some cases where titered virus was not available data is reported as picogram units (pg). Each assay is evaluated by its ability to detect agent strains and differentiate from near neighbors (specificity) and by its range of sensitivity to each strain relative to each signature tested for that agent. Because this method is not aimed at determination of analytical sensitivities (requiring a more extensive sample set tested in clinical sample matrices which is not practical for this stage of development) it does however, provide tentative limits of detection that are used to measure the performance of each assay.

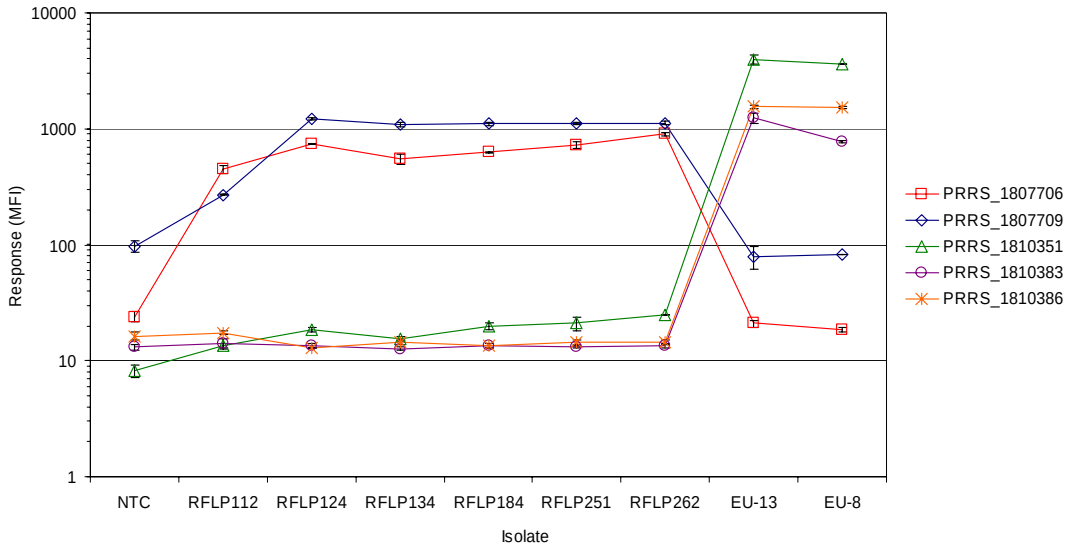

FIG. 51. Porcine multiplex screening data for five PRRS signatures against six North American and two European isolates received from the University of Minnesota. Total nucleic acid was Trizol extracted from virus-infected cell culture media then used as a template. Each reaction was spiked with 200pg. Each point represents the mean response (n=2). Error bars indicate ±1σ of the mean.

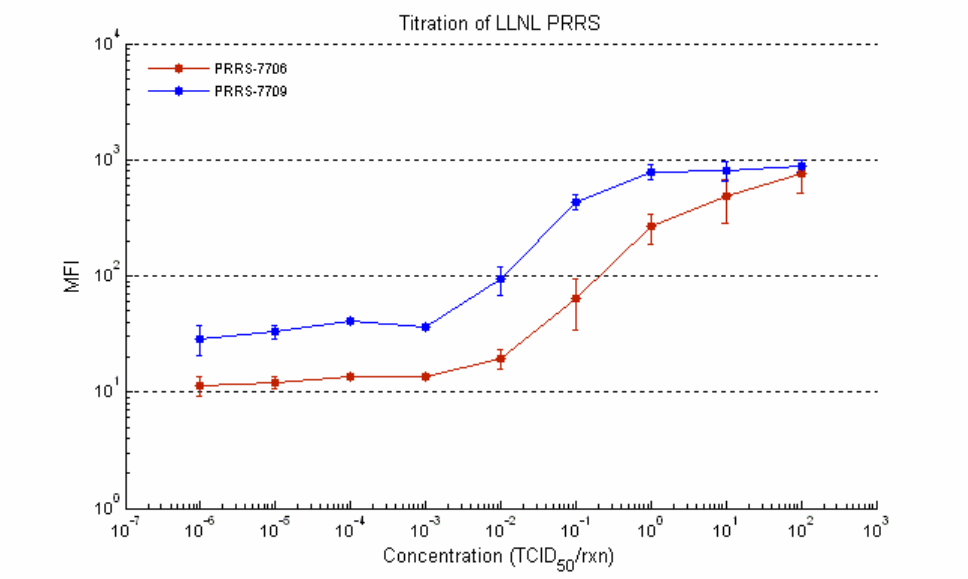

FIG. 52. Porcine multiplex screening data for five PRRS signatures against the North American Strain (NVSL, 1989). Serial dilution of total nucleic acid Trizol extracted from virus-infected cell culture. Total nucleic acid was Trizol extracted from non-infected (no template control, NTC) and virus-infected cell culture media then used as template. Each point represents the mean response (n=2). Error bars indicate ±1σ of the mean. Figure shows that both signatures PRRS_1807706 and PRRS_1807709 responded as expected to the North American strain over four orders of magnitude in concentration. The signatures PRRS_1810351, PRRS_1810383 and PRRS_1810386 designed to detect European strains did not respond to the North American strain.

RESULTS: Of the five PRRS signatures screened in multiplex, 2 of these, specific for the PRRS North American strain, were screened against the North American strain and shown to respond to this isolate. Further screening of the European strain is required to determine functionality of the other candidate signatures that have been added to the porcine multiplex. **This work is a gap that can not be completed this year.**

APPENDIX I: Definitions

Definitions

Alien RNA: Positive control for RT-PCR. Essentially an armored RNA (RNA that is packaged in a protein coat for added stability). This material is added to the RT-PCR reaction and verifies that both RT and PCR have occurred in the reaction.

Amine modified probe: Probe, or internal oligonucleotide sequence, that is modified with a 5 prime amine and carbon spacer for the attachment to Luminex carboxylated microspheres. Hybridizes to amplified nucleic acid with complimentary sequence.

Amplicon: The sequence that results from PCR or RT-PCR amplification that incorporates the primers and replicated sequence.

Background screening: describes the process of signature down-selection where signatures are screened against various environmental confounders for signature interference or cross-reactivity.

Bead number/ID: indicates the bead type used. More specifically, corresponds to a specific bead in the set of 100 in the Luminex xMAP, based on fluorescent properties.

Biotinylated primer: Forward primer that is modified with a 5 prime biotin and internal biotins for multiplexed bead-based assays.

Bt: *Bacillus thuringiensis*, bacterial DNA used as positive control in real-time assays.

Denaturation: Describes the step in nucleic acid amplification where the reaction is heated to "denature" or separate the double stranded nucleic acid in preparation for the amplification process. May also be used to destroy or remove unwanted reagent components at a given step in the process.

Extension: Describes the step in nucleic acid amplification where the primers "extend" along the template sequence to replicate.

FCP: Forward compliment probe. Describes the orientation of the probe sequence that is used for the reaction hybridization. Forward compliment indicates that the sequence is the compliment to the forward primer strand.

Fluorescent control: Control designed to detect stability of the fluorescent label (streptavidin phycoerythrin) or to verify that it was added to the reaction.

Hybridization: Describes process by which PCR product is introduced to microspheres that have oligonucleotide probes attached and heated slightly for hybridization to occur.

Internal controls: Describes the in-built assays that are incorporated into the reaction process to verify that particular steps of the reaction occurred properly, without failures.

Kpathrun ID: LLNL internal identification number that references internal database of signature generation information.

Labeled amplicon: The sequence that results from PCR or RT-PCR amplification that incorporates the primers and replicated sequence; includes the forward primer that is labeled with a biotin. Synonymous with "PCR product"; plural reference.

Limit of detection: described as the point at which the signal crosses the pre-established threshold for that signature. Threshold determination is described herewith.

Luminex: The Luminex instrument is a high-tech flow cytometer with in-built optics used to detect the fluorescent signal from both the microspheres and the fluorescently labeled reaction that is hybridized to the beads. Software allows detection measurements to be semi-quantifiable. Instrument is also called "Bioplex" (Biorad), "Liquiplex" (Qiagen), etc.

MFI: Median fluorescence intensity. Describes fluorescence reported on the Luminex/Bioplex systems that indicate the amount of reporter label is on the bead.

Microsphere: Luminex polystyrene beads (5.5 micron in size), optically encoded with precise ratios of fluorescent dye designed for use in the Luminex Xmap technology. Surface modification of beads allow for covalent attachment to substrate. Synonymous with "beads".

Multiplex: A PCR reaction contains multiple primer sets including the internal control assay(s).

MUX: Abbreviation used for Multiplexed bead-based PCR.

Negative control: Designed such that under "normal" (in the absence of nonspecific binding) assay conditions there will be no signal detected.

Oligonucleotides: Synthesized sequences of nucleic acids that are used for PCR reactions (primers, probes).

PCR product: Describes the resulting product of several cycles of PCR/RT-PCR amplification. The sequence that results from PCR or RT-PCR amplification that incorporates the primers and replicated sequence. Synonymous with "amplicon"; singular reference).

PFU: Plaque forming unit.

Positive control: Control designed to verify that each step in the reaction process occurred correctly. Verifies either RT-PCR, PCR or both.

RCP: Reverse compliment probe. Describes the orientation of the probe sequence that is used for the reaction hybridization. Reverse compliment indicates that the sequence is the compliment to the reverse primer strand.

Real-time PCR: Taqman-based PCR utilizing a fluorescent probe and quencher for real-time reporting.

RT-PCR: Reverse transcriptase polymerase chain reaction. Process of nucleic acid amplification of RNAs that requires a reverse transcriptase step to convert RNA into cDNA and subsequently to be amplified by polymerase chain reaction.

Singleplex: Indicates that the PCR reaction contains only one primer set with an added primer set for the internal control assay.

Spot check or "pre-screen": Describes process by which multiplex assay candidates are rapidly screened in singleplex format to determine relative reactivities across all signatures. This process is called also called "spot checking" because the assays are subjected to a single concentration of target DNA, rather than in a dilution series.

Streptavidin phycoerythrin: Flourophore used in labeling that binds with high affinity to biotin.

Taqman PCR: Synonymous to Real-time polymerase chain reaction (PCR)

Target/Near-Neighbor (NN) screening: describes the process of signature down-selection where signatures are screened against target nucleic acids and nucleic acids from organism that are phylogenically nearest to the target organism.

$TCID_{50}$ or tissue culture infective dose 50% endpoint
This represents the viral dose that causes cytopathic effect in 50% of the inoculated cell cultures. The 50% endpoint may be calculated by either the method of Reed and Muench or Kärber. Author et al. 1989. J Virol. 63. 5046-5053.

Wash assay: Describes process of taking beads with hybridized PCR product attached and washing with buffer in a 96-well filter plate format using a vacuum manifold station. In this process the beads are washed several times to remove unbound nucleic acid and labeled with streptavidin phycoerythrin before they are transferred to the Luminex instrument for processing.

APPENDIX II: Taqsim Reports

Taqsim description

We used a computational Real-time RT- PCR simulator program, "Taqsim", to identify all potential targets for each signature from all sequences available in genbank. Taqsim is a BLAST-based comparison of each signature as a triplet against all sequences in genbank to identify the targets that are predicted to produce a Real-time RT- PCR reaction at 57 degrees primer annealing and 67 degrees for probe annealing (these temperatures are according to Primer 3 oligo Tm calculations). The first column of the excel spreadsheets list the signatures by the name, and the following columns report the predicted genbank targets, coordinates of signature region, amplicon size, and accession #'s for each target.

BLUETONGUE VIRUS SIGNATURES

sig_candidate_1759930_Bluetongue+virus+segment+1+LLNL_0	gi|438509|gb|L20447.1|BTVVP1D_Bluetongue virus RNA-directed RNA polymerase (VP1) gene, complete cds	966	1230	265
sig_candidate_1759930_Bluetongue+virus+segment+1+LLNL_0	gi|58745|emb|X12819.1|BTV10VP1_Bluetongue virus gene for capsid protein VP1, genomic RNA	966	1230	265
sig_candidate_1759930_Bluetongue+virus+segment+1+LLNL_0	gi|438501|gb|L20508.1|BTVCOREPRO_Bluetongue virus core protein (VP1) RNA, complete cds	966	1230	265
5 total amplicons	gi|438505|gb|L20445.1|BTVVP1B_Bluetongue virus RNA-directed RNA polymerase (VP1) gene, complete cds	966	1230	265
	gi|438507|gb|L20446.1|BTVVP1C_Bluetongue virus RNA-directed RNA polymerase (VP1) gene, complete cds	966	1230	265
[redacted]				
sig_candidate_1759931_Bluetongue+virus+segment+1+LLNL_1	gi|438509|gb|L20447.1|BTVVP1D_Bluetongue virus RNA-directed RNA polymerase (VP1) gene, complete cds	1881	2243	363
sig_candidate_1759931_Bluetongue+virus+segment+1+LLNL_1	gi|438505|gb|L20445.1|BTVVP1B_Bluetongue virus RNA-directed RNA polymerase (VP1) gene, complete cds	1881	2243	363
sig_candidate_1759931_Bluetongue+virus+segment+1+LLNL_1				
2 total amplicons				
[redacted]				
sig_candidate_1759932_Bluetongue+virus+segment+1+LLNL_2	gi|438509|gb|L20447.1|BTVVP1D_Bluetongue virus RNA-directed RNA polymerase (VP1) gene, complete cds	3276	3547	272
sig_candidate_1759932_Bluetongue+virus+segment+1+LLNL_2	gi|58745|emb|X12819.1|BTV10VP1_Bluetongue virus gene for capsid protein VP1, genomic RNA	3276	3547	272
sig_candidate_1759932_Bluetongue+virus+segment+1+LLNL_2	gi|438507|gb|L20446.1|BTVVP1C_Bluetongue virus RNA-directed RNA polymerase (VP1) gene, complete cds	3276	3547	272
3 total amplicons				
[redacted]				
sig_candidate_1759933_Bluetongue+virus+segment+8+LLNL_0	gi|323180|gb|L08674.1|BTVS2B_Bluetongue virus mRNA sequence	218	405	188
sig_candidate_1759933_Bluetongue+virus+segment+8+LLNL_0	gi|323182|gb|L08676.1|BTVS2D_Bluetongue virus mRNA sequence	218	405	188

sig_candidate_1759933_Bluetongue+virus+segment+8+LLNL_0	gi|221074|dbj|D00500.1|BTVS8NS2_Bluetongue virus gene for NS2 protein, complete cds	218	405	188
3 total amplicons				

sig_candidate_1759935_Bluetongue+virus+segment+9+LLNL_0	gi|210844|gb|L08670.1|BTV11VP6_Bluetongue virus segment s3 core protein VP6 gene, complete cds	50	382	333
sig_candidate_1759935_Bluetongue+virus+segment+9+LLNL_0	gi|210848|gb|L08672.1|BTV17VP6_Bluetongue virus segment s3 core protein VP6 gene, complete cds	50	382	333
sig_candidate_1759935_Bluetongue+virus+segment+9+LLNL_0	gi|22252937|gb|AY124373.1|_Corsican bluetongue virus serotype 2 VP6 (S9) gene, complete cds	35	367	333
42 total amplicons	gi|15321641|gb|AF403420.1|_Bluetongue virus clone BTV2_OnaA_S9 VP6 gene, complete cds	50	382	333
	gi|1497968|gb|U55796.1|BVU55796_Bluetongue virus strain BTV 11 VP6 (S3) gene, complete cds	50	382	333
	gi|1497948|gb|U55786.1|BVU55786_Bluetongue virus strain 11UC2 VP6 (S3) gene, complete cds	50	382	333
	gi|221080|dbj|D10905.1|BTVVP6_Bluetongue virus genome segment 9 for VP6, complete sequence	50	382	333
	gi|1497960|gb|U55792.1|BVU55792_Bluetongue virus strain 17B90Z VP6 (S3) gene, complete cds	50	382	333
	gi|1497950|gb|U55787.1|BVU55787_Bluetongue virus strain 11UC8 VP6 (S3) gene, complete cds	50	382	333
	gi|1497942|gb|U55783.1|BVU55783_Bluetongue virus strain 10O90Z VP6 (S3) gene, complete cds	50	382	333
	gi|210846|gb|L08671.1|BTV13VP6_Bluetongue virus segment s3 core protein VP6 gene, complete cds	50	382	333
	gi|111054385|gb|DQ832170.1|_Bluetongue virus isolate BTVVACSA serotype 4 VP6 gene, complete cds	35	367	333
	gi|210850|gb|L08668.1|BTV2VP6_Bluetongue virus segment s3 core protein VP6 gene, complete cds	50	382	333
	gi|1497956|gb|U55790.1|BVU55790_Bluetongue virus strain 13B89Y VP6 (S3) gene, complete cds	50	382	333
	gi|1497978|gb|U55801.1|BVU55801_Bluetongue virus strain BTV 10 VP6 (S3) gene, complete cds	50	382	333
	gi|1497962|gb|U55793.1|BVU55793_Bluetongue virus strain 17C81W VP6 (S3) gene, complete cds	50	382	333
	gi|15320543|gb|AF403423.1|_Bluetongue virus clone BTV4SA_S9 VP6 gene, complete cds	50	382	333
	gi|1497952|gb|U55788.1|BVU55788_Bluetongue virus strain 13B81V VP6 (S3) gene, complete cds	50	382	333
	gi|15321639|gb|AF403418.1|_Bluetongue virus clone BTV17_S9 VP6 gene, complete cds	50	382	333
	gi|1497932|gb|U55778.1|BVU55778_Bluetongue virus strain 10B80Y VP6 (S3) gene, complete cds	50	382	333
	gi|1497972|gb|U55798.1|BVU55798_Bluetongue virus strain BTV 17 VP6 (S3) gene, complete cds	50	382	333
	gi|1497934|gb|U55779.1|BVU55779_Bluetongue virus strain 10B90Z VP6 (S3) gene, complete cds	50	382	333
	gi|1497974|gb|U55799.1|BVU55799_Bluetongue virus strain BTV 2 VP6 (S3) gene, complete cds	50	382	333
	gi|1497944|gb|U55784.1|BVU55784_Bluetongue virus strain 11O81Z VP6 (S3) gene, complete cds	50	382	333
	gi|1497938|gb|U55781.1|BVU55781_Bluetongue virus strain 10O80Z VP6 (S3) gene, complete cds	50	382	333
	gi|1497940|gb|U55782.1|BVU55782_Bluetongue virus strain 10O90H VP6 (S3) gene, complete cds	50	382	333
	gi|110809930|gb|DQ825671.1|_Bluetongue virus from Turkey segment 9 VP6 gene, complete cds	35	367	333
	gi|15320539|gb|AF403419.1|_Bluetongue virus clone BTV1SA-ABADRL VP6 gene, complete cds	50	382	333
	gi|1497954|gb|U55789.1|BVU55789_Bluetongue virus strain 13B81X VP6 (S3) gene, complete cds	50	382	333
	gi|1497976|gb|U55800.1|BVU55800_Bluetongue virus vaccine strain VP6 (S3) gene, complete cds	50	382	333
	gi|15320541|gb|AF403421.1|_Bluetongue virus clone BTV2_OnaB_S9 VP6 gene, complete cds	50	382	333
	gi|15321643|gb|AF403422.1|_Bluetongue virus clone BTV2_SA_S9 VP6 gene, complete cds	50	382	333
	gi|1497958|gb|U55791.1|BVU55791_Bluetongue virus strain 13B89Z VP6 (S3) gene, complete cds	50	382	333
	gi|1497970|gb|U55797.1|BVU55797_Bluetongue virus strain BTV 13 VP6 (S3) gene, complete cds	50	382	333
	gi|110809924|gb|DQ825668.1|_Bluetongue virus from France segment 9 VP6 gene, complete cds	35	367	333
	gi|110809926|gb|DQ825669.1|_Bluetongue virus from Greece segment 9 VP6 gene, complete cds	35	367	333
	gi|1497966|gb|U55795.1|BVU55795_Bluetongue virus strain 17O90X VP6 (S3) gene, complete cds	50	382	333
	gi|1497946|gb|U55785.1|BVU55785_Bluetongue virus strain 11C81Z VP6 (S3) gene, complete cds	50	382	333
	gi|221076|dbj|D00509.1|BTVS9VP6_Bluetongue virus gene for VP6, complete cds	50	382	333
	gi|22218291|gb|AF530066.1|_Bluetongue virus VP6 gene, complete cds	35	367	333
	gi|1497936|gb|U55780.1|BVU55780_Bluetongue virus strain 10O80V VP6 (S3) gene, complete cds	50	382	333

	gi|1497964|gb|U55794.1|BVU55794_Bluetongue virus strain 17O81Y VP6 (S3) gene, complete cds	50	382	333

BOVINE HERPES VIRUS 1 SIGNATURES

sig_candidate_644761_BHV_1_ignore_1_no_exclude.000004	gi|73920558|gb|DQ173735.1|_Bovine herpesvirus type 1.1 isolate UY1999 glycoprotein C (gC) gene, partial cds	93	233	141
sig_candidate_644761_BHV_1_ignore_1_no_exclude.000004	gi|73920522|gb|DQ173717.1|_Bovine herpesvirus type 1.1 isolate EVI123 glycoprotein C (gC) gene, partial cds	43	183	141
sig_candidate_644761_BHV_1_ignore_1_no_exclude.000004	gi|73920544|gb|DQ173728.1|_Bovine herpesvirus type 1.1 isolate PG2560 glycoprotein C (gC) gene, partial cds	52	192	141
10 total amplicons	gi|73920560|gb|DQ173736.1|_Bovine herpesvirus type 1.1 isolate T3 glycoprotein C (gC) gene, partial cds	36	176	141
	gi|2653291|emb|AJ004801.1|BHV1CGEN_Bovine herpesvirus type 1.1 complete genome	17101	17241	141
	gi|73920536|gb|DQ173724.1|_Bovine herpesvirus 1 isolate LAM glycoprotein C (gC) gene, partial cds	93	233	141
	gi|73920554|gb|DQ173733.1|_Bovine herpesvirus type 1.1 isolate COOPER glycoprotein C (gC) gene, partial cds	90	230	141
	gi|330754|gb|M27491.1|HSBGPG3A_Bovine herpesvirus type 1 glycoprotein gIII gene, complete cds	1129	1269	141
	gi|995626|emb|Z54206.1|BVH1LFT31_Bovine herpesvirus type 1 31-kb DNA (left genome end)	17101	17241	141
	gi|75709303|gb|DQ184913.1|_Bovine herpesvirus 1 isolate LA glycoprotein gene, partial cds	277	417	141
[redacted]				
sig_candidate_644766_BHV_1_ignore_1_no_exclude.000009	gi|2653291|emb|AJ004801.1|BHV1CGEN_Bovine herpesvirus type 1.1 complete genome	17977	18091	115
sig_candidate_644766_BHV_1_ignore_1_no_exclude.000009	gi|330754|gb|M27491.1|HSBGPG3A_Bovine herpesvirus type 1 glycoprotein gIII gene, complete cds	279	393	115
sig_candidate_644766_BHV_1_ignore_1_no_exclude.000009	gi|995626|emb|Z54206.1|BVH1LFT31_Bovine herpesvirus type 1 31-kb DNA (left genome end)	17977	18091	115
3 total amplicons				
[redacted]				
sig_candidate_644797_BHV_1_ignore_1_no_exclude.000040	gi|1491620|emb|Z78205.1|BHT1UL_Bovine herpesvirus type 1 UL22-35 genes	27390	27576	187
sig_candidate_644797_BHV_1_ignore_1_no_exclude.000040	gi|340858|gb|M23257.1|HSB1GPB_Herpesvirus type 1 glycoprotein gene, complete cds	3162	3347	186
sig_candidate_644797_BHV_1_ignore_1_no_exclude.000040	gi|537304|gb|U14106.1|_Bovine herpesvirus 1 BHV1.2 isolate K22 glycoprotein (gI) gene, partial cds	183	366	184
8 total amplicons	gi|2653291|emb|AJ004801.1|BHV1CGEN_Bovine herpesvirus type 1.1 complete genome	58190	58376	187
	gi|537306|gb|U14107.1|_Bovine herpesvirus 1 BHV1.2 isolate M glycoprotein (gI) gene, partial cds	183	369	187
	gi|537302|gb|U14105.1|_Bovine herpesvirus 1 BHV1.2 isolate Fi glycoprotein (gI) gene, partial cds	183	369	187
	gi|537300|gb|U14104.1|_Bovine herpesvirus 1 BHV1.1 glycoprotein (gI) gene, partial cds	183	369	187
	gi|330756|gb|M21474.1|HSBGPI_Bovine herpesvirus type 1 glycoprotein 1 gene, complete cds	3180	3366	187
[redacted]				
sig_candidate_644869_BHV_1_ignore_1_no_exclude.000112	gi|971311|emb|Z48053.1|BHV130KB_Bovine herpesvirus type 1 (Cooper) DNA (30 kb)	29254	29453	200
sig_candidate_644869_BHV_1_ignore_1_no_exclude.000112	gi|2653291|emb|AJ004801.1|BHV1CGEN_Bovine herpesvirus type 1.1 complete genome	96154	96353	200

sig_candidate_644869_BHV_1_ignore_1_no_exclude.000112

2 total amplicons

BOVINE PAPULAR STOMATITIS VIRUS SIGNATURES

sig_candidate_935544_BPSV_and_Orf_virus__three_strains__no_exclude_IGNORE_1.000003	gi|40019122|gb|AY386263.1|_Orf virus strain OV-IA82, complete genome	109808	109986	179
sig_candidate_935544_BPSV_and_Orf_virus__three_strains__no_exclude_IGNORE_1.000003	gi|74230714|gb|DQ184476.1|_Orf virus strain NZ2, complete genome	110210	110388	179
sig_candidate_935544_BPSV_and_Orf_virus__three_strains__no_exclude_IGNORE_1.000003	gi|40019124|gb|AY386265.1|_Bovine papular stomatitis virus strain BV-AR02, complete genome	110778	110956	179
4 total amplicons	gi|40019123|gb|AY386264.1|_Orf virus strain OV-SA00, complete genome	110975	111153	179

sig_candidate_935545_BPSV_and_Orf_virus__three_strains__no_exclude_IGNORE_1.000004	gi|40019122|gb|AY386263.1|_Orf virus strain OV-IA82, complete genome	24423	24518	96
sig_candidate_935545_BPSV_and_Orf_virus__three_strains__no_exclude_IGNORE_1.000004	gi|74230714|gb|DQ184476.1|_Orf virus strain NZ2, complete genome	24827	24922	96
sig_candidate_935545_BPSV_and_Orf_virus__three_strains__no_exclude_IGNORE_1.000004	gi|40019124|gb|AY386265.1|_Bovine papular stomatitis virus strain BV-AR02, complete genome	25826	25921	96
5 total amplicons	gi|1236945|gb|U49979.1|OVU49979_Orf virus E10R homolog gene, partial cds, and DNA polymerase gene, complete cds	2078	2173	96
	gi|40019123|gb|AY386264.1|_Orf virus strain OV-SA00, complete genome	25588	25683	96

sig_candidate_935546_BPSV_and_Orf_virus__three_strains__no_exclude_IGNORE_1.000005	gi|30230664|gb|AY267342.1|_Orf virus strain Orf-11 D5R-like protein mRNA, partial cds	1037	1183	147
sig_candidate_935546_BPSV_and_Orf_virus__three_strains__no_exclude_IGNORE_1.000005	gi|40019122|gb|AY386263.1|_Orf virus strain OV-IA82, complete genome	69311	69457	147
sig_candidate_935546_BPSV_and_Orf_virus__three_strains__no_exclude_IGNORE_1.000005	gi|74230714|gb|DQ184476.1|_Orf virus strain NZ2, complete genome	69683	69829	147
5 total amplicons	gi|40019124|gb|AY386265.1|_Bovine papular stomatitis virus strain BV-AR02, complete genome	70546	70692	147
	gi|40019123|gb|AY386264.1|_Orf virus strain OV-SA00, complete genome	70444	70590	147

sig_candidate_935549_BPSV_and_Orf_virus__three_strains__no_exclude_IGNORE_1.000008	gi|40019122|gb|AY386263.1|_Orf virus strain OV-IA82, complete genome	85715	85882	168
sig_candidate_935549_BPSV_and_Orf_virus__three_strains__no_exclude_IGNORE_1.000008	gi|74230714|gb|DQ184476.1|_Orf virus strain NZ2, complete genome	86120	86287	168
sig_candidate_935549_BPSV_and_Orf_virus__three_strains__no_exclude_IGNORE_1.000008	gi|40019124|gb|AY386265.1|_Bovine papular stomatitis virus strain BV-AR02, complete genome	86710	86877	168
4 total amplicons	gi|40019123|gb|AY386264.1|_Orf virus strain OV-SA00, complete genome	86867	87034	168

BOVINE VIRAL DIARRHEA DISEASE VIRUS SIGNATURES

Sig ID/Name (F/IO/R)	Verification/Cross Rxn	Start	Stop	Amp Size
bvd_ucd	gi|30841828|gb|AY279527.1|_Bovine viral diarrhea virus strain Osloss isolate Ind S-1171 polyprotein gene, 5' untranslated region and partial cds	80	273	194
bvd_ucd	gi|29028552|gb|AY159548.1|_Bovine viral diarrhea virus 3478/00 5' untranslated region, partial sequence	51	246	196
bvd_ucd	gi|32745468|gb|AY323877.1|_Bovine viral diarrhea virus 1 isolate I-1709/00-24 nonstructural protein Npro gene, partial cds	83	278	196
132 total amplicons	gi|30692273|gb|AY279087.1|_Pestivirus type 1 isolate Ind 446, 5' UTR	80	273	194
	gi|76781922|gb|AF526381.3|_Bovine viral diarrhea virus 1 strain ZM-95, complete genome	132	328	197
	gi|32746133|gb|AY323891.1|_Bovine viral diarrhea virus 1 isolate M-MT/00 polyprotein gene, partial cds	83	278	196
	gi|14112656|gb|AF049222.2|AF049222_Bovine viral diarrhea virus strain Trangie Y546 polyprotein gene, partial cds	70	265	196
	gi|857388|dbj|D50823.1|PESU937L_Bovine viral diarrhea virus 1 genomic RNA, 5'UTR, strain: U937(CRL1593)	54	249	196
	gi|29028545|gb|AY159541.1|_Bovine viral diarrhea virus 2343/01 5' untranslated region, partial sequence	51	246	196
	gi|38260458|gb|AY363096.1|_Bovine viral diarrhea virus 1 isolate VAM_nb_02 N-terminal autoprotease gene, 5'UTR and partial cds	71	265	195
	gi|29028542|gb|AY159538.1|_Bovine viral diarrhea virus 3417/00 5' untranslated region, partial sequence	51	246	196
	gi|2780417|dbj|AB010146.1|_Bovine viral diarrhea virus 1 gene, 5'UTR, partial sequence	1	195	195
	gi|32746416|gb|AY323895.1|_Bovine viral diarrhea virus 1 isolate O-1897/00-175 nonstructural protein Npro gene, partial cds	83	274	192
	gi|28071151|dbj|AB078952.1|_Bovine viral diarrhea virus-1 gene for polyprotein, complete cds, strain:KS86-1cp	190	385	196
	gi|124441703|gb|EF210356.1|_Bovine viral diarrhea virus 1 isolate 10 polyprotein mRNA, 5' UTR	82	276	195
	gi|30908843|gb|AY278460.1|_Bovine viral diarrhea virus isolate Ind S-1166 5' UTR, partial sequence; and polyprotein gene, partial cds	80	273	194
	gi|9049956|gb|AF268278.1|AF268278_Pestivirus type 1, complete genome	188	384	197
	gi|487966|gb|U03912.1|BVU03912_Bovine viral diarrhea virus polyprotein gene, partial cds	140	335	196
	gi|299173|gb|L20926.1|BVDSEQI_Bovine viral diarrhea virus mRNA sequence with 5' flank	55	251	197
	gi|32745937|gb|AY323888.1|_Bovine viral diarrhea virus 1 isolate H-645/97 polyprotein gene, partial cds	83	278	196
	gi|38260442|gb|AY363083.1|_Bovine viral diarrhea virus 1 isolate OLG_ca1_02 N-terminal autoprotease gene, 5'UTR and partial cds	70	266	197
	gi|29028535|gb|AY159531.1|_Bovine viral diarrhea virus 561/01 5' untranslated region, partial sequence	51	246	196
	gi|32746047|gb|AY323890.1|_Bovine viral diarrhea virus 1 isolate M-573/99-15 polyprotein gene, partial cds	83	278	196
	gi|30841826|gb|AY279526.1|_Bovine viral diarrhea virus strain Osloss isolate Ind S-1170 polyprotein gene, 5' untranslated region and partial cds	80	273	194
	gi|299177|gb|L20930.1|BVDSEQM_Bovine viral diarrhea virus mRNA sequence with 5' flank	55	250	196
	gi|32745763|gb|AY323886.1|_Bovine viral diarrhea virus 1 isolate V-804/98 polyprotein gene, partial cds	83	278	196
	gi|38260445|gb|AY363085.1|_Bovine viral diarrhea virus 1 isolate OST_ca_02 N-terminal autoprotease gene, 5'UTR and partial cds	69	264	196
	gi|29028525|gb|AY159521.1|_Bovine viral diarrhea virus 3425/01 5' untranslated region, partial sequence	51	246	196
	gi|32745443|gb|AY323876.1|_Bovine viral diarrhea virus 1 isolate G-1703/99-43 nonstructural protein Npro gene, partial cds	83	276	194
	gi|29028529|gb|AY159525.1|_Bovine viral diarrhea virus 2218/01 5' untranslated region, partial sequence	51	246	196
	gi|77799881|dbj|AB111964.2|_Bovine viral diarrhea virus 839cp gene for poryprotein, partai cds, strain: 839cp	43	238	196

gi|42516769|gb|AY451338.1|_Bovine viral diarrhea virus-1 strain 1/A/00 polyprotein gene, 5'UTR and partial cds	81	276	196
gi|857387|dbj|D50822.1|PESMOLTK_Bovine viral diarrhea virus 1 genomic RNA, 5'UTR, strain: MOLT-4(CRL1582)	54	249	196
gi|29028554|gb|AY159550.1|_Bovine viral diarrhea virus 3251/01 5' untranslated region, partial sequence	51	246	196
gi|1518835|gb|U63479.1|BVU63479_Bovine viral diarrhea virus 1-CP7 polyprotein gene, complete cds	172	367	196
gi|7960753|emb|AJ133738.1|BVI133738_Bovine viral diarrhea virus complete RNA genome, isolate NADL	188	384	197
gi|857381|dbj|D50815.1|PESCV1D_Bovine viral diarrhea virus 1 genomic RNA, 5'UTR, strain: CV-1(CCL70)	54	249	196
gi|857383|dbj|D50818.1|PESHHG_Bovine viral diarrhea virus 1 genomic RNA, 5'UTR, strain: HH(JCRB0099)	54	249	196
gi|2149468|gb|U86600.1|PTU86600_Pestivirus type 1 noncytopathic genomic RNA, complete genome	188	383	196
gi|299166|gb|L20919.1|BVDSEQB_Bovine viral diarrhea virus mRNA sequence with 5' flank	54	249	196
gi|14113964|gb|AF049221.2|AF049221_Bovine viral diarrhea virus strain Bega polyprotein gene, partial cds	141	336	196
gi|124441698|gb|EF210353.1|_Bovine viral diarrhea virus 1 isolate 7 polyprotein mRNA, 5' UTR and partial cds	4	199	196
gi|29028523|gb|AY159519.1|_Bovine viral diarrhea virus 4071/00 5' untranslated region, partial sequence	51	246	196
gi|323205|gb|M31182.1|BVDCG_Bovine viral diarrhea virus 1-NADL, complete genome	188	384	197
gi|32745321|gb|AY323872.1|_Bovine viral diarrhea virus 1 isolate E-1411/00-9 nonstructural protein Npro gene, partial cds	83	274	192
gi|38260429|gb|AY363072.1|_Bovine viral diarrhea virus 1 isolate HLB_nb_02 N-terminal autoprotease gene, 5'UTR and partial cds	87	282	196
gi|61807962|gb|AY954693.1|_Bovine viral diarrhea virus 1 5' UTR and nonstructural protein Npro gene, partial cds	81	277	197
gi|299172|gb|L20925.1|BVDSEQH_Bovine viral diarrhea virus mRNA sequence with 5' flank	56	252	197
gi|29028556|gb|AY159552.1|_Bovine viral diarrhea virus 1372/01 5' untranslated region, partial sequence	53	245	193
gi|29028540|gb|AY159536.1|_Bovine viral diarrhea virus 2708/01 5' untranslated region, partial sequence	51	246	196
gi|29028534|gb|AY159530.1|_Bovine viral diarrhea virus 368/02 5' untranslated region, partial sequence	51	246	196
gi|32746263|gb|AY323893.1|_Bovine viral diarrhea virus 1 isolate U-363/99-54 nonstructural protein Npro gene, partial cds	83	274	192
gi|32746156|gb|AY323892.1|_Bovine viral diarrhea virus 1 isolate X-159/01 nonstructural protein Npro gene, partial cds	83	274	192
gi|124441689|gb|EF210348.1|_Bovine viral diarrhea virus 1 isolate 2 polyprotein mRNA, 5' UTR and partial cds	82	278	197
gi|145309047|gb|DQ088995.2|_Bovine viral diarrhea virus 1 strain Singer_Arg, complete genome	177	373	197
gi|29028553|gb|AY159549.1|_Bovine viral diarrhea virus 3336/00 5' untranslated region, partial sequence	51	246	196
gi|124441696|gb|EF210352.1|_Bovine viral diarrhea virus 1 isolate 6 polyprotein mRNA, 5' UTR and partial cds	82	277	196
gi|3786386|gb|AF039181.1|AF039181_Pestivirus type 1 strain BVDVNADL 5' untranslated region	188	384	197
gi|29028557|gb|AY159553.1|_Bovine viral diarrhea virus 2555/01 5' untranslated region, partial sequence	51	247	197
gi|29028527|gb|AY159523.1|_Bovine viral diarrhea virus 107/01 5' untranslated region, partial sequence	51	246	196
gi|436771|dbj|D26051.1|BVDD_Bovine viral diarrhea virus 1 mRNA, 5' non-coding region, strain: No. 12	78	273	196
gi|32745384|gb|AY323874.1|_Bovine viral diarrhea virus 1 isolate N-1753/00-66 nonstructural protein Npro gene, partial cds	83	276	194
gi|29028531|gb|AY159527.1|_Bovine viral diarrhea virus 4283/00 5' untranslated region, partial sequence	51	247	197
gi|29028528|gb|AY159524.1|_Bovine viral diarrhea virus 4050/00 5' untranslated region, partial sequence	51	246	196
gi|857385|dbj|D50820.1|PESMDBKI_Bovine viral diarrhea virus 1 genomic RNA, 5'UTR, strain: MDBK(CCL-22)	54	249	196
gi|40286660|gb|AY453631.1|_Bovine viral diarrhea virus Tunisia 294 isolate 6 5' UTR	6	201	196
gi|124441687|gb|EF210347.1|_Bovine viral diarrhea virus 1 isolate 1 polyprotein mRNA, 5' UTR and partial cds	82	278	197
gi|42516771|gb|AY451339.1|_Bovine viral diarrhea virus-1 strain 1/B/01 polyprotein gene, 5'UTR and partial cds	83	277	195
gi|32745344|gb|AY323873.1|_Bovine viral diarrhea virus 1 isolate F-1562/99-6 nonstructural protein Npro gene, partial cds	83	276	194
gi|124441694|gb|EF210351.1|_Bovine viral diarrhea virus 1 isolate 5 polyprotein mRNA, 5' UTR and partial cds	82	277	196
gi|299171|gb|L20924.1|BVDSEQG_Bovine viral diarrhea virus mRNA sequence with 5' flank	62	257	196
gi|299169|gb|L20922.1|BVDSEQE_Bovine viral diarrhea virus mRNA sequence with 5' flank	62	257	196
gi|299176|gb|L20929.1|BVDSEQL_Bovine viral diarrhea virus mRNA sequence with 5' flank	64	259	196
gi|32745506|gb|AY323878.1|_Bovine viral diarrhea virus 1 isolate C-1332/00-41 nonstructural protein Npro gene, partial cds	83	278	196
gi|3786383|gb|AF039178.1|AF039178_Pestivirus type 1 strain BVDVNY1 5' untranslated region	185	380	196
gi|857386|dbj|D50821.1|PESMDCKJ_Bovine viral diarrhea virus 1 genomic RNA, 5'UTR, strain: MDCK(CCL34)	54	249	196
gi|299165|gb|L20918.1|BVDSEQA_Bovine viral diarrhea virus mRNA sequence with 5'flank	50	245	196
gi|14485185|gb|AF376001.1|AF376001_Bovine viral diarrhea virus-1 strain C86 polyprotein gene, 5' UTR and partial cds	60	254	195
gi|7960755|emb|AJ133739.1|BVI133739_Bovine viral diarrhea virus complete RNA genome, non-cytopathic isolate NADL	188	384	197
gi|77799875|dbj|AB111961.2|_Bovine viral diarrhea virus 799ncp gene for poryprotein, partai cds, strain: 799ncp	43	238	196

gi|299178|gb|L20931.1|BVDSEQN_Bovine viral diarrhea virus mRNA sequence	64	258	195
gi|32745802|gb|AY323887.1|_Bovine viral diarrhea virus 1 isolate U-278/00-37 polyprotein gene, partial cds	83	278	196
gi|124441691|gb|EF210349.1|_Bovine viral diarrhea virus 1 isolate 3 polyprotein mRNA, 5' UTR and partial cds	82	278	197
gi|29028526|gb|AY159522.1|_Bovine viral diarrhea virus 832/01 5' untranslated region, partial sequence	51	246	196
gi|299179|gb|L20932.1|BVDSEQO_Bovine viral diarrhea virus sequence	64	258	195
gi|29028530|gb|AY159526.1|_Bovine viral diarrhea virus 4325/01 5' untranslated region, partial sequence	51	246	196
gi|30523235|gb|AY273154.1|_Bovine viral diarrhea virus 1 isolate Ind S-1230 Npro gene, partial cds	83	277	195
gi|29028541|gb|AY159537.1|_Bovine viral diarrhea virus 2032/01 5' untranslated region, partial sequence	51	246	196
gi|29028539|gb|AY159535.1|_Bovine viral diarrhea virus 720/02 5' untranslated region, partial sequence	50	245	196
gi|857389|dbj|D50824.1|PESVEROM_Bovine viral diarrhea virus 1 genomic RNA, 5'UTR, strain: Vero(CCL81)	54	249	196
gi|32746343|gb|AY323894.1|_Bovine viral diarrhea virus 1 isolate P-1760/99-81 nonstructural protein Npro gene, partial cds	83	274	192
gi|38260418|gb|AY363064.1|_Bovine viral diarrhea virus 1 isolate ARS_sh_93 N-terminal autoprotease gene, 5'UTR and partial cds	87	282	196
gi|3661565|gb|AF091605.1|AF091605_Bovine viral diarrhea virus strain Oregon C24V, complete genome	189	384	196
gi|857380|dbj|D50814.1|PESCRFKC_Bovine viral diarrhea virus 1 genomic RNA, 5'UTR, strain: CRFK(CCL94)	54	251	198
gi|34596504|gb|AY182136.1|AY182136S1_Pestivirus type 1 strain CP8 polyprotein gene, partial cds	64	259	196
gi|857391|dbj|D50826.1|PESWIDRO_Bovine viral diarrhea virus 1 genomic RNA, 5'UTR, strain: WiDr(JCRB0224)	54	249	196
gi|857384|dbj|D50819.1|PESMDBKH_Bovine viral diarrhea virus 1 genomic RNA, 5'UTR, strain: HeLa(CCL-2)	54	249	196
gi|29028555|gb|AY159551.1|_Bovine viral diarrhea virus 4163/00 5' untranslated region, partial sequence	53	246	194
gi|299170|gb|L20923.1|BVDSEQF_Bovine viral diarrhea virus mRNA sequence with 5' flank	54	249	196
gi|32745293|gb|AY323871.1|_Bovine viral diarrhea virus 1 isolate B-1085/00 nonstructural protein Npro gene, partial cds	83	274	192
gi|29028546|gb|AY159542.1|_Bovine viral diarrhea virus 1041/01 5' untranslated region, partial sequence	51	245	195
gi|29028558|gb|AY159554.1|_Bovine viral diarrhea virus 228/02 5' untranslated region, partial sequence	53	247	195
gi|32745964|gb|AY323889.1|_Bovine viral diarrhea virus 1 isolate M-374/00-16 polyprotein gene, partial cds	83	278	196
gi|29028544|gb|AY159540.1|_Bovine viral diarrhea virus 438/02 5' untranslated region, partial sequence	50	245	196
gi|32745688|gb|AY323884.1|_Bovine viral diarrhea virus 1 isolate M-1193/98-18 polyprotein gene, partial cds	83	278	196
gi|32745410|gb|AY323875.1|_Bovine viral diarrhea virus 1 isolate L-1753/00-21 nonstructural protein Npro gene, partial cds	83	276	194
gi|289507|gb|M96751.1|BVDPOLYPRO_Bovine viral diarrhea virus 1-SD1 polyprotein gene, complete cds	188	384	197
gi|38260422|gb|AY363067.1|_Bovine viral diarrhea virus 1 isolate BRO_ca_02 N-terminal autoprotease gene, 5'UTR and partial cds	69	264	196
gi|30908803|gb|AY278459.1|_Bovine viral diarrhea virus isolate Ind S-1222 5' UTR, partial sequence; and polyprotein gene, partial cds	80	275	196
gi|38260461|gb|AY363098.1|_Bovine viral diarrhea virus 1 isolate VID_nb_02 N-terminal autoprotease gene, 5'UTR and partial cds	69	264	196
gi|124441701|gb|EF210355.1|_Bovine viral diarrhea virus 1 isolate 9 polyprotein mRNA, 5' UTR and partial cds	82	278	197
gi|77799879|dbj|AB111963.2|_Bovine viral diarrhea virus 839ncp gene for poryprotein, partai cds, strain: 839ncp	43	238	196
gi|30692265|gb|AY279086.1|_Pestivirus type 1 isolate Ind S-1168, 5' UTR	80	273	194
gi|9836967|gb|AF220247.1|AF220247_Bovine viral diarrhea virus-1, complete genome	186	381	196
gi|30523357|gb|AY273159.1|_Bovine viral diarrhea virus 1 isolate Ind S-1210 Npro gene, partial cds	83	277	195
gi|857390|dbj|D50825.1|PESWI38N_Bovine viral diarrhea virus 1 genomic RNA, 5'UTR, strain: WI-38(CCL75)	54	249	196
gi|2789676|gb|AF041040.1|AF041040_Pestivirus type 1 polyprotein gene, complete cds	173	368	196
gi|77799877|dbj|AB111962.2|_Bovine viral diarrhea virus 799cp gene for poryprotein, partai cds, strain: 799cp	43	238	196
gi|29028522|gb|AY159518.1|_Bovine viral diarrhea virus bo2340/01 5' untranslated region, partial sequence	51	246	196
gi|118498778|gb|EF101530.1|_Bovine viral diarrhea virus 1 strain KE9, complete genome	171	366	196
gi|32745545|gb|AY323880.1|_Bovine viral diarrhea virus 1 isolate T-482/99 polyprotein gene, partial cds	83	278	196
gi|299167|gb|L20920.1|BVDSEQC_Bovine viral diarrhea virus mRNA sequence with 5' flank	64	258	195
gi|29028536|gb|AY159532.1|_Bovine viral diarrhea virus 3310/01 5' untranslated region, partial sequence	51	246	196
gi|37693100|emb|AJ585412.1|_Bovine viral diarrhea virus VEDEVAC ORF1 for polyprotein, complete genome, genomic RNA, strain VEDEVAC	185	380	196
gi|29028532|gb|AY159528.1|_Bovine viral diarrhea virus 4629/01 5' untranslated region, partial sequence	51	246	196
gi|61992163|gb|AY948435.1|_Bovine viral diarrhea virus 1 5' UTR and polyprotein gene, partial cds	10	205	196
gi|38260433|gb|AY363075.1|_Bovine viral diarrhea virus 1 isolate HOL_ca_02 N-terminal autoprotease gene, 5'UTR and partial cds	69	264	196
gi|29028538|gb|AY159534.1|_Bovine viral diarrhea virus 133/02 5' untranslated region, partial sequence	51	246	196
gi|299180|gb|L20933.1|BVDSEQP_Bovine viral diarrhea virus mRNA sequence with 5' flank	63	257	195

gi|29028543|gb|AY159539.1|_Bovine viral diarrhea virus 1946/01 5' untranslated region, partial sequence	51	246	196
gi|29028533|gb|AY159529.1|_Bovine viral diarrhea virus 4382/01 5' untranslated region, partial sequence	51	246	196
gi|28071149|dbj|AB078951.1|_Bovine viral diarrhea virus-1 gene for polyprotein, complete cds, strain:Nose	190	385	196

MALIGNANT CATARRHAL FEVER VIRUS SIGNATURES

Sig ID/Name	Verification/Cross Rxn	Accession	Start	Stop	Amp Size	Strand (F - I - R)	Amp Seq	Type
emcf_94975:	>gb|AF005370.1| Alcelaphine herpesvirus 1 L-DNA, complete sequence	AF005370.1	21141	21216	76	Plus - Plus - Minus	ATGCCAGTCACTGGCTCTCA AGAGGGGTACCAGGGTGCCA CCGTGATCAACCCCATTTCA GGATTCTACAACACCC	TAQMAN
emcf_95059:	>emb|X79895.1|AHER1DNA Alcelaphine Herpesvirus 1 DNA	X79895.1	2151	2251	101	Plus - Plus - Minus	GTTCTGGAAACTGACCAAAC AGTGTGTTCTTATGTGCACTT ATTTTGTATTTCCCTTATGCC TGCCAGAGTGCTCAATAAAA GTTACACTCAAGTGCCACT	TAQMAN
	>emb|X80112.1|AHV1DNA Alcelaphine Herpesvirus 1 DNA	X80112.1	1516	1616	101	Plus - Plus - Minus	GTTCTGGAAACTGACCAAAC AGTGTGTTCTTATGTGCACTT ATTTTGTATTTCCCTTATGCC TGCCAGAGTGCTCAATAAAA GTTACACTCAAGTGCCACT	TAQMAN
4 total sequence hits	>emb|X80691.1|AHV1GEN Alcelaphine herpesvirus 1 genomic sequence	X80691.1	1516	1616	101	Plus - Plus - Minus	GTTCTGGAAACTGACCAAAC AGTGTTCTTATGTGCACTTAT TTTGTATTTCCCTTATGCCTG CCAGAGTGCTCAATAAAAGT TACACTCAAGTGCCACT	TAQMAN
	>gb|AF005370.1| Alcelaphine herpesvirus 1 L-DNA, complete sequence	AF005370.1	75080	75178	99	Plus - Plus - Minus	CCCTGGAAGCTGTCATACAA AAAGCTTTCCTGCCAGGAGA CCCAGCTGAGGCTCTAAACA GTAGCCAGTTTTGTGAGACA ACTGCAGCCCTGGACTCTAC TGCACCTTGCAAGATATGCC AATGTTT	TAQMAN
emcf_95155:	>gb|AF005370.1| Alcelaphine herpesvirus 1 L-DNA, complete sequence	AF005370.1	106827	106953	127	Plus - Plus - Minus	TGGCCTACTTAAATGCTACT GTATCAAAACCTGTCATTAG TTTGCTTTCACTTTCCAAGAA GGTACTTAAATTTGAGCACT GTGGGGGAGAGGGTCAGTGT TTGGGGCTGATAACAGAGTT TGTAATACATCCTGCAGCTA TGGGCACTTTGTGTGTTAGT ATT	TAQMAN
emcf_95416:	>gb|U18243.1|AHU18243 Alcelaphine herpesvirus 1 putative semaphorin homolog (AHV-sema) and putative membrane antigen genes, complete cds, and major ssDNA-binding protein gene, partial cds	U18243.1	3574	3737	164	Plus - Plus - Minus	TGGCCTACTTAAATGCTACT GTATCAAAACCTGTCATTAG TTTGCTTTCACTTTCCAAGAA GGTACTTAAATTTGAGCACT GTGGGGGAGAGGGTCAGTGT TTGGGGCTGATAACAGAGTT TGTAATACATCCTGCAGCTA TGGGCACTTTGTGTGTTAGT ATT	TAQMAN
2 total sequence hits								
emcf_95476:	>gb|AF005370.1| Alcelaphine herpesvirus 1 L-DNA, complete sequence	AF005370.1	40851	40989	139	Plus - Plus - Minus	CAAAACTGGACAGATGTCTT TAGTTTGATCACATCCCACA CTGTGGGAGAATTGGCACAG ATTTTACAGACCTCAGTGGT TGACTTTGCTAGGAAGGGTA GGTGTCGCTCAGTGCATTTT AACTCACACTTTTTAACCA	TAQMAN

RINDERPEST VIRUS SIGNATURES

Sig ID/Name	Verification/Cross Rxn	Accession	Start	Stop	Amp Size	Strand (F - I - R)	Amp Seq	Type
1811628	>gb|S44819.1| phosphoprotein, C protein [rinderpest virus RV, Kabete "O", mRNA, 1651 nt]	S44819.1	396	489	94	Plus - Plus - Minus	CGGTGAAAAGGTTGAGGGAGTCGAAGATGCTGACTCTA TCCTGGTTCAATCAGGCGCTGATGATGGTGTCGAAGTCT GGGGAGGAGATGAGGAA	TAQMAN
	>emb|X68311.1|RVNPVCA Rinderpest virus mRNA for proteins N, P, V and C	X68311.1	2075	2168	94	Plus - Plus - Minus	CGGTGAAAAGGTTGAGGGAGTCGAAGATGCTGACTCTA TCCTGGTTCAATCAGGCGCTGATGATGGTGTCGAAGTCT GGGGAGGAGATGAGGAA	TAQMAN
7 total sequence hits	>emb|X98291.3|RVLGENPOL Rinderpest virus (strain Kabete O) complete genome, genomic RNA	X98291.3	2154	2247	94	Plus - Plus - Minus	CGGTGAAAAGGTTGAGGGAGTCGAAGATGCTGACTCTA TCCTGGTTCAATCAGGCGCTGATGATGGTGTCGAAGTCT GGGGAGGAGATGAGGAA	TAQMAN
	>emb|Z30697.2|RVPCMFH Rinderpest virus (RBOK) RNA for N, P, C, M, F, H, L proteins	Z30697.2	2154	2247	94	Plus - Plus - Minus	CGGTGAAAAGGTTGAGGGAGTCGAAGATGCTGACTCTA TCCTGGTTCAATCAGGCGCTGATGATGGTGTCGAAGTCT GGGGAGGAGATGAGGAA	TAQMAN
	>gb|AY948430.1| Rinderpest virus isolate 8 phosphoprotein (P) gene, partial cds	AY948430.1	410	317	94	Minus - Minus - Plus	TTCCTCATCTCCTCCCCAGAATTCGACACCATCATCAGC GCCTGATTGAACCAGGATAGAGTCAGCATCTTCGACTCC CTCAACCTTTTCACCG	TAQMAN
	>gb|AY948433.1| Rinderpest virus isolate 7 phosphoprotein (P) gene, partial cds	AY948433.1	410	317	94	Minus - Minus - Plus	TTCCTCATCTCCTCCCCAGAATTCGACACCATCATCAGC GCCTGATTGAACCAGGATAGAGTCAGCATCTTCGACTCC CTCAACCTTTTCACCG	TAQMAN
	>gb|AY948434.1| Rinderpest virus phosphoprotein (P) gene, partial cds	AY948434.1	410	317	94	Minus - Minus - Plus	TTCCTCATCTCCTCCCCAGACTTCGACACCATCATCAGC GCCTGATTGAACCAGGATAGAGTCAGCATCTTCGACTCC CTCAACCTTTTCACCG	TAQMAN
1814853	>gb|U02679.1|RVU02679 Rinderpest virus virulent Kabete O nucleocapsid protein (N) mRNA, complete cds	U02679.1	786	972	187	Plus - Plus - Minus	GGATCGCTGAAATGATCTGTGACATTGATACCTACATAG TGGAGGCAGGGTTGGCCAGTTTTATACTCACTATCAAAT TTGGTATAGAAACGATGTACCCAGCACTGGGCCTGCAT GAATTCGCCGGAGAGCTCTCCACAATCGAGTCTCTTATG AATCTGTACCAGCAAATGGGTGAACTGGCTCC	TAQMAN
	>emb|X68311.1|RVNPVCA Rinderpest virus mRNA for proteins N, P, V and C	X68311.1	774	960	187	Plus - Plus - Minus	GGATCGCTGAAATGATCTGTGACATTGATACCTACATAG TGGAGGCAGGGTTGGCCAGTTTTATACTCACTATCAAAT TTGGTATAGAAACGATGTACCCAGCACTGGGCCTGCAT GAATTCGCCGGAGAGCTCTCCACAATCGAGTCTCTTATG AATCTGTACCAGCAAATGGGTGAACTGGCTCC	TAQMAN
7 total sequence hits	>emb|X98291.3|RVLGENPOL Rinderpest virus (strain Kabete O) complete genome, genomic RNA	X98291.3	853	1039	187	Plus - Plus - Minus	GGATCGCTGAAATGATCTGTGACATTGATACCTACATAG TGGAGGCAGGGTTGGCCAGTTTTATACTCACTATCAAAT TTGGTATAGAAACGATGTACCCAGCACTGGGCCTGCAT GAATTCGCCGGAGAGCTCTCCACAATCGAGTCTCTTATG AATCTGTACCAGCAAATGGGTGAACTGGCTCC	TAQMAN
	>emb|Z30697.2|RVPCMFH Rinderpest virus (RBOK) RNA for N, P, C, M, F, H, L proteins	Z30697.2	853	1039	187	Plus - Plus - Minus	GGATCGCTGAAATGATCTGTGACATTGATACCTACATAG TGGAGGCAGGGTTGGCCAGTTTTATACTCACTATCAAAT TTGGTATAGAAACGATGTACCCAGCACTGGGCCTGCAT GAATTCGCCGGAGAGCTCTCCACAATCGAGTCTCTTATG AATCTGTACCAGCAAATGGGTGAACTGGCTCC	TAQMAN
	>gb|EF186057.1| Rinderpest virus isolate Egypt/84 nucleoprotein (N) gene, complete cds	EF186057.1	746	932	187	Plus - Plus - Minus	GGATCGCTGAAATGATCTGTGACATTGATACATACATAG TGGAGGCAGGGTTGGCCAGTTTCATACTAACTATTAAAT TCGGTATAGAAACTATGTACCCAGCACTGGGCCTGCATG AATTCGCCGGAGAGCTCTCCACGATAGAGTCTCTTATGA ATCTGTACCAACAAATGGGTGAACTGGCTCC	TAQMAN
	>gb|EF186061.1| Rinderpest virus isolate Buffalo nucleoprotein (N) gene, complete cds	EF186061.1	746	932	187	Plus - Plus - Minus	GGATCGCTGAAATGATCTGTGACATTGATACATACATAG TGGAGGCAGGGTTGGCCAGTTTCATACTAACTATTAAAT TCGGTATAGAAACTATGTACCCAGCACTGGGCCTGCATG AATTCGCCGGAGAGCTCTCCACGATAGAGTCTCTTATGA ATCTGTACCAACAAATGGGTGAACTGGCTCC	TAQMAN
	>gb|EF186062.1| Rinderpest virus isolate Sokoto nucleoprotein (N) gene, complete cds	EF186062.1	746	932	187	Plus - Plus - Minus	GGATCGCTGAAATGATCTGTGATATTGATACTTACATAG TGGAGGCAGGGTTGGCCAGCTTTATACTCACTATCAAAT TTGGTATAGAAACTATGTATCCAGCGCTGGGCCTTCATG AATTCGCCGGGGAACTCTCCACAATAGAGTCTCTTATGA ATCTGTACCAGCAAATGGGTGAACTGGCTCC	TAQMAN
1814855	>emb|X98291.3|RVLGENPOL Rinderpest virus (strain Kabete O) complete genome, genomic RNA	X98291.3	12750	12947	198	Plus - Minus - Minus	TGCATCTTATGTGACTTTGGTTCAGCCAATTATGGTTGG TTTTTTGTACCATCGAACTGTCAGTTGGATGACATAGAT AGAGAGACGTCAGCACTCAGGGTCCCCTACATCGGATC GACAACAGATGAGAGGACTGATATGAAGCTCGCATTTG TTAAGTCACCCAGTCGAACCCTGCGGTCAGCTGTGCGGA TAGCC	TAQMAN
	>emb|Z30697.2|RVPCMFH Rinderpest virus (RBOK) RNA for N, P, C, M, F, H, L proteins	Z30697.2	12750	12947	198	Plus - Minus - Minus	TGCATCTTATGTGACTTTGGTTCAGCCAATTATGGTTGG TTTTTTGTACCATCGAACTGTCAGTTGGATGACATAGAT AGAGAGACGTCAGCACTCAGGGTCCCCTACATCGGATC GACAACAGATGAGAGGACTGATATGAAGCTCGCATTTG TTAAGTCACCCAGTCAATCCCTGCGGTCAGCTGTGCGGA TAGCC	TAQMAN
3 total sequence hits	>emb|Z30698.1|RVLPROT Rinderpest virus (RBOK) RNA for RNA polymerase (L) protein	Z30698.1	3554	3751	198	Plus - Minus - Minus	TGCATCTTATGTGACTTTGGTTCAGCCAATTATGGTTGG TTTTTTGTACCATCGAACTGTCAGTTGGATGACATAGAT AGAGAGACGTCAGCACTCAGGGTCCCCTACATCGGATC GACAACAGATGAGAGGACTGATATGAAGCTCGCATTTG TTAAGTCACCCAGTCAATCCCTGCGGTCAGCTGTGCGGA TAGCC	TAQMAN
1814856	>emb|X98291.3|RVLGENPOL Rinderpest virus (strain Kabete O) complete genome, genomic RNA	X98291.3	13756	13870	115	Plus - Minus - Minus	AACTCCTGACCTCATTCCTTGCAAGGATGAGTAAGAGCG TATTCAAGGTCTTTGTGAATGCACTGAGCCACCCCAAGA TTTACAGGAAGTTCTGGCATAGTGGGATTATAGAGCC	TAQMAN
	>emb|Z30697.2|RVPCMFH Rinderpest virus (RBOK) RNA for N, P, C, M, F, H, L proteins	Z30697.2	13756	13870	115	Plus - Minus - Minus	AACTCCTGACCTCATTCCTTGCAAGGATGAGTAAGAGCG TATTCAAGGTCTTTGTGAATGCACTGAGCCACCCCAAGA TTTACAGGAAGTTCTGGCATAGTGGGATTATAGAGCC	TAQMAN
3 total sequence hits	>emb|Z30698.1|RVLPROT Rinderpest virus (RBOK) RNA for RNA polymerase (L) protein	Z30698.1	4560	4674	115	Plus - Minus - Minus	AACTCCTGACCTCATTCCTTGCAAGGATGAGTAAGAGCG TATTCAAGGTCTTTGTGAATGCACTGAGCCACCCCAAGA TTTACAGGAAGTTCTGGCATAGTGGGATTATAGAGCC	TAQMAN
1814893	>gb|U02679.1|RVU02679 Rinderpest virus virulent Kabete O nucleocapsid protein (N) mRNA, complete cds	U02679.1	776	1014	239	Plus - Plus - Minus	AATAAACCAAGGATCGCTGAAATGATCTGTGACATTGA TACCTACATAGTGGAGGCAGGGTTGGCCAGTTTTATACT CACTATCAAATTTGGTATAGAAACGATGTACCCAGCACT GGGCCTGCATGAATTCGCCGGAGAGCTCTCCACAATCG AGTCTCTTATGAATCTGTACCAGCAAATGGGTGAACTGG CTCCTTATATGGTGATCTTAGAGAACTCAATCCAGAACA AGTTCAG	TAQMAN
	>emb|X68311.1|RVNPVCA Rinderpest virus mRNA for proteins N, P, V and C	X68311.1	764	1002	239	Plus - Plus - Minus	AATAAACCAAGGATCGCTGAAATGATCTGTGACATTGA TACCTACATAGTGGAGGCAGGGTTGGCCAGTTTTATACT CACTATCAAATTTGGTATAGAAACGATGTACCCAGCACT GGGCCTGCATGAATTCGCCGGAGAGCTCTCCACAATCG AGTCTCTTATGAATCTGTACCAGCAAATGGGTGAACTGG CTCCTTATATGGTGATCTTAGAGAACTCAATCCAGAACA AGTTCAG	TAQMAN
7 total sequence hits	>emb|X98291.3|RVLGENPOL Rinderpest virus (strain Kabete O) complete genome, genomic RNA	X98291.3	843	1081	239	Plus - Plus - Minus	AATAAACCAAGGATCGCTGAAATGATCTGTGACATTGA TACCTACATAGTGGAGGCAGGGTTGGCCAGTTTTATACT CACTATCAAATTTGGTATAGAAACGATGTACCCAGCACT GGGCCTGCATGAATTCGCCGGAGAGCTCTCCACAATCG	TAQMAN

	>emb|Z30697.2|RVPCMFH Rinderpest virus (RBOK) RNA for N, P, C, M, F, H, L proteins	Z30697.2	843	1081	239	Plus - Plus - Minus	AGTCTCTTATGAATCTGTACCAGCAAATGGGTGAACTGG CTCCTTATATGGTGATCTTAGAGAACTCAATCCAGAACA AGTTCAG AATAAACCAAGGATCGCTGAAATGATCTGTGACATTGA TACCTACATAGTGGAGGCAGGGTTGGCCAGTTTTATACT CACTATCAAATTTGGTATAGAAACGATGTACCCAGCACT GGGCCTGCATGAATTCGCCGGAGAGCTCTCCACAATCG AGTCTCTTATGAATCTGTACCAGCAAATGGGTGAACTGG CTCCTTATATGGTGATCTTAGAGAACTCAATCCAGAACA AGTTCAG AATAAACCGAGGATCGCTGAAATGATCTGTGACATTGA TACATACATAGTGGAGGCAGGGTTGGCCAGTTTCATACT AACTATTAAATTCGGTATAGAAACTATGTACCCAGCACT GGGCCTGCATGAATTCGCCGGAGAGCTCTCCACGATAG AGTCTCTTATGAATCTGTACCAACAAATGGGTGAACTGG CTCCTTACAAGGTGATCTTAGAAAACTCAATCCAGAACA AATTCAG AAACCGAGGATCGCCGAAATGATCTGTGACATTGATAC CTACATAGTGGAGGCAGGATTAGCCAGTTTTATACTCAC TATCAAATTTGGTATAGAAACTATGTACCCAGCACTGGG CCTGCATGAATTCGCCGGAGAGCTCTCCACAATAGAGTC TCTTATGAATATGTACCAACAAATGGGTGAACTGGCTCC TTATATGGTGATCTTAGAGAACTCAATCCAGAACAAATT CAG AATAAACCGAGGATCGCTGAAATGATCTGTGACATTGA TACATACATAGTGGAGGCAGGGTTGGCCAGTTTCATACT AACTATTAAATTCGGTATAGAAACTATGTACCCAGCACT GGGCCTGCATGAATTCGCCGGAGAGCTCTCCACGATAG AGTCTCTTATGAATCTGTACCAACAAATGGGTGAACTGG CTCCTTACATGGTGATCTTAGAAAACTCAATCCAGAACA AATTCAG	TAQMAN
	>gb|EF186057.1| Rinderpest virus isolate Egypt/84 nucleoprotein (N) gene, complete cds	EF186057.1	736	974	239	Plus - Plus - Minus		TAQMAN
	>gb|EF186058.1| Rinderpest virus isolate RBT1 nucleoprotein (N) gene, complete cds	EF186058.1	739	974	236	Plus - Plus - Minus		TAQMAN
	>gb|EF186061.1| Rinderpest virus isolate Buffalo nucleoprotein (N) gene, complete cds	EF186061.1	736	974	239	Plus - Plus - Minus		TAQMAN

VESICULAR STOMATITIS VIRUS SIGNATURES

Sig ID/Name	Verification/Cross Rxn	Accession	Start	Stop	Amp Size	Strand (F - I - R)	Amp Seq	Type
sig_candidate_1798941	>gb|AF473864.1| Vesicular stomatitis Indiana virus strain 98COE, complete genome	AF473864.1	439	543	105	Plus - Minus - Minus	AGAACCAGCGCAGATGACA AATGGCTGCCTTTGTATCTAC TTGGCTTATACAGAGTGGGC AGAACACAAATGCCTGAATA CAGAAAAAAGCTCATGGATG GGCTG	TAQMAN
5 total sequence hits	>gb|AF473865.1| Vesicular stomatitis Indiana virus strain 85CLB, complete genome	AF473865.1	439	543	105	Plus - Minus - Minus	AGAACCAGCGCAGATGACA AATGGCTACCTTTGTATCTAC TTGGCTTATACAGAGTGGGC AGAACACAAATGCCTGAATA CAGAAAAAAGCTCATGGATG GGCTG	TAQMAN
	>gb|AF473866.1| Vesicular stomatitis Indiana virus strain 94GUB, complete genome	AF473866.1	439	543	105	Plus - Minus - Minus	AGAACCAGCGCAGATGACA AATGGTTGCCTTTGTATCTAC TTGGCTTATACAGAGTGGGC AGAACACAAATGCCTGAATA CAGAAAAAAGCTCATGGATG GGCTG	TAQMAN
	>gb|J02428.1|VSVCG Vesicular stomatitis Indiana virus, complete genome	J02428.1	439	543	105	Plus - Minus - Minus	AGAACCAGAGCAGATGACA AATGGTTGCCTTTGTATCTAC TTGGCTTATACAGAGTGGGC AGAACACAAATGCCTGAATA CAGAAAAAAGCTCATGGATG GGCTG	TAQMAN
	>gb|M15213.1|VSVGINJ Vesicular stomatitis Indiana virus nucleocapsid protein (N) gene, complete cds	M15213.1	389	493	105	Plus - Minus - Minus		TAQMAN
sig_candidate_1798942	>gb|AF473864.1| Vesicular stomatitis Indiana virus strain 98COE, complete genome	AF473864.1	842	1015	174	Plus - Plus - Minus	AAATGATGCTTCCAGGCCAA GAAATTGACAAGGCCGATTC ATACATGCCTTATTTGATCG ACTTTGGATTGTCTTCTAAGT CTCCATATTCTTCCGTCAAAA ACCCTGCCTTCCACTTCTGGG GGCAATTGACAGCTCTTCTG CTCAGATCTACTAGAGCAAG GAATGCCCGAC	TAQMAN
6 total sequence hits	>gb|AF473865.1| Vesicular stomatitis Indiana virus strain 85CLB, complete genome	AF473865.1	842	1015	174	Plus - Plus - Minus	AAATGATGCTTCCAGGCCAA GAAATTGACAAGGCCGATTC ATACATGCCTTATTTGATCG ACTTTGGATTGTCTTCTAAGT CTCCATATTCTTCCGTCAAAA ACCCTGCCTTCCACTTCTGGG GGCAATTAACAGCTCTTCTG CTCAGATCCACCAGAGCAAG GAATGCCCGAC	TAQMAN
	>gb|AF473866.1| Vesicular stomatitis Indiana virus strain 94GUB, complete genome	AF473866.1	842	1015	174	Plus - Plus - Minus	AAATGATGCTTCCAGGCCAA GAAATTGACAAGGCCGATTC ATACATGCCTTATTTAATCG ACTTTGGATTGTCTTCCAAGT CTCCATATTCTTCTGTCAAAA ACCCTGCCTTTCACTTCTGGG GGCAATTGACAGCTCTTCTG CTCAGATCCACCAGAGCAAG GAATGCCCGAC	TAQMAN

	>gb|J02428.1|VSVCG Vesicular stomatitis Indiana virus, complete genome	J02428.1	842	1015	174	Plus - Plus - Minus	AAATGATGCTTCCAGGCCAA GAAATTGACAAGGCCGATTC ATACATGCCTTATTTGATCG ACTTTGGATTGTCTTCTAAGT CTCCATATTCTTCCGTCAAAA ACCCTGCCTTCCACTTCTGGG GGCAATTGACAGCTCTTCTG CTCAGATCCACCAGAGCAAG GAATGCCCGAC	TAQMAN
	>gb|U12967.1|VSU12967 Vesicular stomatitis Indiana virus nucleocapsid (N) and phosphoprotein (P) genes, complete cds	U12967.1	792	965	174	Plus - Plus - Minus	AAATGATGCTTCCAGGCCAA GAAATTGACAAGGCCGATTC ATACATGCCTTATTTGATCG ACTTTGGATTGTCTTCTAAGT CTCCATATTCTTCCGTCAAAA ACCCTGCCTTCCACTTCTGGG GGCAATTGACAGCCCTTCTG CTCAGATCCACCAGAGCAAG GAATGCCCGAC	TAQMAN
	>gb|U13898.1|VSU13898 Vesicular stomatitis Indiana virus strain tsW16B nucleocapsid (N) and phosphoprotein (P) genes, complete cds	U13898.1	792	965	174	Plus - Plus - Minus	AAATGATGCTTCCAGGCCAA GAAATTGACAAGGCCGATTC ATACATGCCTTATTTGATCG ACTTAGGATTGTCTTCTAAGT CTCCATATTCTTCCGTCAAAA ACCCTGCCTTCCACTTCTGGG GGCAATTGACAGCTCTTCTG CTCAGATCCACCAGAGCAAG GAATGCCCGAC	TAQMAN
[redacted]								
sig_candidate_1798943	>gb|U13898.1|VSU13898 Vesicular stomatitis Indiana virus strain tsW16B nucleocapsid (N) and phosphoprotein (P) genes, complete cds	U13898.1	1119	1277	159	Plus - Plus - Minus	CGCCACAAGGCAGAGATGTG GTCGAATGGCTCGGATGGTT TGAAGATCAAAACAGAAAA CCGACTCCTGATATGATGCA GTATGCGAAACGAGCAGTCA TGTCACTGCAAGGCCTAAGA GAGAAGACAATTGGCAAGTA TGCTAAGTCAGAATTTGACA	TAQMAN
	>gb|AF473864.1| Vesicular stomatitis Indiana virus strain 98COE, complete genome	AF473864.1	1169	1327	159	Plus - Plus - Minus	CGCCACAAGGCAGAGATGTG GTCGAATGGCTAGGATGGTT TGAAGATCAAAACAGAAAA CCGACTCCTGATATGATGCA GTATGCAAAACGAGCAGTCA TGTCATTGCAAGGCCTGAGA GAGAAGACAATTGGCAAGTA TGCTAAGTCAGAATTTGACA	TAQMAN
7 total sequence hits	>gb|AF473865.1| Vesicular stomatitis Indiana virus strain 85CLB, complete genome	AF473865.1	1169	1327	159	Plus - Plus - Minus	CGCCACAAGGCAGAGATGTG GTCGAATGGCTCGGATGGTT TGAAGATCAAAACAGAAAA CCGACTCCTGATATGATGCA GTATGCGAAAAGAGCAGTCA TGTCATTGCAAGGCCTGAGG GAGAAAACAATTGGCAAGTA TGCTAAGTCAGAATTTGACA	TAQMAN
	>gb|AF473866.1| Vesicular stomatitis Indiana virus strain 94GUB, complete genome	AF473866.1	1169	1327	159	Plus - Plus - Minus	CGCCACAAGGCAGAGATGTA GTCGAATGGCTCGGATGGTT TGAAGATCAGAACAGAAAA CCGACTCCTGATATGATGCA GTATGCGAAAAGAGCAGTCA TGTCATTGCAAGGCCTGAGA GAGAAGACAATCGGCAAGT ATGCTAAGTCAGAATTTGAC A	TAQMAN
	>gb|J02428.1|VSVCG Vesicular stomatitis Indiana virus, complete genome	J02428.1	1169	1327	159	Plus - Plus - Minus	CGCCACAAGGCAGAGATGTG GTCGAATGGCTCGGATGGTT TGAAGATCAAAACAGAAAA CCGACTCCTGATATGATGCA GTATGCGAAAAGAGCAGTCA TGTCACTGCAAGGCCTAAGA GAGAAGACAATTGGCAAGTA TGCTAAGTCAGAATTTGACA	TAQMAN
	>gb|M15213.1|VSVGINJ Vesicular stomatitis Indiana virus nucleocapsid protein (N) gene, complete cds	M15213.1	1119	1277	159	Plus - Plus - Minus	CGCCACAAGGCAGAGATGTG GTCGAATGGCTCGGATGGTT TGAAGATCAAAACAGAAAA CCGACTCCTGATATGATGCA GTATGCGAAACGAGCAGTCA TGTCACTGCAAGGCCTAAGA GAGAAGACAATTGGCAAGTA TGCTAAGTCAGAATTTGACA	TAQMAN
	>gb|U12967.1|VSU12967 Vesicular stomatitis Indiana virus nucleocapsid (N) and phosphoprotein (P) genes, complete cds	U12967.1	1119	1277	159	Plus - Plus - Minus	CGCCACAAGGCAGAGATGTG GTCGAATGGCTCGGATGGTT TGAAGATCAAAACAGAAAA CCGACTCCTGATATGATGCA GTATGCGAAACGAGCAGTCA TGTCACTGCAAGGCCTAAGA GAGAAGACAATTGGCAAGTA TGCTAAGTCAGAATTTGACA	TAQMAN
[redacted]								
sig_candidate_1798944	>emb|X00939.1|RHVSVPOL Vesicular Stomatitis Virus gene for polymerase (L)	X00939.1	1259	1449	191	Plus - Plus - Minus	CCTCATGATCATCCCTTTAAA AGTCATGTTAAAGAAAATAC ATGGCCCACAGCTGCTCAAG TTCAAGATTTTGGAGATAAA TGGCATGAACTTCCGCTGAT TAAATGTTTTGAAATACCCG ACTTACTAGACCCATCGATA ATATACTCTCACAAAAGTCA TTCAATGAATAGGTCAGAGG TGTTGAAACA	TAQMAN
	>gb|AF473864.1| Vesicular stomatitis Indiana virus strain 98COE, complete genome	AF473864.1	5982	6172	191	Plus - Plus - Minus	CCTCATGATCATCCCTTTAAA AGTCATGTTAAAGAAAATAC ATGGCCCACAGCTGCCCAAG TTCAAGATTTTGGAGATAAA TGGCATGAACTTCCACTGAT TAAATGTTTTGAAATACCAG ACTTACTAGACCCATCGATA ATATACTCTGACAAAAGTCA TTCAATGAATAGGTCAGAGG TGTTGAAACA	TAQMAN

6 total sequence hits	>gb|AF473865.1| Vesicular stomatitis Indiana virus strain 85CLB, complete genome	AF473865.1	5975	6165	191	Plus - Plus - Minus	CCTCATGATCATCCCTTTAAA AGTCATGTTAAAGAAAATAC ATGGCCCACAGCTGCTCAAG TTCAAGATTTTGGAGATAAA TGGCATGAACTTCCACTGAT TAAATGTTTTGAAATACCCG ACTTACTGGACCCATCGATA ATATACTCTGACAAAAGTCA TTCAATGAATAGGTCAGAGG TGTTGAAACA	TAQMAN
	>gb|AF473866.1| Vesicular stomatitis Indiana virus strain 94GUB, complete genome	AF473866.1	6156	6346	191	Plus - Plus - Minus	CCTCATGATCATCCCTTTAAA AGTCATGTTAAAGAAAATAC ATGGCCTACTGCCGCTCAAA TTCAAGATTTTGGAGATAAA TGGCATGAACTTCCCCTGAT CAAATGTTTTGAAATACCCG ACTTATTAGACCCATCAATA ATATACTCTGACAAAAGTCA TTCGATGAATAGGTCAGAGG TGTTGAAACA	TAQMAN
	>gb|J02428.1|VSVCG Vesicular stomatitis Indiana virus, complete genome	J02428.1	5981	6171	191	Plus - Plus - Minus	CCTCATGATCATCCCTTTAAA AGTCATGTTAAAGAAAATAC ATGGCCCACAGCTGCTCAAG TTCAAGATTTTGGAGATAAA TGGCATGAACTTCCGCTGAT TAAATGTTTTGAAATACCCG ACTTACTAGACCCATCGATA ATATACTCTGACAAAAGTCA TTCAATGAATAGGTCAGAGG TGTTGAAACA	TAQMAN
	>gb|K02378.1|VSVLMS Vesicular stomatitis Indiana virus polymerase (L) gene, complete cds	K02378.1	1259	1449	191	Plus - Plus - Minus	CCTCATGATCATCCCTTTAAA AGTCATGTTAAAGAAAATAC ATGGCCCACAGCTGCTCAAG TTCAAGATTTTGGAGATAAA TGGCATGAACTTCCGCTGAT TAAATGTTTTGAAATACCCG ACTTACTAGACCCATCGATA ATATACTCTGACAAAAGTCA TTCAATGAATAGGTCAGAGG TGTTGAAACA	TAQMAN
sig_candidate_1798945	>emb|X00939.1|RHVSVPOL Vesicular Stomatitis Virus gene for polymerase (L)	X00939.1	1966	2157	192	Plus - Plus - Minus	ACCAGACTTGATGCGTGTTC ACAACAACACACTGATCAAT TCAACCTCCCAACCAGTTTG TTGGCAAGGACAAGAGGGTG GACTGGAAGGTCTACGGCAA AAAGGATGGACTATCCTCAA TCTACTGGTTATTCAAAGAG AGGCTAAAATCAGAAACACT GCTGTCAAAGTCTTGGCACA AGGTGATAATCA	TAQMAN
6 total sequence hits	>gb|AF473864.1| Vesicular stomatitis Indiana virus strain 98COE, complete genome	AF473864.1	6689	6880	192	Plus - Plus - Minus	ACCAGACTTGATGCGTGTTC AAAATAACACACTGATCAAT TCAACCTCCCAACGAGTTTG TTGGCAAGGACAAGAGGGTG GACTGGAAGGTCTACGGCAA AAAGGATGGAGTATCCTCAA TCTACTGGTTATTCAAAGAG AGGCTAAAATCAGAAACACT GCTGTCAAAGTCTTGGCACA AGGTGATAATCA	TAQMAN
	>gb|AF473865.1| Vesicular stomatitis Indiana virus strain 85CLB, complete genome	AF473865.1	6682	6873	192	Plus - Plus - Minus	ACCAGACTTGATGCGTGTTC ACAACAACACACTGGTCAAT TCAACCTCCCAACGAGTTTG TTGGCAAGGACAGGAAGGTG GACTAGAAGGTCTACGGCAA AAAGGATGGAGTATCCTTAA TCTACTGGTTATTCAAAGAG AGGCTAAAATCAGAAACACT GCTGTCAAAGTCTTGGCACA AGGTGATAATCA	TAQMAN
	>gb|AF473866.1| Vesicular stomatitis Indiana virus strain 94GUB, complete genome	AF473866.1	6863	7054	192	Plus - Plus - Minus	ACCAGACTTGATGCGTGTTC ATAACAACACACTGGTCAAT TCAACCTCCCAACGAGTTTG TTGGCAAGGACAAGAAGGTG GACTGGAAGGTCTACGCCAA AAAGGATGGAGTATCCTCAA TCTACTGGTTATTCAAAGAG AGGCTAAAATCAGAAACACT GCTGTTAAAGTCTTGGCACA AGGTGATAATCA	TAQMAN
	>gb|J02428.1|VSVCG Vesicular stomatitis Indiana virus, complete genome	J02428.1	6688	6879	192	Plus - Plus - Minus	ACCAGACTTGATGCGTGTTC ACAACAACACACTGATCAAT TCAACCTCCCAACGAGTTTG TTGGCAAGGACAAGAGGGTG GACTGGAAGGTCTACGGCAA AAAGGATGGACTATCCTCAA TCTACTGGTTATTCAAAGAG AGGCTAAAATCAGAAACACT GCTGTCAAAGTCTTGGCACA AGGTGATAATCA	TAQMAN
	>gb|K02378.1|VSVLMS Vesicular stomatitis Indiana virus polymerase (L) gene, complete cds	K02378.1	1966	2157	192	Plus - Plus - Minus	ACCAGACTTGATGCGTGTTC ACAACAACACACTGATCAAT TCAACCTCCCAACGAGTTTG TTGGCAAGGACAAGAGGGTG GACTGGAAGGTCTACGGCAA AAAGGATGGACTATCCTCAA TCTACTGGTTATTCAAAGAG AGGCTAAAATCAGAAACACT GCTGTCAAAGTCTTGGCACA AGGTGATAATCA	TAQMAN
sig_candidate_1798946	>emb|X00939.1|RHVSVPOL Vesicular Stomatitis Virus gene for polymerase (L)	X00939.1	2348	2428	81	Plus - Minus - Minus	CCGATTTCCGTGGAGTGAT TAGAGGGTTAGAGACCAAGA GATGGTCACGAGTGACTTGT GTCACCAATGACCAAATACC C	TAQMAN

6 total sequence hits	>gb|AF473864.1| Vesicular stomatitis Indiana virus strain 98COE, complete genome	AF473864.1	7071	7151	81	Plus - Minus - Minus	CCGATTTTCCGTGGAGTGAT TAGAGGGTTAGAGACCAAGA GATGGTCACGAGTGACTTGT GTCACCAATGACCAAATACC C	TAQMAN
	>gb|AF473865.1| Vesicular stomatitis Indiana virus strain 85CLB, complete genome	AF473865.1	7064	7144	81	Plus - Minus - Minus	CCGATTTTCCGTGGAGTGAT TAGAGGGTTAGAGACCAAGA GATGGTCACGAGTGACTTGT GTCACCAATGACCAAATACC C	TAQMAN
	>gb|AF473866.1| Vesicular stomatitis Indiana virus strain 94GUB, complete genome	AF473866.1	7245	7325	81	Plus - Minus - Minus	CCGATTTTCCGTGGAGTGAT TAGAGGGTTAGAGACCAAGA GATGGTCACGAGTAACTTGT GTCACCAATGACCAAATACC C	TAQMAN
	>gb|J02428.1|VSVCG Vesicular stomatitis Indiana virus, complete genome	J02428.1	7070	7150	81	Plus - Minus - Minus	CCGATTTTCCGTGGAGTGAT TAGAGGGTTAGAGACCAAGA GATGGTCACGAGTGACTTGT GTCACCAATGACCAAATACC C	TAQMAN
	>gb|K02378.1|VSVLMS Vesicular stomatitis Indiana virus polymerase (L) gene, complete cds	K02378.1	2348	2428	81	Plus - Minus - Minus	CCGATTTTCCGTGGAGTGAT TAGAGGGTTAGAGACCAAGA GATGGTCACGAGTGACTTGT GTCACCAATGACCAAATACC C	TAQMAN
sig_candidate_1798947	>emb|X00939.1|RHVSVPOL Vesicular Stomatitis Virus gene for polymerase (L)	X00939.1	2494	2666	173	Plus - Minus - Minus	CCCAATCAATGCCATGATAC AGTACAATTATTTTGGGACA TTTGCTAGACTCTTGTTGATG ATGCATGATCCTGCTCTTCGT CAATCATTGTATGAAGTTCA AGATAAGATACCGGGCTTGC ACAGTTCTACTTTCAAATAC GCCATGTTGTATTTGGACCCT TCCATTGGAG	TAQMAN
	>gb|AF473864.1| Vesicular stomatitis Indiana virus strain 98COE, complete genome	AF473864.1	7217	7389	173	Plus - Minus - Minus	CCCAATCAATGCCATGATAC AGTACAATTATTTTGGGACA TTTGCTAGACTCTTGTTGATG ATGCATGATCCTGCTCTTCGT CAATCATTGTATGAAGTTCA AGATAAGATACCGGGCTTGC ACAGTTCTACTTTCAAATAC GCCATGTTGTATTTGGACCCT TCCATTGGAG	TAQMAN
6 total sequence hits	>gb|AF473865.1| Vesicular stomatitis Indiana virus strain 85CLB, complete genome	AF473865.1	7210	7382	173	Plus - Minus - Minus	CCCAATCAATGCCATGATAC AGTACAATTACTTTGGGACA TTTGCTAGACTCTTGTTGATG ATGCATGATCCTGCTCTTCGT CAATCATTGTATGAGGTTCA AGATAAGATACCGGGCTTGC ACAGTTCTACTTTCAAATAC GCCATGTTGTATTTGGACCCT TCCATTGGAG	TAQMAN
	>gb|AF473866.1| Vesicular stomatitis Indiana virus strain 94GUB, complete genome	AF473866.1	7391	7563	173	Plus - Minus - Minus	CCCAATCAATGCCATGATAC AATACAATTATTTCGGGACA TTTGCTCGACTCTTGTTGATG ATGCATGATCCAGCTCTTCG TCAATCATTGTATGAAGTTC AAGACAAGATACCGGGCTTG CACAGTTCTACTTTCAAATAT GCAATGTTGTATTTGGACCC TTCCATTGGAG	TAQMAN
	>gb|J02428.1|VSVCG Vesicular stomatitis Indiana virus, complete genome	J02428.1	7216	7388	173	Plus - Minus - Minus	CCCAATCAATGCCATGATAC AGTACAATTATTTTGGGACA TTTGCTAGACTCTTGTTGATG ATGCATGATCCTGCTCTTCGT CAATCATTGTATGAAGTTCA AGATAAGATACCGGGCTTGC ACAGTTCTACTTTCAAATAC GCCATGTTGTATTTGGACCCT TCCATTGGAG	TAQMAN
	>gb|K02378.1|VSVLMS Vesicular stomatitis Indiana virus polymerase (L) gene, complete cds	K02378.1	2494	2666	173	Plus - Minus - Minus	CCCAATCAATGCCATGATAC AGTACAATTATTTTGGGACA TTTGCTAGACTCTTGTTGATG ATGCATGATCCTGCTCTTCGT CAATCATTGTATGAAGTTCA AGATAAGATACCGGGCTTGC ACAGTTCTACTTTCAAATAC GCCATGTTGTATTTGGACCCT TCCATTGGAG	TAQMAN
sig_candidate_1798948	>gb|K02378.1|VSVLMS Vesicular stomatitis Indiana virus polymerase (L) gene, complete cds	K02378.1	4147	4319	173	Plus - Minus - Minus	TCATGCCGAGGACAGTTCTC TATTTCCTCTATCTATACAAG GTCGTATTAGAGGTCGAGGT TTCTTAAAAGGGTTGCTAGA CGGATTAATGAGAGCAAGTT GCTGCCAAGTAATACACCGG AGAAGTCTGGCTCATTTGAA GAGGCCGGCCAACGCAGTGT ACGGAGGTTTGA	TAQMAN
	>emb|X00939.1|RHVSVPOL Vesicular Stomatitis Virus gene for polymerase (L)	X00939.1	4147	4319	173	Plus - Minus - Minus	TCATGCCGAGGACAGTTCTC TATTTCCTCTATCTATACAAG GTCGTATTAGAGGTCGAGGT TTCTTAAAAGGGTTGCTAGA CGGATTAATGAGAGCAAGTT GCTGCCAAGTAATACACCGG AGAAGTCTGGCTCATTTGAA GAGGCCCGCCAACGCAGTGT ACGGAGGTTTGA	TAQMAN
7 total sequence hits	>gb|AF473864.1| Vesicular stomatitis Indiana virus strain 98COE, complete genome	AF473864.1	8870	9042	173	Plus - Minus - Minus	TCATGCCGAGGACAGTTCTC TATTTCCTCTATCTATACAAA GTCGTATTAGAGGTCGAGGT TTCTTAAAAGGGTTGCTAGA CGGATTAATGAGAGCAAGTT GCTGCCAAGTAATACACCGA AGAAGTCTGGCTCATTTGAA GAGGCCGGCCAACGCAGTGT	TAQMAN

							ACGGAGGTTTGA	
	>gb|AF473865.1| Vesicular stomatitis Indiana virus strain 85CLB, complete genome	AF473865.1	8863	9035	173	Plus - Minus - Minus	TCATGCCGAGGACAGTTCTC TATTTCCTCTATCTATACAAA GTCGTATTAGAGGTCGAGGT TTCTTGAAAGGGTTGCTAGA CGGACTAATGAGAGCCAGTT GCTGCCAAGTGATACATAGA AGAAGTCTGGCTCATTTGAA GAGGCCGGCAAACGCAGTGT ACGGAGGTTTGA	TAQMAN
	>gb|AF473866.1| Vesicular stomatitis Indiana virus strain 94GUB, complete genome	AF473866.1	9044	9216	173	Plus - Minus - Minus	TCATGCCGAGGACAGTTCTC TATTTCCTCTATCTATACAAA ATCGTGTCCGAGGTCGAGGT TTCTTAAAAGGGTTACTAGA CGGATTAATGAGGGCTAGTT GTTGCCAAGTAATACACCGA AGAAGTCTGGCTCATTTGAA GAGGCCGGCTAACGCAGTGT ACGGAGGTTTGA	TAQMAN
	>gb|AY102920.1| Vesicular stomatitis virus serotype Indiana panhandle-type defective interfering particle Ind-ST	AY102920.1	367	539	173	Plus - Minus - Minus	TCATGCCGAGGACAGTTCTC TATTTCCTCTATCTATACAAG GTCGTATTAGAGGTCGAGGT TTCTTAAAAGGGTTGGTAGA CGGATTAATGAGAGCAAGTT GCTGCCAAGTAATACACCGG AGAAGTCTGGCTGATTTGAA GAGGCCGGCCAACGCAGTGT AGGGAGGTTTGA	TAQMAN
	>gb|J02428.1|VSVCG Vesicular stomatitis Indiana virus, complete genome	J02428.1	8869	9041	173	Plus - Minus - Minus	TCATGCCGAGGACAGTTCTC TATTTCCTCTATCTATACAAG GTCGTATTAGAGGTCGAGGT TTCTTAAAAGGGTTGCTAGA CGGATTAATGAGAGCAAGTT GCTGCCAAGTAATACACCGG AGAAGTCTGGCTCATTTGAA GAGGCCGGCCAACGCAGTGT ACGGAGGTTTGA	TAQMAN
sig_candidate_1798949	>emb|X00939.1|RHVSVPOL Vesicular Stomatitis Virus gene for polymerase (L)	X00939.1	4949	5064	116	Plus - Plus - Minus	GGCGCTCATTATAAAATTCG GAGTATATTACATGGAATGG GAATCCATTACAGGGACTTC TTGAGTTGTGGAGACGGCTC CGGAGGGATGACTGCTGCAT TACTACGAGAAAATGT	TAQMAN
	>gb|AF473864.1| Vesicular stomatitis Indiana virus strain 98COE, complete genome	AF473864.1	9672	9787	116	Plus - Plus - Minus	GGCGCTCATTATAAAATTCG GAGTATATTACATGGAATGG GAATCCATTACAGGGACTTC TTAAGTTGTGGAGACGGTTC CGGAGGGATGACTGCTGCAT TACTACGAGAAAATGT	TAQMAN
6 total sequence hits	>gb|AF473865.1| Vesicular stomatitis Indiana virus strain 85CLB, complete genome	AF473865.1	9665	9780	116	Plus - Plus - Minus	GGCGCTCATTATAAAATTCG GAGTATATTACATGGAATGG GAATCCATTACAGGGACTTC TTAAGTTGTGGAGACGGTTC CGGCGGGATGACTGCTGCAT TACTACGAGAAAATGT	TAQMAN
	>gb|AF473866.1| Vesicular stomatitis Indiana virus strain 94GUB, complete genome	AF473866.1	9846	9961	116	Plus - Plus - Minus	GGCGCTCATTATAAAATTCG GAGTATATTACATGGAATGG GAATCCATTACAGGGACTTC CTGAGTTGTGGAGACGGCTC CGGAGGGATTACTGCTGCAT TACTACGAGAAAATGT	TAQMAN
	>gb|J02428.1|VSVCG Vesicular stomatitis Indiana virus, complete genome	J02428.1	9671	9786	116	Plus - Plus - Minus	GGCGCTCATTATAAAATTCG GAGTATATTACATGGAATGG GAATCCATTACAGGGACTTC TTGAGTTGTGGAGACGGCTC CGGAGGGATGACTGCTGCAT TACTACGAGAAAATGT	TAQMAN
	>gb|K02378.1|VSVLMS Vesicular stomatitis Indiana virus polymerase (L) gene, complete cds	K02378.1	4949	5064	116	Plus - Plus - Minus	GGCGCTCATTATAAAATTCG GAGTATATTACATGGAATGG GAATCCATTACAGGGACTTC TTGAGTTGTGGAGACGGCTC CGGAGGGATGACTGCTGCAT TACTACGAGAAAATGT	TAQMAN
sig_candidate_1811405	NONE	none				N - N - N		
0 total sequence hits								
sig_candidate_1811406	NONE	none				N - N - N		
0 total sequence hits								
sig_candidate_1811407	>gb|AF473864.1| Vesicular stomatitis Indiana virus strain 98COE, complete genome	AF473864.1	1146	1211	66	Plus - Plus - Minus	CGGAGGATTGACGACTAATG CACCGCCACAAGGCAGAGAT GTGGTCGAATGGCTAGGATG GTTTGA	
	>gb|J02428.1|VSVCG Vesicular stomatitis Indiana virus, complete genome	J02428.1	1146	1211	66	Plus - Plus - Minus	CGGAGGATTGACGACTAATG CACCGCCACAAGGCAGAGAT GTGGTCGAATGGCTCGGATG GTTTGA	
5 total sequence hits	>gb|M15213.1|VSVGINJ Vesicular stomatitis Indiana virus nucleocapsid protein (N) gene, complete cds	M15213.1	1096	1161	66	Plus - Plus - Minus	CGGAGGATTGACGACTAATG CACCGCCACAAGGCAGAGAT GTGGTCGAATGGCTCGGATG GTTTGA	
	>gb|U12967.1|VSU12967 Vesicular stomatitis Indiana virus nucleocapsid (N) and phosphoprotein (P) genes, complete cds	U12967.1	1096	1161	66	Plus - Plus - Minus	CGGAGGATTGACGACTAATG CACCGCCACAAGGCAGAGAT	

	>gb|U13898.1|VSU13898 Vesicular stomatitis Indiana virus strain tsW16B nucleocapsid (N) and phosphoprotein (P) genes, complete cds	U13898.1	1096	1161	66	Plus - Plus - Minus	GTGGTCGAATGGCTCGGATG GTTTGA CGGAGGATTGACGACTAATG CACCGCCACAAGGCAGAGAT GTGGTCGAATGGCTCGGATG GTTTGA
sig_candidate_1811408	NONE	none				N - N - N	

0 total sequence hits

FOOT-AND-MOUTH DISEASE VIRUS SIGNATURES

Sig ID/Name (F/IO/R)	Verification/Cross Rxn	Start	Stop	Amp Size
FMDV_TC	gi|46810910|gb|AY593827.1|_Foot-and-mouth disease virus O isolate o3venezuela iso15, complete genome	7827	7934	108
FMDV_TC	gi|46810916|gb|AY593830.1|_Foot-and-mouth disease virus O isolate o7poland iso49, complete genome	7865	7972	108
FMDV_TC	gi|89280872|gb|DQ409189.1|_Foot-and-mouth disease virus - type C isolate C-S8p460d417, complete genome	7378	7485	108
161 total amplicons	gi|6572136|emb|AJ251473.1|FDI251473_Foot-and-mouth disease virus SAT2 genomic RNA for L, VP4, VP2, VP3, VP1, 2A, 2B, 2C, 3A, VPg1, VPg2, VPg3, pro coding polpolyprotein, strain KEN/3/57	7455	7562	108
	gi|3676162|emb|AJ010871.1|FVAJ0871_Foot-and-mouth disease virus type A subtype A5, isolate A5Ww RNA for partial 3D gene and 3'UTR	80	187	108
	gi|27464334|gb|AF524946.1|_Foot-and-mouth disease virus O RNA polymerase (3D) gene, partial cds	182	289	108
	gi|46810876|gb|AY593810.1|_Foot-and-mouth disease virus C isolate cwald iso32, complete genome	7775	7882	108
	gi|46810848|gb|AY593796.1|_Foot-and-mouth disease virus Asia 1 isolate asia1-2isrl3-63 iso6, complete genome	7858	7965	108
	gi|46810900|gb|AY593822.1|_Foot-and-mouth disease virus O isolate o1m11 iso57, complete genome	7829	7936	108
	gi|30145782|emb|AJ539141.1|FOO539141_Foot-and-mouth disease virus O, strain UKG/35/2001, complete genome	7863	7970	108
	gi|210306|gb|M10975.1|APHA12CDR_Foot & mouth disease virus A12; L, P2, and P3 polypeptide coding region	7392	7499	108
	gi|46810862|gb|AY593803.1|_Foot-and-mouth disease virus A isolate avenceslau iso70, complete genome	7868	7975	108
	gi|46810902|gb|AY593823.1|_Foot-and-mouth disease virus O isolate o1manisa iso87, complete genome	7870	7977	108
	gi|61186|emb|V01136.1|PIVM03_FMDV gene encoding RNA polymerase (FMDV viral-infection associated antigen)	1189	1296	108
	gi|46810810|gb|AY593777.1|_Foot-and-mouth disease virus A isolate a4 W Germany iso42, complete genome	7827	7934	108
	gi|46810864|gb|AY593804.1|_Foot-and-mouth disease virus C1 isolate c1noville iso56, complete genome	7807	7914	108
	gi|46810946|gb|AY593845.1|_Foot-and-mouth disease virus SAT 1 isolate sat1bot iso47, complete genome	7839	7946	108
	gi|46810760|gb|AY593752.1|_Foot-and-mouth disease virus A isolate a12valle 119 iso20, complete genome	7868	7975	108
	gi|46810866|gb|AY593805.1|_Foot-and-mouth disease virus C1 isolate c1ober iso88, complete genome	7807	7914	108
	gi|46810874|gb|AY593809.1|_Foot-and-mouth disease virus C5 isolate c5arg iso60, complete genome	7857	7964	108
	gi|46810930|gb|AY593837.1|_Foot-and-mouth disease virus O isolate ouruguay-51 iso51, complete genome	7866	7973	108
	gi|46810800|gb|AY593772.1|_Foot-and-mouth disease virus A isolate a28 Turkey iso44, complete genome	7824	7931	108
	gi|46810792|gb|AY593768.1|_Foot-and-mouth disease virus A isolate a24cruzeiro iso71, complete genome	7823	7930	108
	gi|46810840|gb|AY593792.1|_Foot-and-mouth disease virus A isolate aparma iso55, complete genome	7864	7971	108
	gi|46810816|gb|AY593780.1|_Foot-and-mouth disease virus A isolate a5allier iso45, complete genome	7864	7971	108
	gi|89280868|gb|DQ409187.1|_Foot-and-mouth disease virus - type C isolate C-S8p360d951, complete genome	6844	6951	108
	gi|46810776|gb|AY593760.1|_Foot-and-mouth disease virus A isolate a20ussr iso10, complete genome	7818	7925	108
	gi|5031481|gb|AF154271.1|AF154271_Foot-and-mouth disease virus polyprotein precursor, mRNA, complete cds	7420	7527	108
	gi|30145772|emb|AJ539136.1|FOO539136_Foot-and-mouth disease virus O, strain TAW/2/99 TC, complete genome	7863	7970	108
	gi|46810786|gb|AY593765.1|_Foot-and-mouth disease virus A isolate a22turkey iso66, complete genome	7868	7975	108
	gi|46810818|gb|AY593781.1|_Foot-and-mouth disease virus A isolate a5westerwald iso73, complete genome	7882	7989	108
	gi|46810892|gb|AY593818.1|_Foot-and-mouth disease virus O isolate o1campos iso96, complete genome	7865	7972	108
	gi|89213430|gb|DQ404163.1|_Foot-and-mouth disease virus - type O strain UKG/14339/2001, complete genome	7861	7968	108
	gi|46810772|gb|AY593758.1|_Foot-and-mouth disease virus A isolate a18zulia iso40, complete genome	7867	7974	108

gi|89213464|gb|DQ404180.1|_Foot-and-mouth disease virus - type O strain UKG/11/2001, complete genome	7863	7970	108
gi|18074008|emb|AJ320488.1|FDI320488_Foot-and-mouth disease virus O genomic RNA, isolate O1Campos, complete genome	7853	7960	108
gi|33348772|gb|AY317098.1|_Foot-and-mouth disease virus HKN/2002, complete genome	7784	7891	108
gi|46810854|gb|AY593799.1|_Foot-and-mouth disease virus Asia 1 isolate asia1leb4 iso4, complete genome	7859	7966	108
gi|46810890|gb|AY593817.1|_Foot-and-mouth disease virus O isolate o1brugge iso79, complete genome	7865	7972	108
gi|61076|emb|X00871.1|PIFMDV2_Foot and mouth disease virus (FMDV-O1K) RNA for polyprotein precursor	7485	7592	108
gi|22004049|dbj|AB079061.1|_Foot-and-mouth disease virus O genomic RNA, strain:O/JPN/2000, L-fragment	7485	7592	108
gi|46810852|gb|AY593798.1|_Foot-and-mouth disease virus Asia 1 isolate asia1leb-89 iso89, complete genome	7859	7966	108
gi|89213458|gb|DQ404177.1|_Foot-and-mouth disease virus - type O strain UKG/128/2001, complete genome	7863	7970	108
gi|6456593|gb|AF189157.1|AF189157_Foot-and-mouth disease virus (strain O1) polyprotein gene, complete cds	6769	6876	108
gi|46810770|gb|AY593757.1|_Foot-and-mouth disease virus A isolate a17 Aguarulbos iso83, complete genome	7867	7974	108
gi|89213444|gb|DQ404169.1|_Foot-and-mouth disease virus - type O strain UKG/7038/2001, complete genome	7863	7970	108
gi|46810814|gb|AY593779.1|_Foot-and-mouth disease virus A isolate a4wg iso72, complete genome	7827	7934	108
gi|46810908|gb|AY593826.1|_Foot-and-mouth disease virus O isolate o2brescia iso17, complete genome	7829	7936	108
gi|46810948|gb|AY593846.1|_Foot-and-mouth disease virus SAT 1 isolate sat1rhod iso33, complete genome	7800	7907	108
gi|46810888|gb|AY593816.1|_Foot-and-mouth disease virus O isolate o1bfs46 iso46, complete genome	7865	7972	108
gi|22770783|gb|AF536536.1|_Foot-and-mouth disease virus C isolate Noville/Switzerland/65 RNA polymerase (3D) gene, partial cds	182	289	108
gi|46810768|gb|AY593756.1|_Foot-and-mouth disease virus A isolate a16belem iso80, complete genome	7780	7887	108
gi|4007043|emb|AJ007572.1|FMV7572_Foot-and-mouth disease virus, derived from C3Arg85, clone 15	7841	7948	108
gi|210476|gb|M11027.1|APHP61_Foot-and-mouth disease virus - type C polyprotein (P81) gene, partial cds	1365	1472	108
gi|22770785|gb|AF536537.1|_Foot-and-mouth disease virus SAT 1 isolate Rhodesia/11/37 RNA polymerase (3D) gene, partial cds	182	289	108
gi|32307408|gb|AY312587.1|AY312586S2_Foot-and-mouth disease virus O isolate O/SKR/2000 L fragment, complete sequence	7482	7589	108
gi|89280862|gb|DQ409184.1|_Foot-and-mouth disease virus - type C isolate C-S8p260d999, complete genome	6796	6903	108
gi|6318191|emb|AJ133359.1|FAN133359_Foot-and-mouth disease virus (FMDV) strain C, isolate rp146, genomic RNA	7795	7902	108
gi|89213426|gb|DQ404161.1|_Foot-and-mouth disease virus - type O strain UKG/14391/2001, complete genome	7859	7966	108
gi|46810934|gb|AY593839.1|_Foot-and-mouth disease virus SAT 1 isolate sat1-20 iso11, complete genome	7800	7907	108
gi|89213442|gb|DQ404170.1|_Foot-and-mouth disease virus - type O strain UKG/7675/2001, complete genome	7863	7970	108
gi|111114742|gb|DQ119643.2|_Foot-and-mouth disease virus - type O strain HLJOC12/03 polyprotein gene, complete cds	7447	7554	108
gi|46810914|gb|AY593829.1|_Foot-and-mouth disease virus O isolate o6pirbright iso58, complete genome	7866	7973	108
gi|732696|emb|X85493.1|FMDV3D_Foot and mouth disease virus 3D gene	1186	1293	108
gi|46810912|gb|AY593828.1|_Foot-and-mouth disease virus O isolate o5india iso34, complete genome	7823	7930	108
gi|89213456|gb|DQ404176.1|_Foot-and-mouth disease virus - type O strain UKG/150/2001, complete genome	7863	7970	108
gi|89280870|gb|DQ409188.1|_Foot-and-mouth disease virus - type C isolate C-S8p360p5d, complete genome	7795	7902	108
gi|46810790|gb|AY593767.1|_Foot-and-mouth disease virus A isolate a24 argentina iso9, complete genome	7863	7970	108
gi|46810932|gb|AY593838.1|_Foot-and-mouth disease virus SAT 1 isolate sat1-1bech iso30, complete genome	7841	7948	108
gi|46810798|gb|AY593771.1|_Foot-and-mouth disease virus A isolate a27columbia iso78, complete genome	7864	7971	108
gi|11493905|gb|AF207520.1|AF207520_Foot-and-mouth disease virus Asia 1 strain Asia1/IND 63/72 polyprotein gene, partial cds	1186	1293	108
gi|46810806|gb|AY593775.1|_Foot-and-mouth disease virus A isolate a32ven iso36, complete genome	7870	7977	108
gi|46810920|gb|AY593832.1|_Foot-and-mouth disease virus O isolate O UK2001-FB, complete genome	7865	7972	108
gi|46810778|gb|AY593761.1|_Foot-and-mouth disease virus A isolate a21kenya iso77, complete genome	7824	7931	108
gi|89213448|gb|DQ404172.1|_Foot-and-mouth disease virus - type O strain UKG/621/2001, complete genome	7863	7970	108
gi|46810838|gb|AY593791.1|_Foot-and-mouth disease virus A isolate airan iso105, complete genome	7933	8040	108
gi|46810882|gb|AY593813.1|_Foot-and-mouth disease virus O isolate o11indonesia iso52, complete genome	7781	7888	108
gi|134274528|emb|AM409325.1|_Foot-and-mouth disease virus - type C complete genome, genomic RNA, isolate H595	7795	7902	108
gi|46810872|gb|AY593808.1|_Foot-and-mouth disease virus C4 isolate C4 Tierra del Fuego iso2, complete genome	7853	7960	108
gi|46810850|gb|AY593797.1|_Foot-and-mouth disease virus Asia 1 isolate asia1-3kimron iso61, complete genome	7802	7909	108
gi|30145776|emb|AJ539138.1|FOO539138_Foot-and-mouth disease virus O, strain Tibet/CHA/99, complete genome	7863	7970	108
gi|46810894|gb|AY593819.1|_Foot-and-mouth disease virus O isolate o1campos94 iso94, complete genome	7865	7972	108
gi|46810870|gb|AY593807.1|_Foot-and-mouth disease virus C3 isolate c3resende iso1, complete genome	7857	7964	108

gi|6318187|emb|AJ133357.1|FDI133357_Foot-and-mouth disease virus (FMDV) strain C, isolate c-s8c1, genomic RNA	7795	7902	108
gi|37223495|gb|AY390432.1|_Foot-and-mouth disease virus Asia1 strain YNBS/58, complete genome	7823	7930	108
gi|89213450|gb|DQ404173.1|_Foot-and-mouth disease virus - type O strain UKG/220/2001, complete genome	7863	7970	108
gi|89280874|gb|DQ409190.1|_Foot-and-mouth disease virus - type C isolate C-S8p460d951, complete genome	6844	6951	108
gi|89213432|gb|DQ404164.1|_Foot-and-mouth disease virus - type O strain UKG/11676/2001, complete genome	7863	7970	108
gi|46810904|gb|AY593824.1|_Foot-and-mouth disease virus O isolate o1skr iso85, complete genome	7864	7971	108
gi|46810844|gb|AY593794.1|_Foot-and-mouth disease virus A isolate asabana iso68, complete genome	7866	7973	108
gi|89213446|gb|DQ404171.1|_Foot-and-mouth disease virus - type O strain UKG/4569/2001, complete genome	7863	7970	108
gi|89213420|gb|DQ404158.1|_Foot-and-mouth disease virus - type O strain UKG/15101/2001, complete genome	7857	7964	108
gi|46810944|gb|AY593844.1|_Foot-and-mouth disease virus SAT 1 isolate sat1-7isrl iso12, complete genome	7854	7961	108
gi|46810820|gb|AY593782.1|_Foot-and-mouth disease virus A isolate a argentina 2000 iso104, complete genome	7862	7969	108
gi|81248484|gb|DQ248888.1|_Foot-and-mouth disease virus - type O isolate lz, complete genome	7783	7890	108
gi|51340579|gb|AY687333.1|_Foot-and-mouth disease virus - type Asia 1 isolate IND 321/01, complete genome	7847	7954	108
gi|89213434|gb|DQ404165.1|_Foot-and-mouth disease virus - type O strain UKG/9964/2001, complete genome	7863	7970	108
gi|46810918|gb|AY593831.1|_Foot-and-mouth disease virus O isolate O UK2001-ED, complete genome	7865	7972	108
gi|89280860|gb|DQ409183.1|_Foot-and-mouth disease virus - type C isolate C-S8p260d417, complete genome	7378	7485	108
gi|33332022|gb|AF540910.1|_Foot-and-mouth disease virus SAT 2 clone ZIM/7/83, complete genome	7838	7945	108
gi|46810940|gb|AY593842.1|_Foot-and-mouth disease virus SAT 1 isolate sat1-5sa iso13, complete genome	7842	7949	108
gi|46810884|gb|AY593814.1|_Foot-and-mouth disease virus O isolate o1argentina iso5, complete genome	7861	7968	108
gi|15216732|gb|AF377945.1|_Foot-and-mouth disease virus O/SKR/2000, complete genome	7484	7591	108
gi|24711609|gb|AY149620.1|_Foot-and-mouth disease virus O polyprotein gene, partial cds	592	699	108
gi|89213460|gb|DQ404178.1|_Foot-and-mouth disease virus - type O strain UKG/127/2001, complete genome	7863	7970	108
gi|105873298|gb|DQ533483.1|_Foot-and-mouth disease virus - type Asia 1, complete genome	7856	7963	108
gi|12018088|gb|AF308157.1|AF308157_Foot-and-mouth disease virus, complete genome	7800	7907	108
gi|46810762|gb|AY593753.1|_Foot-and-mouth disease virus A isolate a13brazil iso75, complete genome	7868	7975	108
gi|30145780|emb|AJ539140.1|FOO539140_Foot-and-mouth disease virus O, strain SAR/19/2000, complete genome	7863	7970	108
gi|46810898|gb|AY593821.1|_Foot-and-mouth disease virus O isolate o1caseros iso35, complete genome	7865	7972	108
gi|37575129|gb|AY333431.1|_Foot-and-mouth disease virus O isolate O/NY00, complete genome	7484	7591	108
gi|6318189|emb|AJ133358.1|FAN133358_Foot-and-mouth disease virus (FMDV) strain C, isolate rp99, genomic RNA	7795	7902	108
gi|46810950|gb|AY593847.1|_Foot-and-mouth disease virus SAT 2 isolate sat2-1rhod iso26, complete genome	7785	7892	108
gi|46810758|gb|AY593751.1|_Foot-and-mouth disease virus A isolate a10holland iso82, complete genome	7826	7933	108
gi|89213440|gb|DQ404168.1|_Foot-and-mouth disease virus - type O strain UKG/9011/2001, complete genome	7863	7970	108
gi|32140992|gb|AY304994.1|_Foot-and-mouth disease virus Asia 1 IND 63/72, complete genome	7818	7925	108
gi|46810796|gb|AY593770.1|_Foot-and-mouth disease virus A isolate a26arg iso74, complete genome	7781	7888	108
gi|45725010|emb|AJ633821.1|_Foot-and-mouth disease virus polyprotein gene, genomic RNA, serotype O, isolate FRA/1/2001, complete genome	7866	7973	108
gi|5921457|gb|AF026168.2|AF026168_Foot-and-mouth disease virus O strain Chu-Pei complete genome	7403	7510	108
gi|89280876|gb|DQ409191.1|_Foot-and-mouth disease virus - type C isolate C-S8p460p5d, complete genome	7795	7902	108
gi|51104937|gb|AY686687.1|_Foot-and-mouth disease virus O/ES/2001 isolate O/ES/2001, complete genome	7826	7933	108
gi|4007041|emb|AJ007347.1|FMV7347_Foot-and-mouth disease virus polyprotein, isolate C3Arg85	7841	7948	108
gi|89280864|gb|DQ409185.1|_Foot-and-mouth disease virus - type C isolate C-S8p260p3d, complete genome	7795	7902	108
gi|22770789|gb|AF536539.1|_Foot-and-mouth disease virus SAT 3 isolate Rhodesia/7/34 RNA polymerase (3D) gene, partial cds	182	289	108
gi|22770791|gb|AF536540.1|_Foot-and-mouth disease virus Asia 1 isolate Lebanon/88 RNA polymerase (3D) gene, partial cds	182	289	108
gi|89213422|gb|DQ404159.1|_Foot-and-mouth disease virus - type O strain UKG/14603/2001, complete genome	7857	7964	108
gi|30145774|emb|AJ539137.1|FOO539137_Foot-and-mouth disease virus O, strain TAW/2/99 BOV, complete genome	7863	7970	108
gi|46810936|gb|AY593840.1|_Foot-and-mouth disease virus SAT 1 isolate sat1-3swa iso14, complete genome	7843	7950	108
gi|10334811|gb|AF274010.1|AF274010_Foot-and-mouth disease virus C strain C-S8 clone MARLS, complete genome	7795	7902	108
gi|89280866|gb|DQ409186.1|_Foot-and-mouth disease virus - type C isolate C-S8p360d417, complete genome	7378	7485	108
gi|89213454|gb|DQ404175.1|_Foot-and-mouth disease virus - type O strain UKG/173/2001, complete genome	7863	7970	108
gi|89213428|gb|DQ404162.1|_Foot-and-mouth disease virus - type O strain UKG/14476/2001, complete genome	7861	7968	108

gi|121592384|gb|EF175732.1|_Foot-and-mouth disease virus - type O isolate WFL, complete genome	7801	7908	108
gi|46810922|gb|AY593833.1|_Foot-and-mouth disease virus O isolate openghu iso108, complete genome	7796	7903	108
gi|134286059|emb|AM409190.1|_Foot-and-mouth disease virus mRNA for polyprotein, genomic RNA, isolate H51	7795	7902	108
gi|46810886|gb|AY593815.1|_Foot-and-mouth disease virus O isolate o1bfs iso18, complete genome	7865	7972	108
gi|397965|emb|X74812.1|FMDVALF_Foot and Mouth Disease Virus A L-fragment of RNA genome	7487	7594	108
gi|22770779|gb|AF536534.1|_Foot-and-mouth disease virus O isolate Manisa/Turkey/69 RNA polymerase (3D) gene, partial cds	182	289	108
gi|61063|emb|X00429.1|PIFMDV1_FMDV RNA of primary translation product (Foot and Mouth Disease Virus)	6775	6882	108
gi|89213438|gb|DQ404167.1|_Foot-and-mouth disease virus - type O strain UKG/9327/2001, complete genome	7863	7970	108
gi|46810928|gb|AY593836.1|_Foot-and-mouth disease virus O isolate ouk2001x iso84, complete genome	7864	7971	108
gi|46810804|gb|AY593774.1|_Foot-and-mouth disease virus A isolate a2spain iso7, complete genome	7827	7934	108
gi|30145778|emb|AJ539139.1|FOO539139_Foot-and-mouth disease virus O, strain SKR/2000, complete genome	7862	7969	108
gi|51340581|gb|AY687334.1|_Foot-and-mouth disease virus - type Asia 1 strain IND 491/97, complete genome	7843	7950	108
gi|89213462|gb|DQ404179.1|_Foot-and-mouth disease virus - type O strain UKG/126/2001, complete genome	7863	7970	108
gi|46810926|gb|AY593835.1|_Foot-and-mouth disease virus O isolate otaiwan97 iso106/112, complete genome	7796	7903	108
gi|46810802|gb|AY593773.1|_Foot-and-mouth disease virus A isolate a29peru iso37, complete genome	7870	7977	108
gi|46810856|gb|AY593800.1|_Foot-and-mouth disease virus Asia 1 isolate asia1leb83 iso28, complete genome	7859	7966	108
gi|46810766|gb|AY593755.1|_Foot-and-mouth disease virus A isolate a15thailand iso43, complete genome	7863	7970	108
gi|89213452|gb|DQ404174.1|_Foot-and-mouth disease virus - type O strain UKG/438/2001, complete genome	7863	7970	108
gi|46810896|gb|AY593820.1|_Foot-and-mouth disease virus O isolate o1canefa iso59, complete genome	7865	7972	108
gi|46810954|gb|AY593849.1|_Foot-and-mouth disease virus SAT 2 isolate sat2-3kenya-21, complete genome	7851	7958	108
gi|46810938|gb|AY593841.1|_Foot-and-mouth disease virus SAT 1 isolate sat1-4srhod iso24, complete genome	7842	7949	108
gi|21542501|gb|AF506822.2|_Foot-and-mouth disease virus O strain China/1/99(Tibet), complete genome	7853	7960	108
gi|89213424|gb|DQ404160.1|_Foot-and-mouth disease virus - type O strain UKG/14524/2001, complete genome	7857	7964	108
gi|22770787|gb|AF536538.1|_Foot-and-mouth disease virus SAT 2 isolate Rhodesia/1/48 RNA polymerase (3D) gene, partial cds	140	247	108
gi|46810952|gb|AY593848.1|_Foot-and-mouth disease virus SAT 2 isolate sat2-2 iso25, complete genome	7786	7893	108
gi|29502081|gb|AY250077.1|AH012646S2_Foot-and-mouth disease virus Asia 1 polymerase 3D gene, partial cds	240	347	108
gi|122056479|gb|EF149010.1|_Foot-and-mouth disease virus - type Asia 1 strain Asia 1/HNK/CHA/05, complete genome	7853	7960	108
gi|89213436|gb|DQ404166.1|_Foot-and-mouth disease virus - type O strain UKG/9788/2001, complete genome	7863	7970	108
gi|46810868|gb|AY593806.1|_Foot-and-mouth disease virus C3 isolate c3ind iso19, complete genome	7853	7960	108

Sig ID/Name (F/IO/R)	Verification/Cross Rxn	Start	Stop	Amp Size
FMDV_Pirbright	gi|46810816|gb|AY593780.1|_Foot-and-mouth disease virus A isolate a5allier iso45, complete genome	894	991	98
FMDV_Pirbright	gi|46810776|gb|AY593760.1|_Foot-and-mouth disease virus A isolate a20ussr iso10, complete genome	851	948	98
FMDV_Pirbright	gi|46810834|gb|AY593789.1|_Foot-and-mouth disease virus A isolate acanefa iso48, complete genome	821	918	98
5 total amplicons	gi|46810794|gb|AY593769.1|_Foot-and-mouth disease virus A isolate a25 argentina iso38, complete genome	821	918	98
	gi|46810820|gb|AY593782.1|_Foot-and-mouth disease virus A isolate a argentina 2000 iso104, complete genome	888	985	98

****As a result of degeneracies, Taqsim results for FMDV.Pir are limited.**

SWINE VESICULAR DISEASE VIRUS SIGNATURES

Sig ID/Name (F/IO/R)	Verification/Cross Rxn	Start	Stop	Amp Size
sig_candidate_1739038_SVD+TEST+FOR+ORACLE+CONSERVED+400+amp+Ignore+1_0	gi|402536|db|D16364.1|SVDMPS_Swine vesicular disease virus gene for polyprotein, complete cds	2430	2779	350
sig_candidate_1739038_SVD+TEST+FOR+ORACLE+CONSERVED+400+amp+Ignore+1_0	gi|61167|emb|X54521.1|PISVDV_Swine vesicular disease virus complete genomic RNA	2429	2778	350

sig_candidate_1739038_SVD+TEST+FOR+ORACLE+CONSERVED+400+amp+Ignore+1_0	gi|2745829|gb|AF039166.1|AF039166_Swine vesicular disease virus polyprotein capsid protein precursor (1ABCD) gene, partial cds	1687	2036	350
7 total amplicons	gi|1228947|dbj|D00435.1|SVDG_Swine vesicular disease virus (STRAIN H/3 '76) genomic RNA, complete genome	2430	2779	350
	gi|24061818|gb|AY157625.1|_Swine vesicular disease virus polyprotein P1 (1ABCD) gene, partial cds	1687	2036	350
	gi|8896132|gb|AF268065.1|_Swine vesicular disease virus strain NET/1/92, complete genome	2430	2779	350
	gi|37993797|gb|AY429470.1|_Swine vesicular disease virus strain HK'70, complete genome	2430	2779	350
sig_candidate_1739039_SVD+TEST+FOR+ORACLE+CONSERVED+400+amp+Ignore+1_1	gi|402536|dbj|D16364.1|SVDMPS_Swine vesicular disease virus gene for polyprotein, complete cds	3711	3992	282
sig_candidate_1739039_SVD+TEST+FOR+ORACLE+CONSERVED+400+amp+Ignore+1_1	gi|61167|emb|X54521.1|PISVDV_Swine vesicular disease virus complete genomic RNA	3710	3991	282
sig_candidate_1739039_SVD+TEST+FOR+ORACLE+CONSERVED+400+amp+Ignore+1_1	gi|1228947|dbj|D00435.1|SVDG_Swine vesicular disease virus (STRAIN H/3 '76) genomic RNA, complete genome	3711	3992	282
5 total amplicons	gi|8896132|gb|AF268065.1|_Swine vesicular disease virus strain NET/1/92, complete genome	3711	3992	282
	gi|37993797|gb|AY429470.1|_Swine vesicular disease virus strain HK'70, complete genome	3711	3992	282
sig_candidate_1739040_SVD+TEST+FOR+ORACLE+CONSERVED+400+amp+Ignore+1_2	gi|402536|dbj|D16364.1|SVDMPS_Swine vesicular disease virus gene for polyprotein, complete cds	6408	6656	249
sig_candidate_1739040_SVD+TEST+FOR+ORACLE+CONSERVED+400+amp+Ignore+1_2	gi|61167|emb|X54521.1|PISVDV_Swine vesicular disease virus complete genomic RNA	6407	6655	249
sig_candidate_1739040_SVD+TEST+FOR+ORACLE+CONSERVED+400+amp+Ignore+1_2	gi|1228947|dbj|D00435.1|SVDG_Swine vesicular disease virus (STRAIN H/3 '76) genomic RNA, complete genome	6408	6656	249
6 total amplicons	gi|5738886|emb|AJ245863.1|SVE245863_Swine vesicular disease virus 3D gene for RNA-dependent RNA-polymerase, genomic RNA	496	744	249
	gi|8896132|gb|AF268065.1|_Swine vesicular disease virus strain NET/1/92, complete genome	6408	6656	249
	gi|37993797|gb|AY429470.1|_Swine vesicular disease virus strain HK'70, complete genome	6408	6656	249
sig_candidate_1739041_SVD+TEST+FOR+ORACLE+CONSERVED+400+amp+Ignore+1_3	gi|402536|dbj|D16364.1|SVDMPS_Swine vesicular disease virus gene for polyprotein, complete cds	6980	7093	114
sig_candidate_1739041_SVD+TEST+FOR+ORACLE+CONSERVED+400+amp+Ignore+1_3	gi|61167|emb|X54521.1|PISVDV_Swine vesicular disease virus complete genomic RNA	6979	7092	114
sig_candidate_1739041_SVD+TEST+FOR+ORACLE+CONSERVED+400+amp+Ignore+1_3	gi|1228947|dbj|D00435.1|SVDG_Swine vesicular disease virus (STRAIN H/3 '76) genomic RNA, complete genome	6980	7093	114
6 total amplicons	gi|5738886|emb|AJ245863.1|SVE245863_Swine vesicular disease virus 3D gene for RNA-dependent RNA-polymerase, genomic RNA	1068	1181	114
	gi|8896132|gb|AF268065.1|_Swine vesicular disease virus strain NET/1/92, complete genome	6980	7093	114
	gi|37993797|gb|AY429470.1|_Swine vesicular disease virus strain HK'70, complete genome	6980	7093	114

VESICULAR EXANTHEMAOF SWINE VIRUS SIGNATURES

sig_candidate_643331_Vesicular_exanthema_of_swine_virus__1_genome__ignore_1.000001	gi|10141008|gb|U76874.2|VEU76874_Vesicular exanthema of swine virus stain A48, complete genome	404	557	154
sig_candidate_643331_Vesicular_exanthema_of_swine_virus__1_genome__ignore_1.000001				
sig_candidate_643331_Vesicular_exanthema_of_swine_virus__1_genome__ignore_1.000001				
1 total amplicons				
sig_candidate_643348_Vesicular_exanthema_of_swine_virus__1_genome__ignore_1.000018	gi|10141008|gb|U76874.2|VEU76874_Vesicular exanthema of swine virus stain A48, complete genome	1693	1837	145
sig_candidate_643348_Vesicular_exanthema_of_swine_virus__1_genome__ignore_1.000018				
sig_candidate_643348_Vesicular_exanthema_of_swine_virus__1_genome__ignore_1.000018				
1 total amplicons				
sig_candidate_643349_Vesicular_exanthema_of_swine_virus__1_genome__ignore_1.000019	gi|10141008|gb|U76874.2|VEU76874_Vesicular exanthema of swine virus stain A48, complete genome	1898	2097	200
sig_candidate_643349_Vesicular_exanthema_of_swine_virus__1_genome__ignore_1.000019				
sig_candidate_643349_Vesicular_exanthema_of_swine_virus__1_genome__ignore_1.000019				
1 total amplicons				
sig_candidate_643353_Vesicular_exanthema_of_swine_virus__1_genome__ignore_1.000023	gi|10141008|gb|U76874.2|VEU76874_Vesicular exanthema of swine virus stain A48, complete genome	3274	3398	125
sig_candidate_643353_Vesicular_exanthema_of_swine_virus__1_genome__ignore_1.000023				

sig_candidate_643353_Vesicular_exanthema_of_swine_virus__1_genome__ignore_1.000023				
1 total amplicons				
sig_candidate_643355_Vesicular_exanthema_of_swine_virus__1_genome__ignore_1.000025	gi|10141008|gb|U76874.2|VEU76874_Vesicular exanthema of swine virus stain A48, complete genome	3869	4069	201
sig_candidate_643355_Vesicular_exanthema_of_swine_virus__1_genome__ignore_1.000025				
sig_candidate_643355_Vesicular_exanthema_of_swine_virus__1_genome__ignore_1.000025				
1 total amplicons				
sig_candidate_643363_Vesicular_exanthema_of_swine_virus__1_genome__ignore_1.000033	gi|10141008|gb|U76874.2|VEU76874_Vesicular exanthema of swine virus stain A48, complete genome	5556	5756	201
sig_candidate_643363_Vesicular_exanthema_of_swine_virus__1_genome__ignore_1.000033				
sig_candidate_643363_Vesicular_exanthema_of_swine_virus__1_genome__ignore_1.000033				
1 total amplicons				

PORCINE RESPIRATORY AND REPRODUCTIVE SYDROME VIRUS SIGNATURES

Sig ID/Name	Verification/Cross Rxn	Accession	Start	Stop	Amp Size	Strand (F - I - R)	Amp Seq	Type
sig_candidate_1807661	>dbj|AB023782.1| Porcine reproductive and respiratory syndrome virus ORF 2 to 7 genes, partial and complete cds, strain:Kitasato 93-1	none	1025	1295	271	Plus - Minus - Minus	PARTIAL GACATCAGCTGCCTTAGGCATGGCGACCCGTCCTCTCCG ACGATTCGCAAGAGCCCTCAATGCCGCGCGGCGATAGG GACACCCGTGTACATTACTATCACAGCCAACGTAACAG ATGAGAATTATTTACATTCTTCTGATCTCCTTATGCTCTC TTCTTGCCTTTTCTATGCTTCTGAGATGAGCGAAAAAGG ATTTAAGGTGGTATTTGGCAATGTGTCAGGCATCGTGGC TGTGTGTGTCAATTTTACCAGCTACGTCCAACATGTCA	TAQMAN
	>gb|AF066384.1|AF066384 Porcine reproductive and respiratory syndrome virus RNA-dependent RNA polymerase gene, partial cds; and GP2 envelope protein, GP3 envelope protein, GP4 envelope protein, GP5 envelope protein, M matrix protein, and N nucleocapsid protein genes, complete cds	AF066384.1	4760	5034	275	Plus - Minus - Minus	PARTIAL CCAGGACATCAGCTGCCTTAGGCATGGCGACCCGTCCTC TGCGGCGATTCGCAAAAGCTCTCAGTGCCGCACGGCGA TAGGGACACCCGTGTATATCACCATTACAGCCAATGTGA CAGATGAGAATTATTTACACTCCTCTGATCTCCTCATGC TTTCTTCTTGCCTTTTCTATGCTTCTGAGATGAGTGAAAA GGGATTTAAGGTGGTATTTGGCAATGTGTCAGGCATCGT GGCTGTGTGTGTTAATTTTACCAGCTACGTCCAACATGT CA	TAQMAN
27 total sequence hits	>gb|AF184212.1|AF184212 Porcine reproductive and respiratory syndrome virus strain SP, complete genome	AF184212.1	13490	13764	275	Plus - Minus - Minus	PARTIAL CCAGGACATCAGCTGCCTTAGGCATGGCGACCCGTCCTC TGCGGCGATTCGCAAAAGCTCTCAGTGCCGCACGGCGA TAGGGACACCCGTGTATATCACCATTACAGCCAATGTGA CAGATGAGAATTATTTACACTCCTCTGATCTCCTCATGC TTTCTTCTTGCCTTTTCTATGCTTCTGAGATGAGTGAAAA GGGATTTAAGGTGGTATTTGGCAATGTGTCAGGCATCGT GGCTGTGTGTGTTAATTTTACCAGCTACGTCCAACATGT CA	TAQMAN
	>gb|AF325691.1|AF325691 Porcine reproductive and respiratory syndrome virus isolate NVSL 97-7985 IA 1-4-2, complete genome	AF325691.1	13359	13633	275	Plus - Minus - Minus	PARTIAL CCAGGACATCAGCTGCCTTAGGCATGGCGACCCGTCCTT TCCGGCGATTCGTAAAAGCTCTCAATGCCGCACGGCGAT AGGAACACCCGTGTATATCACCATCACAGCCAATGTTAC AGATGAGAATTATTTACATTCTTCTGATCTCCTCATGCTT TCTTCTTGCCTTTTCTATGCTTCTGAGATGAGTGAAAAG GGGTTCAAGGTGGTATTTGGCAATGTGGCAGGCATCGT GGCTGTGTGTGTCAACTTTACCAGCTACGTCCAACATGT CA	TAQMAN
	>gb|AF396844.1| Porcine	AF396844.1	1309	1583	275	Plus - Minus - Minus	PARTIAL	TAQMAN

reproductive and respiratory syndrome virus isolate JA-142 (252p) GP2 glycoprotein, GP3 glycoprotein, GP4 glycoprotein, GP5 glycosylated envelope protein, membrane protein, and nucleocapsid protein mRNAs, complete cds						CCAGGACATCAGCTGCCTTAGGCATGGCGACTCGTCCTC TCAGACGATTCGCAAAAGCTCTCAGTGCCGCGCGGCGA TAGGGACGCCCGTGTACATCACTGTCACAGCCAATGTCA CAGATGAGAATTATTTACATTCTTCTGATCTCCTTATGCT TTCTTCTTGCCTTTTCTATGCTTCTGAGATGAGTGAAAAG GGATTCAAGGTGATGTTTGGCAATGTGTCAGGCATCGTG GCTGTGTGTGTCAACTTTACCAGCTACGTCCAACATGTC A	
>gb|AF396839.1| Porcine reproductive and respiratory syndrome virus isolate NVSL-14 (E) GP2 glycoprotein, GP3 glycoprotein, GP4 glycoprotein, GP5 glycosylated envelope protein, membrane protein, and nucleocapsid protein mRNAs, complete cds	AF396839.1	1309	1583	275	Plus - Minus - Minus	PARTIAL CCAGGACATCAGCTGCCTTAGGCATGGCGACTCGTCCTC TCCGGCGATTCGCAAAAGCTCTCAGTGCCGCACGGCGA TAGGGACGCCCGTGTATATCACCATCACAGCCAATGTG ACAGATGAGAATTATTTACATTCTTCTGATCTCCTCATG CTTTCTTCTTGCCTTTTCTATGCTTCTGAGATGAGTGAAA AGGGATTCAAGGTGGTATTTGGCAATGTGTCAGGCGTC GTGGCTGTGTGTGTCAATTTTACCAGCTACGTCCAACAT GTCA	TAQMAN
>gb|AF494042.1| Porcine reproductive and respiratory syndrome virus isolate P129, complete genome	AF494042.1	13365	13639	275	Plus - Minus - Minus	PARTIAL CCAGGACATCAGCTGCCTTAGGCATGGCGACTCGTCCTT TCCGACGATTCGCAAAAGCTCTCAATGCCGCACGGCGA TAGGGACACCCGTGTATATCACCATCACAGCCAATGTG ACAGATGAGAATTACTTACATTCTTCTGATCTCCTCATG CTTTCTTCTTGCCTTTTCTATGCTTCTGAGATGAGTGAAA AGGGATTCAAGGTGGTGTTTGGCAATGTGTCAGGCATC GTGGCTGTGTGTGTCAACTTTACCAGCTACGTCCAACAT GTCA	TAQMAN
>gb|DQ473474.1| Porcine respiratory and reproductive syndrome virus isolate LMY, complete genome	DQ473474.1	13381	13655	275	Plus - Minus - Minus	PARTIAL CCAGGACATCAGCTGTCTTAGGCATGGCAACTCAACCTC TGAGGCGTTTCGCAAGATTCCTCAATGCCGCACGGCGAT AGGGACACCAGTGTATATCACCATCACAGCCAATGTAA CAGATGAGAATTATTTACATTCTTCTGACCTTCTCATGCT CTCTGCTTGCCTTTTCTATGCTTCTGAGATGAGCGAAAA GGGATTTAAGGTGGTATTTGGCAATGTGTCAGGCATCGT GGCTGTGTGTGTCAATTTTACCAGCTACGTCCAACACGT CA	TAQMAN
>gb|AF396840.1| Porcine reproductive and respiratory syndrome virus isolate NVSL-14 (251) GP2 glycoprotein, GP3 glycoprotein, GP4 glycoprotein, GP5 glycosylated envelope protein, membrane protein, and nucleocapsid protein mRNAs, complete cds	AF396840.1	1309	1583	275	Plus - Minus - Minus	PARTIAL CCAGGACATCAGCTGCCTTAGGCATGGCGACTCGTCCTC TCCGGCGATTCGCAAAAGTTCTCAGTGCCGCACGGCGAT AGGGACGCCCGTGTATATCACCATCACAGCCAATGTGA CAGATGAGAATTATTTACATTCTTCTGATCTCCTCATGCT TTCTTCTTGCCTTTTCTATGCTTCTGAGATGAGTGAAAAG GGATTCAAGGTGGTATTTGGCAATGTGTCAGGCGTCGTG GCTGTGTGTGTCAATTTTACCAGCTACGTCCAACATGTC A	TAQMAN
>gb|AY032626.1| Porcine reproductive and respiratory syndrome virus strain CH-1a, complete genome	AY032626.1	13382	13656	275	Plus - Minus - Minus	PARTIAL CCAGGACATCAGCTGCCTTAGGCATGGCGACTCGTCCTC TCCGACGATTCGCAAAAGCTCTCAGTGCCGCACGGCGA TAGGGACGCCCGTGTACATCACCATCACAGCCAATGTC ACAGATGAGAATTATTTACATTCTTCTGATCTCCTCATG CTTTCTTCTTGCCTTTTCTATGCTTCTGAGATGAGTGAAA AGGGATTCAAAGTGGTGTTTGGCAATGTGTCAGGCATC GTGGCTGTGTGTATCAACTTTACCAGCTACGTCCAACAT GTCA	TAQMAN
>gb|DQ642048.1|DQ642049S2 Porcine respiratory and reproductive syndrome virus strain HB-1(3.9) GP2a, P2b, GP3, GP4 glycoprotein, GP5, and matrix protein (M) genes, complete cds	DQ642048.1	1309	1583	275	Plus - Minus - Minus	PARTIAL CCAGGACATCAGCTGCCTTAGGCATGGCGACTCGTCCTC TCCGGCGATTCGCAAAAGTTCTCAGTGCCGTACGGCGAT AGGGACGCCCGTGTACATCACCATCACTGCCAATGTCAC AGATGAGAATTATCTACATTCTTCTGACCTCCTCATGCT TTCTTCTTGCCTTTTCTATGCTTCCGAGATGAGTGAAAA GGGATTCAAAGTGGTGTTTGGCAATGTGTCAGGCATCGT GGCTGTGTGTATCAACTTTACCAGCTACGTCCAACATGT CA	TAQMAN
>gb|AF396841.1| Porcine reproductive and respiratory syndrome virus isolate NVSL-14 (252p) GP2 glycoprotein, GP3 glycoprotein, GP4 glycoprotein, GP5 glycosylated envelope protein, membrane protein, and nucleocapsid protein mRNAs, complete cds	AF396841.1	1309	1583	275	Plus - Minus - Minus	PARTIAL CCAGGACATCAGCTGCCTTAGGCATGGCGACTCGTCCTC TCCGGCGATTCGCAAAAGCTCTCAGTGCCGCACGGCGA TAGGGACGCCCGTGTATATCACCATCACAGCCAATGTG ACAGATGAGAATTATTTACATTCTTCTGATCTCCTCATG CTTTCTTCTTGCCTTTTCTATGCTTCTGAGATGAGTGAAA AGGGATTCAAGGTGGTATTTGGCAATGTGTCAGGCGTC GTGGCTGTGTGTGTCAATTTTACCAGCTACGTCCAACAT GTCA	TAQMAN
>gb|AY150312.1| PRRSV HB-1(sh)/2002, complete genome	AY150312.1	13381	13655	275	Plus - Minus - Minus	PARTIAL CCAGGACATCAGCTGCCTTAGGCATGGCGACTCGTCCTC TCCGACGATTCGCAAAAGCTCTCAGTGCCGCACGGCGA TAGGGACGCCCGTGTACATCACCATCACAGCCAATGTC ACAGATGAGAATTATTTACATTCTTCTGATCTCCTCATG CTTTCTTCTTGCCTTTTCTATGCTTCTGAGATGAGTGAAA AGGGATTCAAAGTGGTGTTTGGCAATGTGTCAGGCATC GTGGCTGTGTGTATCAACTTTACCAGCTACGTCCAACAT GTCA	TAQMAN
>gb|DQ779791.1| Porcine respiratory and reproductive syndrome virus strain Prime Pac, complete genome	DQ779791.1	13490	13764	275	Plus - Minus - Minus	PARTIAL CCAGGACATCAGCTGCCTTAGGCATGGCGACCCGTCCTC TGCGGCGATTCGCAAAAGCTCTCAGTGCCGCACGGCGA TAGGGACACCCGTGTATATCACCATTACAGCCAATGTGA CAGATGAGAATTATTTACACTCCTCTGATCTCCTCATGC TTTCTTCTTGCCTTTTCTATGCTTCTGAGATGAGTGAAAA GGGATTTAAGGTGGTATTTGGCAATGTGTCAGGCATCGT GGCTGTGTGTGTTAATTTTACCAGCTACGTCCAACATGT CA	TAQMAN
>gb|AF396842.1| Porcine reproductive and respiratory syndrome virus isolate JA-142 (E) GP2 glycoprotein, GP3 glycoprotein, GP4 glycoprotein, GP5 glycosylated envelope protein, membrane protein, and nucleocapsid protein mRNAs, complete cds	AF396842.1	1309	1583	275	Plus - Minus - Minus	PARTIAL CCAGGACATCAGCTGCCTTAGGCATGGCGACTCGTCCTC TCAGACGATTCGCAAAAGCTCTCAGTGCCGCGCGGCGA TAGGGACGCCCGTGTACATCACTGTCACAGCCAATGTCA CAGATGAGAATTATTTACATTCTTCTGATCTCCTTATGCT TTCTTCTTGCCTTTTCTATGCTTCTGAGATGAGTGAAAAG GGATTCAAGGTGATATTTGGCAATGTGTCAGGCATCGTG GCTGTGTGTGTCAACTTTACCAGCTACGTCCAACATGTC A	TAQMAN
>gb|AY262352.1| PRRSV HB-2(sh)/2002, complete genome	AY262352.1	13343	13617	275	Plus - Minus - Minus	PARTIAL CCAGGACATCAGCTGCCTTAGGTATGGCGACTCGCCCCC TCCGACGGTTCGCAAGAGCTCTCAGTGCCGCACGGCGA TAGGGACACCCGTGTACATCACCATCACAGCCAATGTC ACAGATGAGAATTATTTACATTCTTCTGATCTCCTCATG CTTTCCTCTTGCCTTTTCTATGCTTCTGAGATGAGTGAAA AGGGATTCAAAGTGGTATTTGGCAATGTGTCAGGCATC GTGGCTGTGTGTGTTAACTTTACCAGCTACGTCCAACAT GTCA	TAQMAN
>gb|DQ988080.1| Porcine respiratory and reproductive syndrome virus	DQ988080.1	13383	13657	275	Plus - Minus - Minus	PARTIAL CCAGGACATCAGCTGCCTTAGGCATGGCGACTCGTCCTC	TAQMAN

	isolate Ingelvac ATP, complete genome						TCAGACGATTCGCAAAAGCTCTCAGTGCCGCGCGGCGA TAGGGACGCCCGTGTACATCACTGTCACAGCCAATGTCA CAGATGAGAATTATTTACATTCTTCTGATCTCCTTATGCT TTCTTCTTGCCTTTTCTATGCTTCTGAGATGAGTGAAAAG GGATTCAAGGTGATGTTTGGCAATGTGTCAGGCATCGTG GCTGTGTGTGTCAACTTTACCAGCTACGTCCAACATGTC A	
	>gb|AF396843.1| Porcine reproductive and respiratory syndrome virus isolate JA-142 (251) GP2 glycoprotein, GP3 glycoprotein, GP4 glycoprotein, GP5 glycosylated envelope protein, membrane protein, and nucleocapsid protein mRNAs, complete cds	AF396843.1	1309	1583	275	Plus - Minus - Minus	PARTIAL CCAGGACATCAGCTGCCTTAGGCATGGCGACTCGTCCTC TCAGACGATTCGCAAAAGCTCTCAGTGCCGCGCGGCGA TAGGGACGCCCGTGTACATCACTGTCACAGCCAATGTCA CAGATGAGAATTATTTACATTCTTCTGATCTCCTTATGCT TTCTTCTTGCCTTTTCTATGCTTCTGAGATGAGTGAAAAG GGATTCAAGGTGATGTTTGGCAATGTGTCAGGCATCGTG GCTGTGTGTGTCAACTTTACCAGCTACGTCCAACATGTC A	TAQMAN
	>gb|AY387693.1| Porcine reproductive and respiratory syndrome virus envelope protein GP4 gene, complete cds	AY387693.1	141	415	275	Plus - Minus - Minus	PARTIAL CCAGGACATCAGCTGCCTTAGGCATGGCGACTCGTCCTC TCCGGCAATTCGCAAAAGCTCTCAATGCCGCACGGCGA TAGGGACGCCCGTGTATATCACCATCACAGCCAATGTG ACAGATGAGAATTATTTACATTCTTCTGATCTCCTCATG CTTTCTTCTTGCCTTTTCTATGCTTCTGAGATGAGTGAAA AGGGATTCAAGGTGGTGTTTGGCAATGTGTCAGGCATC GTGGCTGTGTGTGTTAATTTTACCAGCTACGTCCAACAT GTCA	TAQMAN
	>gb|EF075945.1| Porcine respiratory and reproductive syndrome virus strain HUB1, complete genome	EF075945.1	13291	13565	275	Plus - Minus - Minus	PARTIAL CCAGGACATCAGCTGCCTTAGGCATGGCGACTCGTCCTC TCCGACGATTCGCAAAAGTTCTCAGTGCCGCACGGCGAT AGGGACGCCCGTGTACATCACCATCACTGCCAATGTCAC AGATGAAAATTATCTACATTCTTCTGATCTCCTCATGCTT TCTTCTTGCCTTTTCTATGCTTCCGAGATGAGTGAAAAG GGATTCAAAGTGGTGTTTGGCAATGTGTCAGGCATCGTG GCTGTGTGCGTCAACTTTACCAGCTACGTCCAACACGTC A	TAQMAN
	>gb|AY424271.1| Porcine reproductive and respiratory syndrome virus strain JA142, complete genome	AY424271.1	13383	13657	275	Plus - Minus - Minus	PARTIAL CCAGGACATCAGCTGCCTTAGGCATGGCGACTCGTCCTC TCAGACGATTCGCAAAAGCTCTCAGTGCCGCGCGGCGA TAGGGACGCCCGTGTACATCACTGTCACAGCCAATGTCA CAGATGAGAATTATTTACATTCTTCTGATCTCCTTATGCT TTCTTCTTGCCTTTTCTATGCTTCTGAGATGAGTGAAAAG GGATTCAAGGTGATGTTTGGCAATGTGTCAGGCATCGTG GCTGTGTGTGTCAACTTTACCAGCTACGTCCAACATGTC A	TAQMAN
	>gb|EF112446.1| Porcine respiratory and reproductive syndrome virus strain HUB2, complete genome	EF112446.1	13291	13565	275	Plus - Minus - Minus	PARTIAL CCAGGACATCAGCTGCCTTAGGCATGGCGACTCGTCCTC TCCGACGATTCGCAAAAGTTCTCAGTGCCGCACGGCGAT AGGGACGCCCGTGTACATCACCATCACTGCCAATGTTAC AGATGAAAATTATCTACATTCTTCTGATCTCCTCATGCTT TCTTCTTGCCTTTTCTATGCTTCCGAGATGAGTGAAAAG GGATTCAAAGTGGTGTTTGGCAATGTGTCAGGCATCGTG GCTGTGTGCGTCAACTTTACCAGCTACGTCCAACACGTC A	TAQMAN
	>gb|AY545985.1| Porcine reproductive and respiratory syndrome virus strain NVSL 97-7895, complete genome	AY545985.1	13383	13657	275	Plus - Minus - Minus	PARTIAL CCAGGACATCAGCTGCCTTAGGCATGGCGACTCGTCCTC TCAGACGATTCGCAAAAGCTCTCAGTGCCGCGCGGCGA TAGGGACGCCCGTGTACATCACTGTCACAGCCAATGTCA CAGATGAGAATTATTTACATTCTTCTGATCTCCTTATGCT TTCTTCTTGCCTTTTCTATGCTTCTGAGATGAGTGAAAAG GGATTCAAGGTGATATTTGGCAATGTGTCAGGCATCGTG GCTGTGTGTGTCAACTTTACCAGCTACGTCCAACATGTC A	TAQMAN
	>gb|EF112447.1| Porcine respiratory and reproductive syndrome virus strain HEB1, complete genome	EF112447.1	13291	13565	275	Plus - Minus - Minus	PARTIAL CCAGGACATCAGCTGCCTTAGGCATGGCGACTCGTCCTC TCCGACGATTCGCAAAAGTTCTCAGTGCCGCACGGCGAT AGGGACGCCCGTGTACATCACCATCACTGCCAATGTTAC AGATGAAAATTATCTACATTCTTCTGATCTCCTCATGCTT TCTTCTTGCCTTTTCTATGCTTCCGAGATGAGTGAAAAG GGATTCAAAGTGGTGTTTGGCAATGTGTCAGGCATCGTG GCTGTGTGCGTCAACTTTACCAGCTACGTCCAACACGTC A	TAQMAN
	>gb|AY569973.1| Porcine reproductive and respiratory syndrome virus isolate 25544 envelope glycoprotein GP2, envelope glycoprotein GP3, envelope glycoprotein GP4, envelope glycoprotein GP5, matrix protein, and nucleocapsid protein genes, complete cds	AY569973.1	1309	1583	275	Plus - Minus - Minus	PARTIAL CCAGGACATCAGCTGCCTTAGGCATGGCGACTCGTCCCC TAAGACGATTCGCAAAAGCTCTCAGTGCCGCGCGGCAA TAGGGACGCCCGTGTACATTACCATTACAGCCAATGTCA CAGATGAGAATTATTTACATTCTTCTGATCTCCTCATGCT TTCTTCTTGCCTTTTCTATGCTTCTGAGATGAGTGAAAAG GGATTCAAGGTGGTGTTTGGCAATGTGTCAGGCATCGTG GCTGTGTGTGTCAACTTTACCAGCTACGTCCAACACGTC A	TAQMAN
	>gb|L39363.1|PPSIL1A Porcine respiratory and reproductive syndrome virus (individual isolate IL1), mRNA sequence	L39363.1	1313	1583	271	Plus - Minus - Minus	PARTIAL GACATCAGCTGCCTTAGGCATCGCGACTCGGCCTCTGAG GCGATTCGCAAAATTCCTCAGTGCCGTACGGCGATAGG GACACCTGTTTATATTACCATCACAGCCAATGTGACAGA TGAGAATTATTTACATTCTTCTGATCTCCTCATGCTCTCT TCTTGCCTTTTCTATGCTTCTGAGATGAGTGAAAAGGGA TTTGAGGTGGTATTTGGCAATGTGTCGGGCATCGTGGCT GTGTGTGTCAATTTTACCAGCTACGTCCAACATGTCA	TAQMAN
	>gb|AY569974.1| Porcine reproductive and respiratory syndrome virus isolate 8981 envelope glycoprotein GP2, envelope glycoprotein GP3, envelope glycoprotein GP4, envelope glycoprotein GP5, matrix protein, and nucleocapsid protein genes, complete cds	AY569974.1	1309	1583	275	Plus - Minus - Minus	PARTIAL CCAGGACATCAGCTGCCTTAGGCATGGCGACTCGTCCTC TCAGACGATTCGCAAAAGCTCTCAGTGCCGCACGGCGA TAGGGACGCCCGTGTACATCACCATTACAGCCAATGTCA CAGATGAGAATTATTTGCATTCTTCTGATCTCCTCATGCT TTCTTCTTGCCTTTTCTATGCTTCTGAGATGAGTGAAAAG GGATTCAAGGTGGTGTTTGGCAATGTGTCAGGCATCGTG GCTGTGTGTGTCAACTTTACCAGCTACGTCCAACACGTC A	TAQMAN
sig_candidate_1807662	>gb|AF396835.1| Porcine reproductive and respiratory syndrome virus isolate NADC-8 (252p) GP2 glycoprotein, GP3 glycoprotein, GP4 glycoprotein, GP5 glycosylated envelope protein, membrane protein, and nucleocapsid protein mRNAs, complete cds	AF396835.1	2512	2784	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTCTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTAAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
	>gb|DQ176021.1| Porcine respiratory and reproductive syndrome virus clone VR-2332 V7, complete genome	DQ176021.1	14584	14856	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA	TAQMAN

109 total sequence hits	>gb|AF396836.1| Porcine reproductive and respiratory syndrome virus isolate NADC-9 (E) GP2 glycoprotein, GP3 glycoprotein, GP4 glycoprotein, GP5 glycosylated envelope protein, membrane protein, and nucleocapsid protein mRNAs, complete cds	AF396836.1	2512	2784	273	Plus - Minus - Minus	CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTAAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
	>gb|DQ217415.1| Porcine respiratory and reproductive syndrome virus strain VR-2332 clone pVR-V7, complete genome	DQ217415.1	14570	14842	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTAAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
	>gb|AF396837.1| Porcine reproductive and respiratory syndrome virus isolate NADC-9 (251) GP2 glycoprotein, GP3 glycoprotein, GP4 glycoprotein, GP5 glycosylated envelope protein, membrane protein, and nucleocapsid protein mRNAs, complete cds	AF396837.1	2512	2784	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTAAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
	>gb|DQ246451.1| Porcine respiratory and reproductive syndrome virus strain FJ04A GP2 glycoprotein, GP3 glycoprotein, GP4 glycoprotein, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	DQ246451.1	2512	2784	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTAAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
	>gb|DQ265740.1| Porcine respiratory and reproductive syndrome virus strain SD-2 membrane protein gene, complete cds	DQ265740.1	210	482	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTAAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
	>gb|DQ269472.1| Porcine respiratory and reproductive syndrome virus isolate ZJ2005 matrix protein and nucleocapsid genes, partial cds	DQ269472.1	138	410	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGGGCAGTAGTCGCACTTCTTTG GGGAGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTCGAAAGTGCCGCGGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
	>gb|DQ355796.1| Porcine respiratory and reproductive syndrome virus isolate R98 GP3 protein, GP4 protein, GP5 protein, M protein, and N protein mRNA, complete cds	DQ355796.1	1889	2161	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTAAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
	>gb|U18750.1|PRU18750 Porcine reproductive and respiratory syndrome virus ISU-3927 putative matrix protein and putative nucleocapsid protein genes, complete cds	U18750.1	210	482	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTCGCACTTCTCTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAGAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTTAACGGCACATTGG TGCCCGGGTTGAGAAGCCTCGTGTTGGGTGGCAAAAAA GC	TAQMAN
	>gb|DQ379480.1| Porcine respiratory and reproductive syndrome virus isolate SCQ ORF6 mRNA, complete cds	DQ379480.1	210	482	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTAAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
	>gb|U18751.1|PRU18751 Porcine reproductive and respiratory syndrome virus ISU-55 putative matrix protein and putative nucleocapsid protein genes, complete cds	U18751.1	210	482	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTTTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATAGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTTAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
	>gb|DQ459471.1| Porcine respiratory and reproductive syndrome virus isolate S1, complete genome	DQ459471.1	14584	14856	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTAAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
	>gb|U18752.1|PRU18752 Porcine reproductive and respiratory syndrome virus ISU-79 putative matrix protein and putative nucleocapsid protein	U18752.1	210	482	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA	TAQMAN

genes, complete cds						TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	
>gb|DQ473474.1| Porcine respiratory and reproductive syndrome virus isolate LMY, complete genome	DQ473474.1	14584	14856	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAGATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCTACTACGGTCAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|U64928.1|PRU64928 Porcine reproductive and respiratory syndrome virus strain IAF-Klop envelope glycoprotein (E), matrix protein (M), and nucleocapsid protein (N) genes, complete cds	U64928.1	798	1069	272	Plus - Minus - Minus	PARTIAL GTCGCGCTCACTATGGGAGCAGTAGTTGCGCTCCTTTGG GGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCAC CTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACAT TCTGGCCCCTGCCCACCACGTTGAGAGTGCCGCAGGCTT TCATCCGATTGCGGCAAGTGATAACCACGCATTTGTCGT CCGGCGTCCCGGCTCCACTACGGTTAACGGCACATTGGT GCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAAG C	TAQMAN
>gb|DQ642048.1|DQ642049S2 Porcine respiratory and reproductive syndrome virus strain HB-1(3.9) GP2a, P2b, GP3, GP4 glycoprotein, GP5, and matrix protein (M) genes, complete cds	DQ642048.1	2512	2784	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTTCTTTG GGGAGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTCGAAAGTGCCGCGGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTTAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|U64929.1|PRU64929 Porcine reproductive and respiratory syndrome virus strain IAF-BAJ envelope glycoprotein (E), matrix protein (M), and nucleocapsid protein (N) genes, complete cds	U64929.1	798	1069	272	Plus - Minus - Minus	PARTIAL GTCGCGCTCACTATGGGAGCAGTAGTTGCGCTCCTTTGG GGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCAC CTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACAT TCTGGCCCCTGCCCACCACGTTGAGAGTGCCGCAGGCTT TCATCCGATTGCGGCAAGTGATAACCACGCATTTGTCGT CCGGCGTCCCGGCTCCACTACGGTTAACGGCACATTGGT GCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAAG C	TAQMAN
>gb|U64931.1|PRU64931 Porcine reproductive and respiratory syndrome virus strain IAF 93-653 envelope glycoprotein (E), matrix protein (M), and nucleocapsid protein (N) genes, complete cds	U64931.1	797	1069	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCGGTGATCGCACTCCTTTG GGGGGTGTATTCAGCCATAGAGACCTGGAGATTCATCA CCTCCAGATGCCGTTTGTGCTTGTTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGACGAGTGATAACCACGCATTTGTCG TTCGGCGTCCCGGCTCCACTACGGTTAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAAAAAA GC	TAQMAN
>gb|U64932.1|PRU64932 Porcine reproductive and respiratory syndrome virus strain IAF 93-2616 envelope glycoprotein (E), matrix protein (M), and nucleocapsid protein (N) genes, complete cds	U64932.1	797	1069	273	Plus - Minus - Minus	PARTIAL GTAATTGCACTCCTCTGGGGAGTGTACTCAGCCATAGAG ACCTGGAGATTCATCACCTCCAGATGCCGCTTGTGCTTG TTAGGTCGCAAGTACATTCTGGCCCCTGCCCACCACGTT GAAAGTGCCGCAGGTTTTCATCCGATTGCGACAAGTGAT AACCACGCATTTGTCGTCCGGCGTCCCGGCTCCACTACG GTTAACGGCACATTGGTGCCCGGGTTGAAAAGCTCTCGTG TTGGGTGGCAGAAAAGC	TAQMAN
>gb|U64935.1|PRU64935 Porcine reproductive and respiratory syndrome virus strain ONT-TS envelope glycoprotein (E), matrix protein (M), and nucleocapsid protein (N) genes, complete cds	U64935.1	797	1069	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAGACCTGGAAATTCATCA CTTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATTCGATTACGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTTAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|U75443.1|PRU75443 Porcine reproductive and respiratory syndrome virus matrix protein (M) and nucleocapsid protein (N) genes, complete cds	U75443.1	225	497	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTAAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|U87392.3|PRU87392 Porcine reproductive and respiratory syndrome virus strain VR-2332, complete genome	U87392.3	14584	14856	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCACGGT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTAAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AF396838.1| Porcine reproductive and respiratory syndrome virus isolate NADC-9 (252p) GP2 glycoprotein, GP3 glycoprotein, GP4 glycoprotein, GP5 glycosylated envelope protein, membrane protein, and nucleocapsid protein mRNAs, complete cds	AF396838.1	2512	2784	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTAAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AF396839.1| Porcine reproductive and respiratory syndrome virus isolate NVSL-14 (E) GP2 glycoprotein, GP3 glycoprotein, GP4 glycoprotein, GP5 glycosylated envelope protein, membrane protein, and nucleocapsid protein mRNAs, complete cds	AF396839.1	2512	2784	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTTCTCTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTCGAAAGTGCCGCGGGCT TTCATCCGATTACGGCAAATGATAACCACGCGTTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGTGGGTGGCAGAAAA GC	TAQMAN
>gb|AF396840.1| Porcine reproductive and respiratory syndrome virus isolate NVSL-14 (251) GP2 glycoprotein, GP3 glycoprotein, GP4 glycoprotein, GP5 glycosylated envelope protein, membrane protein,	AF396840.1	2512	2784	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTCTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTCGAAAGTGCCGCGGGCT TTCATCCGATTACGGCAAATGATAACCACGCGTTTGTCG	TAQMAN

and nucleocapsid protein mRNAs, complete cds						TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	
>gb|AF396841.1| Porcine reproductive and respiratory syndrome virus isolate NVSL-14 (252p) GP2 glycoprotein, GP3 glycoprotein, GP4 glycoprotein, GP5 glycosylated envelope protein, membrane protein, and nucleocapsid protein mRNAs, complete cds	AF396841.1	2512	2784	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTCTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTCGAAAGTGCCGCGGGCT TTCATCCGATTACGGCAAATGATAACCACGTGTTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AF396842.1| Porcine reproductive and respiratory syndrome virus isolate JA-142 (E) GP2 glycoprotein, GP3 glycoprotein, GP4 glycoprotein, GP5 glycosylated envelope protein, membrane protein, and nucleocapsid protein mRNAs, complete cds	AF396842.1	2512	2784	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTTCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAGTTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCCGCCCACCACGTCGAAAGTGCCGCGGGCT TTCATCCGATCGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTTAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AF396843.1| Porcine reproductive and respiratory syndrome virus isolate JA-142 (251) GP2 glycoprotein, GP3 glycoprotein, GP4 glycoprotein, GP5 glycosylated envelope protein, membrane protein, and nucleocapsid protein mRNAs, complete cds	AF396843.1	2512	2784	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTTCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAGTTCATCA CCTCCAGATGTCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCCGCCCACCACGTCGAAAGTGCCGCGGGCT TTCATCCGATTCGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTTAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AF396844.1| Porcine reproductive and respiratory syndrome virus isolate JA-142 (252p) GP2 glycoprotein, GP3 glycoprotein, GP4 glycoprotein, GP5 glycosylated envelope protein, membrane protein, and nucleocapsid protein mRNAs, complete cds	AF396844.1	2512	2784	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTTCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAGTTCATCA CCTCCAGATGTCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCCGCCCACCACGTCGAAAGTGCCGCGGGCT TTCATCCGATTCGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTTAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AF494042.1| Porcine reproductive and respiratory syndrome virus isolate P129, complete genome	AF494042.1	14568	14840	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTTCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTCGAAAGTGCCGCGGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTTAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|DQ779791.1| Porcine respiratory and reproductive syndrome virus strain Prime Pac, complete genome	DQ779791.1	14693	14965	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACTTGGAGGTTCATCA CCTCTAGATGCCGTTTGTGCTTGTTAGGCCGCAGGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTACGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTTAACGGCACATTGG TGCCCGGGTTGAAGAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AY032626.1| Porcine reproductive and respiratory syndrome virus strain CH-1a, complete genome	AY032626.1	14585	14857	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTTCTTTG GGGAGTGTACTCGGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTCGAAAGTGCCGCGGGCT TTCATCCGATTCGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTTAACGGCACATTGG TGCCCGGGTTGAAAGGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|DQ988080.1| Porcine respiratory and reproductive syndrome virus isolate Ingelvac ATP, complete genome	DQ988080.1	14586	14858	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTTCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAGTTCATCA CCTCCAGATGTCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCCGCCCACCACGTCGAAAGTGCCGCGGGCT TTCATCCGATCGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTTAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AY150312.1| PRRSV HB-1(sh)/2002, complete genome	AY150312.1	14584	14856	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTTCTTTG GGGAGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTCGAAAGTGCCGCGGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTTAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|EF014225.1| Porcine respiratory and reproductive syndrome virus strain JX1 GP5, membrane protein, and nucleocapsid protein genes, complete cds	EF014225.1	797	1069	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTTCTTTG GGGAGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTCGAAAGTGCCGCGGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTTCAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|EF075945.1| Porcine respiratory and reproductive syndrome virus strain HUB1, complete genome	EF075945.1	14494	14766	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTTCTTTG GGGAGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTCTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTCGAAAGTGCCGCGGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|EF112445.1| Porcine respiratory and reproductive syndrome virus strain JXA1, complete genome	EF112445.1	14494	14766	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTTCTTTG GGGAGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTCGAAAGTGCCGCGGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG	TAQMAN

>gb|EF112446.1| Porcine respiratory and reproductive syndrome virus strain HUB2, complete genome	EF112446.1	14494	14766	273	Plus - Minus - Minus	TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAAGC PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTTCTTTGGGGAGTGTACTCAGCCATAGAAACCTGGAAATTCATCACCTCCAGATGCCGTCTGTGCTTGCTAGGCCGCAAGTACATTCTGGCCCCTGCCCACCACGTCGAAAGTGCCGCGGGCTTTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCGTCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGGTGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAAGC PARTIAL	TAQMAN
>gb|EF112447.1| Porcine respiratory and reproductive syndrome virus strain HEB1, complete genome	EF112447.1	14494	14766	273	Plus - Minus - Minus	GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTTCTTTGGGGAGTGTACTCAGCCATAGAAACCTGGAAATTCATCACCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACATTCTGGCCCCTGCCCACCACGTCGAAAGTGCCGCGGGCTTTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCGTCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGGTGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAAGC PARTIAL	TAQMAN
>gb|EF150361.1| Porcine respiratory and reproductive syndrome virus strain HLV013 GP5 and matrix protein genes, complete cds	EF150361.1	797	1069	273	Plus - Minus - Minus	GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTGGGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCACCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACATTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCTTTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCGTCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGGTGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAAGC PARTIAL	TAQMAN
>gb|EF153486.1| Porcine respiratory and reproductive syndrome virus isolate CC-1, complete genome	EF153486.1	14584	14856	273	Plus - Minus - Minus	GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTGGGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCACCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACATTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCTTTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCGTCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGGTGCCCGGGTTAAAAAGCCTCGTGTTGGGTGGCAGAAAAGC PARTIAL	TAQMAN
>gb|L39361.1|PPSIA1A Porcine respiratory and reproductive syndrome virus (individual isolate IA1), mRNA sequence	L39361.1	2512	2784	273	Plus - Minus - Minus	GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTGGGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCACCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACATTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCTTTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCGTCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGGTGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAAGC PARTIAL	TAQMAN
>emb|AJ223079.1|PRR223079 Porcine respiratory and reproductive syndrome virus ORFs 2 to 7, isolate Danish DK3506-12	AJ223079.1	2512	2784	273	Plus - Minus - Minus	GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTGGGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCACCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACATTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCTTTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCGTCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGGTGCCCGGGTTAAAAAGCCTCGTGTTGGGTGGCAGAAAAGC PARTIAL	TAQMAN
>emb|AJ223080.1|PRR223080 Porcine respiratory and reproductive syndrome virus ORFs 2 to 7, isolate Danish DK5163-17	AJ223080.1	2512	2784	273	Plus - Minus - Minus	GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTGGGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCACCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACATTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCTTTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCGTCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGGTGCCCGGGTTAAAAAGCCTCGTGTTGGGTGGCAGAAAAGC PARTIAL	TAQMAN
>emb|AJ223081.1|PRR223081 Porcine respiratory and reproductive syndrome virus ORFs 2 to 7, isolate Danish DK5163-23	AJ223081.1	2512	2784	273	Plus - Minus - Minus	GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTGGGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCACCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACATTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCTTTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCGTCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGGTGCCCGGGTTAAAAAGCCTCGTGTTGGGTGGCAGAAAAGC PARTIAL	TAQMAN
>gb|AF066384.1|AF066384 Porcine reproductive and respiratory syndrome virus RNA-dependent RNA polymerase gene, partial cds; and GP2 envelope protein, GP3 envelope protein, GP4 envelope protein, GP5 envelope protein, M matrix protein, and N nucleocapsid protein genes, complete cds	AF066384.1	5963	6235	273	Plus - Minus - Minus	GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTGGGGGGTGTACTCAGCCATAGAAACTTGGAGGTTCATCACCCTCTAGATGCCGTTTGTGCTTGTTAGGCCGCAGGTACATTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCTTTCATCCGATTACGGCAAATGATAACCACGCATTTGTCGTCCGGCGTCCCGGCTCCACTACGGTTAACGGCACATTGGTGCCCGGGTTGAAGAGCCTCGTGTTGGGTGGCAGAAAAGC PARTIAL	TAQMAN
>emb|AJ223082.1|PRR223082 Porcine respiratory and reproductive syndrome virus ORFs 2 to 7, isolate MLV RespPRRS	AJ223082.1	2512	2784	273	Plus - Minus - Minus	GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTGGGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCACCCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACATTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCTTTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCGTCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGGTGCCCGGGTTAAAAAGCCTCGTGTTGGGTGGCAGAAAAGC PARTIAL	TAQMAN
>gb|AF090173.1|AF090173 Porcine reproductive and respiratory syndrome virus glycoprotein, membrane protein, and nucleocapsid protein genes, complete cds	AF090173.1	816	1088	273	Plus - Minus - Minus	GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTGGGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCACCCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACATTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCACGGTTTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCGTCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGGTGCCCGGGTTAAAAAGCCTCGTGTTGGGTGGCAGAAAAGC PARTIAL	TAQMAN
>gb|AF030306.1|AF030306 Porcine reproductive and respiratory syndrome virus envelope proteins, matrix protein, and nucleocapsid genes, complete cds	AF030306.1	2593	2865	273	Plus - Minus - Minus	GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTGGGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCACCCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACATTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCTTTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCGTCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGGTGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA	TAQMAN

>gb|AF121131.1|AF121131 Porcine reproductive and respiratory syndrome virus envelope protein GP2, envelope protein GP3, envelope protein GP4, envelope protein, matrix protein, and nucleocapsid protein genes, complete cds	AF121131.1	2538	2810	273	Plus - Minus - Minus	GC PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAGACCTGGAGATTCATCA CCTCTAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAGTGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTTAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AF035409.1|AF035409 Porcine reproductive and respiratory syndrome virus envelope proteins, matrix protein, and nucleocapsid protein genes, complete cds	AF035409.1	2593	2865	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAGACCTGGAGATTCATCA CCTCTAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAGTGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTTAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AF159149.1|AF159149 Porcine reproductive and respiratory syndrome virus isolate MLV RespPRRS/Repro, complete genome	AF159149.1	1456 0	14832	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTAAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AF046869.1|AF046869 Porcine reproductive and respiratory syndrome virus isolate 16244B, 2/18/97(Nebraska)pass.3, complete genome	AF046869.1	1458 4	14856	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AF176348.2| Porcine reproductive and respiratory syndrome virus isolate PA8 complete genome	AF176348.2	1458 4	14856	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTAAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AF066068.1|PRRSVORF2 Porcine reproductive and respiratory syndrome virus major membrane glycoprotein, membrane protein, and nucleocapsid protein genes, complete cds	AF066068.1	797	1069	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCACGGT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTAAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AF184212.1|AF184212 Porcine reproductive and respiratory syndrome virus strain SP, complete genome	AF184212.1	1469 3	14965	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACTTGGAGGTTCATCA CCTCTAGATGCCGTTTGTGCTTGTTAGGCCGCAGGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTACGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTTAACGGCACATTGG TGCCCGGGTTGAAGAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AF066183.4| Porcine reproductive and respiratory syndrome virus RespPRRS MLV, complete genome	AF066183.4	1458 5	14857	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTAAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AF188680.1|AF188680 Porcine reproductive and respiratory syndrome virus viral envelope protein mRNA, complete cds	AF188680.1	210	482	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTATTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAGAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTGAAAGGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AF299404.1|AF299404 Porcine reproductive and respiratory syndrome virus isolate pig22-7o ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299404.1	2879	3151	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AY150564.1| Porcine reproductive and respiratory syndrome virus isolate VR-2332, complete genome	AY150564.1	1458 5	14857	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTAAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AF299405.1|AF299405 Porcine reproductive and respiratory syndrome virus isolate pig22-84bo ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and	AF299405.1	2720	2992	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA	TAQMAN

nucleocapsid protein N genes, complete cds						GC	
>gb|AY256685.1| Porcine reproductive and respiratory syndrome virus VR-2332 nonfunctional NSP1 and nonfunctional unglycosylated membrane protein, complete sequence; and nucleocapsid protein mRNA, complete cds	AY256685.1	1136	1408	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AF299406.1|AF299406 Porcine reproductive and respiratory syndrome virus isolate pig27-7o ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299406.1	2879	3151	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AY262352.1| PRRSV HB-2(sh)/2002, complete genome	AY262352.1	14546	14818	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGGGCAGTAGTTGCACTTCTCTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTCGAAAGTGCCGCGGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTTAACGGCATATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AY387695.1| Porcine reproductive and respiratory syndrome virus matrix protein M gene, complete cds	AY387695.1	210	482	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTTCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTCGAAAGTGCCGCGGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTTAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGTGGTAGAAAA GC	TAQMAN
>gb|AY424271.1| Porcine reproductive and respiratory syndrome virus strain JA142, complete genome	AY424271.1	14586	14858	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTTCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAGTTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCCGCCCACCACGTCGAAAGTGCCGCGGGCT TTCATCCGATCGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTTAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGTGGCAGAAAA GC	TAQMAN
>gb|AY457635.1| Porcine reproductive and respiratory syndrome virus HN1, complete genome	AY457635.1	14583	14855	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CTTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TACCCGGGTTAAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AY545985.1| Porcine reproductive and respiratory syndrome virus strain NVSL 97-7895, complete genome	AY545985.1	14586	14858	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTTCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAGTTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCCGCCCACCACGTCGAAAGTGCCGCGGGCT TTCATCCGATCGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTTAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGTGGCAGAAAA GC	TAQMAN
>gb|AY569972.1| Porcine reproductive and respiratory syndrome virus isolate 1530B envelope glycoprotein GP2, envelope glycoprotein GP3, envelope glycoprotein GP4, envelope glycoprotein GP5, matrix protein, and nucleocapsid protein genes, complete cds	AY569972.1	2512	2784	273	Plus - Minus - Minus	PARTIAL TCAGCCATAGAAACCTGGAAATTCATCACCTCCAGATGC CGTTTGTGCTTGCTAGGCCGCAAGTACATTCTGGCCCCT GCCCACCACGTTGAAAGTGCCGCAGGCTTTCATCCGATT GCGGCAAATGATAACCACGCATTTGTCGTCCGGCGTCCC GGCTCCACTACGGTCAACGGCACATTGGTGCCCGGGTTA AAAAGCCTCGTGTTGGGTGGCAGAAAAGC	TAQMAN
>gb|L39362.1|PPSIA6A Porcine respiratory and reproductive syndrome virus (individual isolate IA6), mRNA sequence	L39362.1	2512	2784	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTTCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTCGAAAGTGCCGCAGGCT TTCATCCGATTACGGCAAACGATAACCACGCATTTGTCG TCCGGTGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGGCCGGGTTGAAAAGCCTCGTTTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AY569973.1| Porcine reproductive and respiratory syndrome virus isolate 25544 envelope glycoprotein GP2, envelope glycoprotein GP3, envelope glycoprotein GP4, envelope glycoprotein GP5, matrix protein, and nucleocapsid protein genes, complete cds	AY569973.1	2512	2784	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTCGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTTTGGCCCCTGCCCACCACGTCGAAAGTGCCGCGGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|L39363.1|PPSIL1A Porcine respiratory and reproductive syndrome virus (individual isolate IL1), mRNA sequence	L39363.1	2512	2784	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTTCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTCGAAAGTGCCGCGGGCT TTCATCCGATTGCGGCAAGTGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCACGGCACATTTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AY569974.1| Porcine reproductive and respiratory syndrome virus isolate 8981 envelope glycoprotein GP2, envelope glycoprotein GP3, envelope glycoprotein GP4, envelope glycoprotein GP5, matrix protein, and	AY569974.1	2512	2784	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTCGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTTTGGCCCCTGCCCACCACGTCGAAAGTGCCGCGGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG	TAQMAN

nucleocapsid protein genes, complete cds						TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAAGC	
>gb|L39364.1|PPSKS1A Porcine respiratory and reproductive syndrome virus (individual isolate KS1), mRNA sequence	L39364.1	2512	2784	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTGGGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCACCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACATTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCTTTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCGTCCGGCGTCCCGGCTCCACTACGGACAACGGCACATTGGTGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAAAAAAGC	TAQMAN
>gb|L39365.1|PPSKY1A Porcine respiratory and reproductive syndrome virus (individual isolate KY1), mRNA sequence	L39365.1	2512	2782	271	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTGGGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCACCTCCAGATGCCGTTTGTGCTTGTTAGGCCGCAAGTACATTCTGGCCCCTGCCCACCACGTTGAAAGTGTCGCAGGCTTTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCGTCCGGCGTCCCGGCTCCACTACGGACAACGGCACATTGGTGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA	TAQMAN
>gb|L39366.1|PPSMN1A Porcine respiratory and reproductive syndrome virus (individual isolate MN1), mRNA sequence	L39366.1	2512	2784	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTGGGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCACCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACATTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCTTTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCGTCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGGTGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAAGC	TAQMAN
>gb|L39367.1|PPSMO1A Porcine respiratory and reproductive syndrome virus (individual isolate MO1), mRNA sequence	L39367.1	2512	2784	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTGGGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCACCTCCAGATGCCGTTTGTGCTTGTTAGGCCGCAAGTACATTCTGGCCCCTGCCCACCACGTTGAAAGTGTCGCAGGCTTTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCGTCCGGCGTCCCGGTTCCACTACGGACAACGGCACATTGGTGCCCGGGTTGAAAAGCCTCGTTTTGGGTGGCAGAAAAGC	TAQMAN
>gb|L39368.1|PPSNE1A Porcine respiratory and reproductive syndrome virus (individual isolate NE1), mRNA sequence	L39368.1	2512	2784	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTGGGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCACCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACATTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCTTTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCGTCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGGTGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAAGC	TAQMAN
>gb|L39369.1|PPSSG1A Porcine respiratory and reproductive syndrome virus (individual isolate SG1), mRNA sequence	L39369.1	2512	2784	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTGGGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCACCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACATTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCTTTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCGTCCGGCGTCCCGGTTCCACTACGGTCAACGGGACATTGGTCCCCGGGTTGAAAAGCCTCGTTTTGGGTGGCAGAAAAGC	TAQMAN
>gb|U03040.1|PRU03040 Porcine reproductive and respiratory syndrome virus putative envelope and nucleocapsid protein genes, complete cds	U03040.1	1222	1494	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTGGGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCACCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACATTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCTTTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCGTCCGGCGTCCCGGCTCCACTACGGTCAACGGGACATTGGTGCCCGGGTTAAAAAGCCTCGTGTTGGGTGGCAGAAAAGC	TAQMAN
>gb|U18748.1|PRU18748 Porcine reproductive and respiratory syndrome virus ISU-1894 putative matrix protein and putative nucleocapsid protein genes, complete cds	U18748.1	210	482	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTGGGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCACCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACATTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCTTTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCGTCCGGCGTCCCGGCTCCACTACGGTCAACGGGACATTGGTGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAAGC	TAQMAN
>gb|AF299407.1|AF299407 Porcine reproductive and respiratory syndrome virus isolate pig27-84bo ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299407.1	2732	3004	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTGGGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCACCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACATTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCTTTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCGTCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGGTGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAAGC	TAQMAN
>gb|AF299408.1|AF299408 Porcine reproductive and respiratory syndrome virus isolate pig3-150bo ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299408.1	2879	3151	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTGGGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCACCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACATTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCTTTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCGTCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGGTGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAAGC	TAQMAN
>gb|AF299409.1|AF299409 Porcine reproductive and respiratory syndrome virus isolate pig3-7o ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299409.1	2879	3151	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTGGGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCACCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACATTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCTTTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCGTCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGGTGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAAGC	TAQMAN
>gb|AF299410.1|AF299410 Porcine reproductive and respiratory syndrome virus isolate pig3-84bo ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3	AF299410.1	2879	3151	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTGGGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCACCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACATTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT	TAQMAN

envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds						TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	
>gb|AF299411.1|AF299411 Porcine reproductive and respiratory syndrome virus isolate pig3-84o ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299411.1	2879	3151	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AF299412.1|AF299412 Porcine reproductive and respiratory syndrome virus isolate pig31-150bo ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299412.1	2879	3151	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AF299413.1|AF299413 Porcine reproductive and respiratory syndrome virus isolate pig31-7o ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299413.1	2879	3151	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AF299414.1|AF299414 Porcine reproductive and respiratory syndrome virus isolate pig31-84bo ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299414.1	2879	3151	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGCCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AF299415.1|AF299415 Porcine reproductive and respiratory syndrome virus isolate pig32-56o ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299415.1	2809	3081	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AY585241.1| Porcine reproductive and respiratory syndrome virus strain PL97-1, complete genome	AY585241.1	14584	14856	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTAAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AF299416.1|AF299416 Porcine reproductive and respiratory syndrome virus isolate pig32-7o ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299416.1	2879	3151	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AY612613.1| Porcine respiratory and reproductive syndrome virus strain PL97-1/LP1, complete genome	AY612613.1	14584	14856	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTAAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AY857635.1| Porcine reproductive and respiratory syndrome virus isolate GDCZ1 GP3 envelope protein (ORF3), GP5 envelope protein (ORF5), and M matrix protein (ORF6) genes, complete cds	AY857635.1	1562	1834	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTTCTTTG GGGAGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGTAAGTACA TTCTGGCCCCTGCCCACCACGTCGAAAGTGCCGCGGGCT TTCATCCGATTACGGCAAATGATAACCACGCGTTTGTCG TCCGGCGTCCCGGCTCCACTACGGTTAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AY857636.1| Porcine reproductive and respiratory syndrome virus isolate GDCZ2 GP3 envelope protein (ORF3), GP5 envelope protein (ORF5), and M matrix protein (ORF6) genes, complete cds	AY857636.1	1562	1834	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTTCTTTG GGGAGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTCGAAAGTGCCGCGGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTTAGCGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AY857637.1| Porcine reproductive and respiratory syndrome virus isolate GDHZ1 GP3 envelope protein (ORF3), GP5 envelope protein (ORF5), and M matrix protein (ORF6) genes, complete cds	AY857637.1	1562	1834	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTTCTTTG GGGAGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCGGGCT TTCATCCGATTGCGGCAAGTGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTTAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA	TAQMAN

>gb|AY858585.1| Porcine reproductive and respiratory syndrome virus isolate GDZC1 GP3 protein, GP5 envelope protein, and M matrix protein genes, complete cds	AY858585.1	1562	1834	273	Plus - Minus - Minus	GC PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTTCTTTG GGGAGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTCGAAAGTGCCGCGGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AY858586.1| Porcine reproductive and respiratory syndrome virus isolate GDDG1 GP3 protein, GP5 envelope protein, and M matrix protein genes, complete cds	AY858586.1	1562	1834	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AY885248.1| Porcine reproductive and respiratory syndrome virus GP2 envelope protein, GP3 envelope glycoprotein, GP4 envelope protein, GP5 envelope protein, GP6 envelope glycoprotein, and GP7 envelope glycoprotein genes, complete cds	AY885248.1	2584	2856	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTAAAGAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|DQ056373.1| Porcine respiratory and reproductive syndrome virus strain 01NP1.2, complete genome	DQ056373.1	14584	14856	273	Plus - Minus - Minus	PARTIAL GGTGTACTCAGCCATAGAAACCTGGAAATTCATCACCT CCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACATTC TGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGGTTTC ATCCGATTGCGGCAAATGATAACCACGCATTTGTCGTCC GGCGTCCCGGCTCCACTACGGTCAACGGCACATTGGTGC CCGGGTTAAAAAGCCTCGTGTTGGGTGGCAGAAAAGC	TAQMAN
>gb|DQ120519.1| Porcine respiratory and reproductive syndrome virus strain YA1 membrane protein (M) mRNA, complete cds	DQ120519.1	210	482	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTTCTTTG GGGAGTCTACTCGGCCATAGGAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTCGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATTACCACGCATTTGTCG TCCGGCGTCCCGGCTCCGCTACGGTTAACGGCACATTGG TGCCCGGGTTGAATGGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AF299417.1|AF299417 Porcine reproductive and respiratory syndrome virus isolate pig32-84bo ORF1b polyprotein gene, partial cds; GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, and membrane protein M genes, complete cds; and nucleocapsid protein N gene, partial cds	AF299417.1	2720	2992	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AF317692.1|AF317692 Porcine reproductive and respiratory syndrome virus structural protein M gene, partial cds; and structural protein N gene, complete cds	AF317692.1	205	477	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AF325691.1|AF325691 Porcine reproductive and respiratory syndrome virus isolate NVSL 97-7985 IA 1-4-2, complete genome	AF325691.1	14562	14834	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTTCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TCCTGGCCCCTGCCCACCACGTCGAAAGTGCCGCGGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTTAACGGCACATTGG TGCCCGGGTTGAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AF331831.1|AF331831 Porcine reproductive and respiratory syndrome virus BJ-4, complete genome	AF331831.1	14582	14854	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTAAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AF355104.1|AF355104 Porcine reproductive and respiratory syndrome virus structural protein M gene, complete cds	AF355104.1	210	482	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAATTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTAAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AF396833.1| Porcine reproductive and respiratory syndrome virus isolate NADC-8 (E) GP2 glycoprotein, GP3 glycoprotein, GP4 glycoprotein, GP5 glycosylated envelope protein, membrane protein, and nucleocapsid protein mRNAs, complete cds	AF396833.1	2512	2784	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTTTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTAAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN
>gb|AF396834.1| Porcine reproductive and respiratory syndrome virus isolate NADC-8 (251) GP2 glycoprotein, GP3 glycoprotein, GP4 glycoprotein, GP5 glycosylated envelope protein, membrane protein, and nucleocapsid protein mRNAs, complete cds	AF396834.1	2512	2784	273	Plus - Minus - Minus	PARTIAL GGTCGCGCTCACTATGGGAGCAGTAGTTGCACTCCTTTG GGGGGTGTACTCAGCCATAGAAACCTGGAAATTCATCA CCTCCAGATGCCGTCTGTGCTTGCTAGGCCGCAAGTACA TTCTGGCCCCTGCCCACCACGTTGAAAGTGCCGCAGGCT TTCATCCGATTGCGGCAAATGATAACCACGCATTTGTCG TCCGGCGTCCCGGCTCCACTACGGTCAACGGCACATTGG TGCCCGGGTTAAAAAGCCTCGTGTTGGGTGGCAGAAAA GC	TAQMAN

sig_candidate_1807703	>gb|AF046869.1|AF046869 Porcine reproductive and respiratory syndrome virus isolate 16244B, 2/18/97(Nebraska)pass.3, complete genome	AF046869.1	5045	5317	273	Plus - Minus - Minus	PARTIAL CTAACCCGTTTGCCGTCCCTGGCTACGGACCTGGCTCTC TGTGCACGTCCAGATTGTGCATTTCCCAACACGGCCTTA CCCTGCCCTTGACAGCACTTGTGGCGGGATTCGGCATTC AAGAAATTGCCTTGGTCGTTTTGATTTTTGTTTCCATCGG AGGCATGGCTCATAGGTTGAGCTGTAAGGCTGACATGC TGTGCATTTTGCTTGCAATTGCCAGCTATGTTTGGGTAC CTCTCACCTGGTTGCTTTGTGTGTTTCCTTGCTGGTTGC	TAQMAN
23 total sequence hits	>gb|AF066183.4| Porcine reproductive and respiratory syndrome virus RespPRRS MLV, complete genome	AF066183.4	5046	5318	273	Plus - Minus - Minus	PARTIAL CTAACCCGTTTGCCGTCCCTGGCTACGGACCTGGCTCTC TCTGCACGTCCAGATTGTGCATTTCCCAACACGGCCTTA CCCTGCCCTTGACAGCACTTGTGGCGGGATTCGGTATTC AAGAAATTGCCTTGGTCGTTTTGATTTTTGTTTCCATCGG AGGCATGGCTCATAGGTTGAGCTGTAAGGCTGACATGC TGTGTGTTTTGCTTGCAATTGCCAGCTATGTTTGGGTACC TCTTACCTGGTTGCTTTGTGTGTTTCCTTGCTGGTTGC	TAQMAN
	>gb|AF159149.1|AF159149 Porcine reproductive and respiratory syndrome virus isolate MLV RespPRRS/Repro, complete genome	AF159149.1	5021	5293	273	Plus - Minus - Minus	PARTIAL CTAACCCGTTTGCCGTCCCTGGCTACGGACCTGGCTCTC TCTGCACGTCCAGATTGTGCATTTCCCAACACGGCCTTA CCCTGCCCTTGACAGCACTTGTGGCGGGATTCGGTATTC AAGAAATTGCCTTGGTCGTTTTGATTTTTGTTTCCATCGG AGGCATGGCTCATAGGTTGAGCTGTAAGGCTGACATGC TGTGTGTTTTGCTTGCAATTGCCAGCTATGTTTGGGTACC TCTTACCTGGTTGCTTTGTGTGTTTCCTTGCTGGTTGC	TAQMAN
	>gb|AF176348.2| Porcine reproductive and respiratory syndrome virus isolate PA8 complete genome	AF176348.2	5045	5317	273	Plus - Minus - Minus	PARTIAL CTAACCCGTTTGCCGTCCCTGGCTACGGACCTGGCTCTC TCTGCACGTCCAGATTGTGCATTTCCCAACACGGCCTTA CCCTGCCCTTGACAGCACTTGTGGCGGGATTCGGTATTC AAGAAATTGCCTTGGTCGTTTTGATTTTTGTTTCCATCGG AGGCATGGCTCATAGGTTGAGCTGTAAGGCTGACATGC TGTGTGTTTTGCTTGCAATTGCCAGCTATGTTTGGGTACC TCTTACCTGGTTGCTTTGTGTGTTTCCTTGCTGGTTGC	TAQMAN
	>gb|AF331831.1|AF331831 Porcine reproductive and respiratory syndrome virus BJ-4, complete genome	AF331831.1	5043	5315	273	Plus - Minus - Minus	PARTIAL CTAACCCGTTTGCCGTCCCTGGCTACGGACCTGGCTCTC TCTGCACGTCCAGATTGTGCATTTCCCAACACGGCCTTA CCCTGCCCTTGACAGCACTTGTGGCGGGATTCGGTATTC AAGAAATTGCCTTGGTCGTTTTGATTTTTGTTTCCATCGG AGGCATGGCTCATAGGTTGAGCTGTAAGGCTGACATGC TGTGTGTTTTGCTTGCAATTGCCAGCTATGTTTGGGTACC TCTTACCTGGCTGCTTTGTGTGTTTCCTTGCTGGTTGC	TAQMAN
	>gb|AF184212.1|AF184212 Porcine reproductive and respiratory syndrome virus strain SP, complete genome	AF184212.1	5156	5426	271	Plus - Minus - Minus	PARTIAL AACCCGTTTGCCGTCCCTGGCTACGGACCTGGCTCTCTC TGCACGTCCAGATTGTGCATCTCCCAACATGGCCTTACC CTACCCTTGACAGCACTTGTGGCGGGATTCGGCTTTCAG GAAATTGCCTTGGTTATTTTGATTTTTGTTTCTATCGGAG GCATGGCTCATAGATTGAGTTGCAAGGCTGATATGCTGT GTATTTTGTTTGCAATCGCCAGCTATGTTTGGGTACCTCT TACCTGGTTGCTTTGTGTGTTTCCTTGCTGGTTGC	TAQMAN
	>gb|AY150564.1| Porcine reproductive and respiratory syndrome virus isolate VR-2332, complete genome	AY150564.1	5046	5318	273	Plus - Minus - Minus	PARTIAL CTAACCCGTTTGCCGTCCCTGGCTACGGACCTGGCTCTC TCTGCACGTCCAGATTGTGCATTTCCCAACACGGCCTTA CCCTGCCCTTGACAGCACTTGTGGCGGGATTCGGTATTC AAGAAATTGCCTTGGTCGTTTTGATTTTTGTTTCCATCGG AGGCATGGCTCATAGGTTGAGCTGTAAGGCTGACATGC TGTGTGTTTTGCTTGCAATTGCCAGCTATGTTTGGGTACC TCTTACCTGGTTGCTTTGTGTGTTTCCTTGCTGGTTGC	TAQMAN
	>gb|DQ459471.1| Porcine respiratory and reproductive syndrome virus isolate S1, complete genome	DQ459471.1	5045	5317	273	Plus - Minus - Minus	PARTIAL CTAACCCGTTTGCCGTCCCTGGCTACGGACCTGGCTCTC TCTGCACGTCCAGATTGTGCATTTCCCAACACGGCCTTA CCCTGCCCTTGACAGCACTTGTGGCGGGATTCGGTATTC AAGAAATTGCCTTGGTCGTTTTGATTTTTGTTTCCATCGG AGGCATGGCTCATAGGTTGAGCTGTAAGGCTGACATGC TGTGTGTTTTGCTTGCAATTGCCAGCTATGTTTGGGTACC TCTTACCTGGTTGCTTTGTGTGTTTCCTTGCTGGTTGC	TAQMAN
	>gb|AF303354.1|AF303354 Porcine reproductive and respiratory syndrome virus strain 17704A polyprotein ORF1ab gene, complete cds	AF303354.1	4856	5128	273	Plus - Minus - Minus	PARTIAL CTAACCCGTTTGCCGTCCCTGGCTACGGACCTGGCTCTC TCTGCACGTCCAGATTGTGCATTTCCCAACACGGCCTTA CCCTGCCCTTGACAGCACTTGTGGCGGGATTCGGTATTC AAGAAATTGCCTTGGTCGTTTTGATTTTTGTTTCCATCGG AGGCATGGCTCATAGGTTGAGCTGTAAGGCTGACATGC TGTGTGTTTTGCTTGCAATTGCCAGCTATGTTTGGGTACC TCTTACCTGGTTGCTTTGTGTGTTTCCTTGCTGGTTGC	TAQMAN
	>gb|AY457635.1| Porcine reproductive and respiratory syndrome virus HN1, complete genome	AY457635.1	5044	5316	273	Plus - Minus - Minus	PARTIAL CTAACCCGTTTGCCGTCCCTGGCTACGGACCTGGCTCTC TCTGCACGTCCAGATTGTGCATTTCCCAACACGGCCTTA CCCTGCCCCTGACAGCACTTGTGGCGGGATTCGGTATTC AAGAAATTGCCTTGGTCGTTTTGATTTTTGTTTCCATCGG AGGCATGGCTCATAGGTTGAGCTGTAAGGCTGACATGC TGTGTGTTTTGCTTGCAATTGCCAGCTATGTTTGGGTACC TCTCACCTGGTTGCTTTGTGTGTTTCCTTGCTGGTTGC	TAQMAN
	>gb|DQ779791.1| Porcine respiratory and reproductive syndrome virus strain Prime Pac, complete genome	DQ779791.1	5156	5426	271	Plus - Minus - Minus	PARTIAL AACCCGTTTGCCGTCCCTGGCTACGGACCTGGCTCTCTC TGCACGTCCAGATTGTGCATCTCCCAACATGGCCTTACC CTACCCTTGACAGCACTTGTGGCGGGATTCGGCTTTCAG GAAATTGCCTTGGTTATTTTGATTTTTGTTTCTATCGGAG GCATGGCTCATAGATTGAGTTGCAAGGCTGATATGCTGT GTATTTTGTTTGCAATCGCCAGCTATGTTTGGGTACCTCT TACCTGGTTGCTTTGTGTGTTTCCTTGCTGGTTGC	TAQMAN
	>gb|AF303355.1|AF303355 Porcine reproductive and respiratory syndrome virus strain 17738B polyprotein ORF1ab gene, complete cds	AF303355.1	4856	5128	273	Plus - Minus - Minus	PARTIAL CTAACCCGTTTGCCGTCCCTGGCTACGGACCTGGCTCTC TCTGCACGTCCAGATTGTGCATTTCCCAACACGGCCTTA CCCTGCCCTTGACAGCACTTGTGGCGGGATTCGGTATTC AAGAAATTGCCTTGGTCGTTTTGATTTTTGTTTCCATCGG AGGCATGGCTCATAGGTTGAGCTGTAAGGCTGACATGC TGTGTGTTTTGCTTGCAATTGCCAGCTATGTTTGGGTACC TCTTACCTGGTTGCTTTGTGTGTTCCCTTGCTGGTTGC	TAQMAN
	>gb|AY585241.1| Porcine reproductive and respiratory syndrome virus strain PL97-1, complete genome	AY585241.1	5045	5317	273	Plus - Minus - Minus	PARTIAL CTAACCCGTTTGCCGTCCCTGGCTACGGACCTGGCTCTC TCTGCACGTCCAGATTGTGCATTTCCCAACACGGCCTTA CCCTGCCCTTGACAGCACTTGTGGCGGGATTCGGTATTC AAGAAATTGCCTTGGTCGTTTTGATTTTTGTTTCCATCGG AGGCATGGCTCATAGGTTGAGCTGTAAGGCTGACATGC TGTGTGTTTTGCTTGCAATTGCCAGCTATGTTTGGGTACC TCTTACCTGGTTGCTTTGTGTGTTTCCTTGCTGGTTGC	TAQMAN
	>gb|EF153486.1| Porcine respiratory and reproductive syndrome virus isolate CC-1, complete genome	EF153486.1	5045	5317	273	Plus - Minus - Minus	PARTIAL CTAACCCGTTTGCCGTCCCTGGCTACGGACCTGGCTCTC TCTGCACGCCCAGATTGTGCATTTCCCAACACGGCCTTA	TAQMAN

	>gb|AF303356.1|AF303356 Porcine reproductive and respiratory syndrome virus strain 19407B polyprotein ORF1ab gene, complete cds	AF303356.1	4844	5116	273	Plus - Minus - Minus	CCCTGCCCTTGACAGCACTTGTGGCGGGATTCGGTATTC AAGAAATTGCCTTGGTCGTTTTGATTTTTGTTTCCATCGG AGGCATGGCTCATAGGTTGAGCTGTAAGGCTGACATGC TGTGTGTTTTGCTTGCAATTGCCAGCTATGTTTGGGTACC TCTTACCTGGTTGCTTTGTGTGTTTCCTTGCTGGTTGC PARTIAL CTAACCCGTTTGCCGTCCCTGGCTACGGACCTGGCTCTC TCTGCACGTCCAGATTGTGCATTTCCCAACACGGCCTTA CCCTGCCCTTGACAGCACTTGTGGCGGGATTCGGTATTC AAGAAATTGCCTTGGTCGTTTTGATTTTTGTTTCCATCGG AGGCATGGCTCATAGGTTGAGCTGTAAGGCTGACATGC TGTGTGTTTTGCTTGCAATTGCCAGCTATGTTTGGGTACC TCTTACCTGGTTGCTTTGTGTGTTTCCTTGCTGGTTGC	TAQMAN
	>gb|AY612613.1| Porcine respiratory and reproductive syndrome virus strain PL97-1/LP1, complete genome	AY612613.1	5045	5317	273	Plus - Minus - Minus	PARTIAL CTAACCCGTTTGCCGTCCCTGGCTACGGACCTGGCTCTC TCTGCACGTCCAGATTGTGCATTTCCCAACACGGCCTTA CCCTGCCCTTGACAGCACTTGTGGCGGGATTCGGTATTC AAGAAATTGCCTTGGTCGTTTTGATTTTTGTTTCCATCGG AGGCATGGCTCATAGGTTGAGCTGTAAGGCTGACATGC TGTGTGTTTTGCTTGCAATTGCCAGCTATGTTTGGGTACC TCTTACCTGGTTGCTTTGTGTGTTTCCTTGCTGGTTGC	TAQMAN
	>gb|U87392.3|PRU87392 Porcine reproductive and respiratory syndrome virus strain VR-2332, complete genome	U87392.3	5045	5317	273	Plus - Minus - Minus	PARTIAL CTAACCCGTTTGCCGTCCCTGGCTACGGACCTGGCTCTC TCTGCACGTCCAGATTGTGCATTTCCCAACACGGCCTTA CCCTGCCCTTGACAGCACTTGTGGCGGGATTCGGTATTC AAGAAATTGCCTTGGTCGTTTTGATTTTTGTTTCCATCGG AGGCATGGCTCATAGGTTGAGCTGTAAGGCTGACATGC TGTGTGTTTTGCTTGCAATTGCCAGCTATGTTTGGGTACC TCTTACCTGGTTGCTTTGTGTGTTTCCTTGCTGGTTGC	TAQMAN
	>gb|AF303357.1|AF303357 Porcine reproductive and respiratory syndrome virus strain Ingelvac PRRS Vet vaccine polyprotein ORF1ab gene, complete cds	AF303357.1	4856	5128	273	Plus - Minus - Minus	PARTIAL CTAACCCGTTTGCCGTCCCTGGCTACGGACCTGGCTCTC TCTGCACGTCCAGATTGTGCATTTCCCAACACGGCCTTA CCCTGCCCTTGACAGCACTTGTGGCGGGATTCGGTATTC AAGAAATTGCCTTGGTCGTTTTGATTTTTGTTTCCATCGG AGGCATGGCTCATAGGTTGAGCTGTAAGGCTGACATGC TGTGTGTTTTGCTTGCAATTGCCAGCTATGTTTGGGTACC TCTTACCTGGTTGCTTTGTGTGTTTCCTTGCTGGTTGC	TAQMAN
	>gb|DQ056373.1| Porcine respiratory and reproductive syndrome virus strain 01NP1.2, complete genome	DQ056373.1	5045	5317	273	Plus - Minus - Minus	PARTIAL CTAACCCGTTTGCCGTCCCTGGCTACGGACCTGGCTCTC TCTGCACGTCCAGATTGTGCATTTACCAACACGGCCTTA CCCTGCCCTTGACAGCACTTGTGGCGGGATTCGGTATTC AAGAAATTGCCTTGGTCGTTTTGATTTTTGTTTCCATCGG AGGCATGGCTCATAGGTTGAGCTGTAAGGCTGACATGC TGTGTGTTTTGCTTGCAATTGCCAGCTATGTTTGGGTACC TCTTACCTGGTTGCTTTGTGTGTTTCCTTGCTGGTTGC	TAQMAN
	>gb|DQ176019.1| Porcine respiratory and reproductive syndrome virus isolate MN184A, complete genome	DQ176019.1	4653	4925	273	Plus - Minus - Minus	PARTIAL CTAACCCGTTTGCCGTCCCTGTCTACGGGCCTGGCTCTC TTTGCACGTCCAGGTTGTGCATCTCCCAGCATGGCCTTA CTCTGCCTTTAACAGCGCTTGTGGCGGGGTTTGGTATTC AGGAAGTTGCTTTGGTTGTTTTAATCTTTGCTTCCATCGG GGGTATGGCTCACAGGTTGAGTTGCAAGGCCGATGTGC TGTGCATTCTGCTTGCAATTGCCAGCTATGTTTGGGTAC CCTTCACCTGGTTGCTTTGTGTGTTTCCTTGCTGGTTGC	TAQMAN
	>gb|DQ176020.1| Porcine respiratory and reproductive syndrome virus isolate MN184B, complete genome	DQ176020.1	4653	4925	273	Plus - Minus - Minus	PARTIAL CTAACCCGTTTGCCGTCCCTGTCTACGGGCCTGGCTCTC TTTGCACGTCCAGGTTGTGCATCTCCCAGCATGGCCTTA CTCTGCCTTTAACAGCGCTTGTGGCGGGGTTTGGCATTC AGGAAGTTGCTTTGGTTGTTTTAATCTTTACTTCCATCGG GGGTATGGCTCACAGGTTGAGCTGCAAGGCCGATGTGC TGTGTATTCTGCTTGCAATTGCCAGCTATGTTTGGGTAC CCTTCACCTGGTTGCTTTGTGTGTTTCCTTGCTGGTTGC	TAQMAN
	>gb|DQ176021.1| Porcine respiratory and reproductive syndrome virus clone VR-2332 V7, complete genome	DQ176021.1	5045	5317	273	Plus - Minus - Minus	PARTIAL CTAACCCGTTTGCCGTCCCTGGCTACGGACCTGGCTCTC TCTGCACGTCCAGATTGTGCATTTCCCAACACGGCCTTA CCCTGCCCTTGACAGCACTTGTGGCGGGATTCGGTATTC AAGAAATTGCCTTGGTCGTTTTGATTTTTGTTTCCATCGG AGGCATGGCTCATAGGTTGAGCTGTAAGGCTGACATGC TGTGTGTCTTGCTTGCAATTGCCAGCTATGTTTGGGTAC CTCTTACCTGGTTGCTTTGTGTGTTTCCTTGCTGGTTGC	TAQMAN
	>gb|DQ217415.1| Porcine respiratory and reproductive syndrome virus strain VR-2332 clone pVR-V7, complete genome	DQ217415.1	5031	5303	273	Plus - Minus - Minus	PARTIAL CTAACCCGTTTGCCGTCCCTGGCTACGGACCTGGCTCTC TCTGCACGTCCAGATTGTGCATTTCCCAACACGGCCTTA CCCTGCCCTTGACAGCACTTGTGGCGGGATTCGGTATTC AAGAAATTGCCTTGGTCGTTTTGATTTTTGTTTCCATCGG AGGCATGGCTCATAGGTTGAGCTGTAAGGCTGACATGC TGTGTGTCTTGCTTGCAATTGCCAGCTATGTTTGGGTAC CTCTTACCTGGTTGCTTTGTGTGTTTCCTTGCTGGTTGC	TAQMAN
[redacted]								
sig_candidate_1807706	>gb|AF331831.1|AF331831 Porcine reproductive and respiratory syndrome virus BJ-4, complete genome	AF331831.1	12228	12513	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAATCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
	>gb|AF396833.1| Porcine reproductive and respiratory syndrome virus isolate NADC-8 (E) GP2 glycoprotein, GP3 glycoprotein, GP4 glycoprotein, GP5 glycosylated envelope protein, membrane protein, and nucleocapsid protein mRNAs, complete cds	AF396833.1	158	443	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTGCGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCCTATGAGGCCTTTC TCTCTCAGTGCCAGGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGTTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAAGCAGGACAGGCTGCCTGGAAACAGGTA GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
69 total sequence hits	>gb|AF396834.1| Porcine reproductive and respiratory syndrome virus isolate NADC-8 (251) GP2 glycoprotein, GP3 glycoprotein, GP4 glycoprotein, GP5 glycosylated envelope protein, membrane protein, and nucleocapsid protein mRNAs, complete cds	AF396834.1	158	443	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTGCGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCCTATGAGGCCTTTC TCTCTCAGTGCCAGGTGGACATTCCCACCTGGGGAACCA AACATCCTTTGGGGATGTTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAAGCAGGACAGGCTGCCTGGAAACAGGTA GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
	>gb|AF396835.1| Porcine reproductive and respiratory syndrome virus isolate NADC-8 (252p) GP2	AF396835.1	158	443	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTGCGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCCTATGAGGCCTTTC	TAQMAN

glycoprotein, GP3 glycoprotein, GP4 glycoprotein, GP5 glycosylated envelope protein, membrane protein, and nucleocapsid protein mRNAs, complete cds						TCTCTCAGTGCCAGGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGTTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAAGCAGGACAGGCTGCCTGGAAACAGGTA GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	
>gb|AF396838.1| Porcine reproductive and respiratory syndrome virus isolate NADC-9 (252p) GP2 glycoprotein, GP3 glycoprotein, GP4 glycoprotein, GP5 glycosylated envelope protein, membrane protein, and nucleocapsid protein mRNAs, complete cds	AF396838.1	158	443	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTGCGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCCTATGAGGCCTTTC TCTCTCAGTGCCAGGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGTTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAAGCAGGACAGGCTGCCTGGAAACAGGTA GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|AY150564.1| Porcine reproductive and respiratory syndrome virus isolate VR-2332, complete genome	AY150564.1	12231	12516	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAAGCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|AY262352.1| PRRSV HB-2(sh)/2002, complete genome	AY262352.1	12197	12480	284	Plus - Minus - Minus	PARTIAL TGGTTTGCTCCGCGATATTCCGTGCGCGCCCTACCGTTC ACCCTGAGCAATTACAGAAGATCCTATGAGGCCTTTCTT TCTCAATGCCAGGTGGACATTCCCACCTGGGGCGTTAAA CACCCTTTGGGGAATACTTTGGCACCATAAGGTGTCAACC CTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCATC ATGGAAAAAGCAGGGCAGGCTGCCTGGAAACAGGTGGT GAGCGAGGCTACACTGTCTCGCATTAGTAATTTGGATGT GGTGGCTCATTT	TAQMAN
>gb|AY387691.1| Porcine reproductive and respiratory syndrome virus nonfunctional envelope protein GP2 gene, partial sequence	AY387691.1	160	443	284	Plus - Minus - Minus	PARTIAL TGGTTTGCTCCGCGATATTCCGTGCGCGCCCTGCCGTTC ACCCTGAGCAATTACAGAAGATCCTATGAGGCCTTTCTT TCTCAGTGCCAGGTGGACATTCCTACCTGGGGAGTTAAA CACCCTTTGGGGATGTTTTGGCACCATAAGGTGTCAACC CTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCATC ATGGAGAAAGCACGGCAGGCTGCCTGGAAACAGGTGGT GAGCGAGGCTACGCTGTTTCGCATTACTAGTTTGGATGT GGTGGCTCATTT	TAQMAN
>gb|AY457635.1| Porcine reproductive and respiratory syndrome virus HN1, complete genome	AY457635.1	12229	12514	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCTCTGCCAT TCACTCTGAACAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATACTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAAATCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|AY569972.1| Porcine reproductive and respiratory syndrome virus isolate 1530B envelope glycoprotein GP2, envelope glycoprotein GP3, envelope glycoprotein GP4, envelope glycoprotein GP5, matrix protein, and nucleocapsid protein genes, complete cds	AY569972.1	158	443	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TCTCTCAGTGCCAGGTGGACATTCCCACCTGGGGGATTA AACATCCTTTAGGGATGTTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGGCA TCATGGAAAAAGCAGGACAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCCACGCTGTCTCGCATTAGTGGTCTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|AY585241.1| Porcine reproductive and respiratory syndrome virus strain PL97-1, complete genome	AY585241.1	12230	12515	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGTTGCTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAATCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|AY612613.1| Porcine respiratory and reproductive syndrome virus strain PL97-1/LP1, complete genome	AY612613.1	12230	12515	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGTTGCTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAATCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|AY885248.1| Porcine reproductive and respiratory syndrome virus GP2 envelope protein, GP3 envelope glycoprotein, GP4 envelope protein, GP5 envelope protein, GP6 envelope glycoprotein, and GP7 envelope glycoprotein genes, complete cds	AY885248.1	230	515	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TTACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATACTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAATCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>emb|AJ223079.1|PRR223079 Porcine respiratory and reproductive syndrome virus ORFs 2 to 7, isolate Danish DK3506-12	AJ223079.1	158	443	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTGCGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACACCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAAGCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>emb|AJ223080.1|PRR223080 Porcine respiratory and reproductive syndrome virus ORFs 2 to 7, isolate Danish DK5163-17	AJ223080.1	158	443	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTGCGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACACCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAAGCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>emb|AJ223081.1|PRR223081 Porcine respiratory and reproductive syndrome virus ORFs 2 to 7, isolate Danish DK5163-23	AJ223081.1	158	443	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTGCGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA	TAQMAN

>gb|AF066384.1|AF066384 Porcine reproductive and respiratory syndrome virus RNA-dependent RNA polymerase gene, partial cds; and GP2 envelope protein, GP3 envelope protein, GP4 envelope protein, GP5 envelope protein, M matrix protein, and N nucleocapsid protein genes, complete cds	AF066384.1	3609	3894	286	Plus - Minus - Minus	AACACCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAAGCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT PARTIAL ATTGGTTTGCTCCGCGATACTCCGTGCACGCCCTGCCTT TCACTCTGAGCAGTTACAGAAGATCCTATGAGGCCTTTC TTTCCCTGTGTCAGGTGGACATTCCCACCTGGGGAATCA AACATCCTCTGGGGGTGCTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAAGCAGGACAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCCACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAAGCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>emb|AJ223082.1|PRR223082 Porcine respiratory and reproductive syndrome virus ORFs 2 to 7, isolate MLV RespPRRS	AJ223082.1	158	443	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTGCGCGCCCTGCCGT TCACTCTGAGCAATTACAGAAGATCCTATGAGGCCTTTC TCTCCCAGTGCCAGGTGGATATCCCCACCTGGGGAACCA AACATCCTTTGGGGATGTTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGGATGTACCGCA TCATGGAAAAAGCAGGACAAGCTGCCTGGAAACAGGTG GTGAGCGAGGCCACGTTGTCTCGCATCAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|AF121131.1|AF121131 Porcine reproductive and respiratory syndrome virus envelope protein GP2, envelope protein GP3, envelope protein GP4, envelope protein, matrix protein, and nucleocapsid protein genes, complete cds	AF121131.1	184	469	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGTTGTGGCACCATAAGGTGTCAA CCCTGATTGATGAGATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAAGCAGGACAGGCTGCCTGGAAACAGGTG GTGAACGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|AF030306.1|AF030306 Porcine reproductive and respiratory syndrome virus envelope proteins, matrix protein, and nucleocapsid genes, complete cds	AF030306.1	239	524	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAATCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|AF159149.1|AF159149 Porcine reproductive and respiratory syndrome virus isolate MLV RespPRRS/Repro, complete genome	AF159149.1	12206	12491	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTGCGCGCCCTGCCGT TCACTCTGAGCAATTACAGAAGATCCTATGAGGCCTTTC TCTCCCAGTGCCAGGTGGATATCCCCACCTGGGGAACCA AACATCCTTTGGGGATGTTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAAGCAGGACAAGCTGCCTGGAAACAGGTG GTGAACGAGGCCACGTTGTCTCGCATCAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|AF035409.1|AF035409 Porcine reproductive and respiratory syndrome virus envelope proteins, matrix protein, and nucleocapsid protein genes, complete cds	AF035409.1	239	524	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGACACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAAGCAGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|AF176348.2| Porcine reproductive and respiratory syndrome virus isolate PA8 complete genome	AF176348.2	12230	12515	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAAGCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|AF046869.1|AF046869 Porcine reproductive and respiratory syndrome virus isolate 16244B, 2/18/97(Nebraska)pass.3, complete genome	AF046869.1	12230	12515	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCTTGATAGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAATCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACACTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|AF184212.1|AF184212 Porcine reproductive and respiratory syndrome virus strain SP, complete genome	AF184212.1	12339	12624	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTGCACGCCCTGCCTT TCACTCTGAGCAGTTACAGAAGATCCTATGAGGCCTTTC TTTCCCAGTGTCAGGTGGACATTCCCACCTGGGGAATCA AACATCCTCTGGGGGTGCTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAAGCAGGACAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCCACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|AF066066.1|PRRSVORF1 Porcine reproductive and respiratory syndrome virus minor membrane glycoprotein and glycoprotein genes, complete cds	AF066066.1	158	443	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGACACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAAGCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|AF188196.1|AF188196 Porcine reproductive and respiratory syndrome virus strain AVC19381 envelope glycoprotein GP2 and ORF2b mRNAs, complete cds	AF188196.1	158	443	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAAATCTTATGAAGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGTTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAAATCAGGACAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACCCTGTCTCGCATTAATAATTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|AF066183.4| Porcine reproductive and respiratory syndrome virus RespPRRS MLV, complete genome	AF066183.4	12231	12516	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA	TAQMAN

>gb|AF188197.1|AF188197 Porcine reproductive and respiratory syndrome virus strain ONT-43697 envelope glycoprotein GP2 and ORF2b mRNAs, complete cds	AF188197.1	158	443	286	Plus - Minus - Minus	CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAAGCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT PARTIAL ATTGGTTTACTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAATCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT PARTIAL	TAQMAN
>gb|AF204291.1|AF204291 Porcine reproductive and respiratory syndrome virus strain P1750-96 envelope glycoprotein 2a and ORF2b genes, complete cds	AF204291.1	158	443	286	Plus - Minus - Minus	ATTGGTTTGCTCCGCGATACTCCGTGCGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGCGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAAGCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT PARTIAL	TAQMAN
>gb|DQ056373.1| Porcine respiratory and reproductive syndrome virus strain 01NP1.2, complete genome	DQ056373.1	12230	12515	286	Plus - Minus - Minus	ATTGGTTTGCTCCGCGATACTCCGTACGCGCTCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAAGCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT PARTIAL	TAQMAN
>gb|AF205183.1|AF205183 Porcine reproductive and respiratory syndrome virus strain DVX355 ORF2b and envelope glycoprotein GP2 genes, complete cds	AF205183.1	158	443	286	Plus - Minus - Minus	ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAAGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGTTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAGATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAAGCAGGACAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT PARTIAL	TAQMAN
>gb|DQ176019.1| Porcine respiratory and reproductive syndrome virus isolate MN184A, complete genome	DQ176019.1	11838	12123	286	Plus - Minus - Minus	ATTCCCACCTGGGGAACTAAACATCCCTTGGGGATGCTC TGGCACCACAAGGTGTCGACCCTAATTGATGAAATGGT GTCGCGTCGAATGTACCGCATCATGGAACAAGCAGGGC AGGCTGCCTGGAAACAGGTGGTGACCGAGGCAACGTTG TCTCGTATTAGTAGCTTGGATGTGGTGGCTCATTT PARTIAL	TAQMAN
>gb|AF205184.1|AF205184 Porcine reproductive and respiratory syndrome virus strain HV-18 ORF2b and envelope glycoprotein GP2 genes, complete cds	AF205184.1	158	443	286	Plus - Minus - Minus	ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAATCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT PARTIAL	TAQMAN
>gb|DQ176020.1| Porcine respiratory and reproductive syndrome virus isolate MN184B, complete genome	DQ176020.1	11838	12123	286	Plus - Minus - Minus	ATTGGTTTGCTCCGCGATACTCCGTTCGCGCTCTGCCGTT CACCCTGAGCAATTACAGAAGATCCTATGAGGCATTTCT CTCCCAGTGCCGGACGGACATTCCCACCTGGGGAACTA AACATCCCTTGGGGATGCTCTGGCACCACAAGGTGTCG ACCCTAATTGATGAAATGGTGTCGCGTCGAATGTACCGC ACCATGGAACAAGCAGGGCAGGCTGCCTGGAGACAGGT GGTGACCGAGGCAACGTTGTCTCGTATTAGTAACTTGGA TGTGGTGGCTCATTT PARTIAL	TAQMAN
>gb|DQ176021.1| Porcine respiratory and reproductive syndrome virus clone VR-2332 V7, complete genome	DQ176021.1	12230	12515	286	Plus - Minus - Minus	ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAAGCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT PARTIAL	TAQMAN
>gb|DQ217415.1| Porcine respiratory and reproductive syndrome virus strain VR-2332 clone pVR-V7, complete genome	DQ217415.1	12216	12501	286	Plus - Minus - Minus	ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAAGCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT PARTIAL	TAQMAN
>gb|DQ246451.1| Porcine respiratory and reproductive syndrome virus strain FJ04A GP2 glycoprotein, GP3 glycoprotein, GP4 glycoprotein, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	DQ246451.1	158	443	286	Plus - Minus - Minus	ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TTACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATACTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAATCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT PARTIAL	TAQMAN
>gb|DQ380248.1| Porcine respiratory and reproductive syndrome virus isolate SQQ nonfunctional ORF2 mRNA, partial sequence	DQ380248.1	158	443	286	Plus - Minus - Minus	ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGTTGCTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA CCATGGAAAAATCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT PARTIAL	TAQMAN
>gb|DQ438982.1| Porcine respiratory and reproductive syndrome virus isolate SCQ ORF2 mRNA, complete cds	DQ438982.1	158	443	286	Plus - Minus - Minus	ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGTTGCTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA CCATGGAAAAATCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN

>gb|DQ459471.1| Porcine respiratory and reproductive syndrome virus isolate S1, complete genome	DQ459471.1	12230	12515	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATACTTTGGCACCATAAGGTGCCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAATCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|DQ779791.1| Porcine respiratory and reproductive syndrome virus strain Prime Pac, complete genome	DQ779791.1	12339	12624	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTGCACGCCCTGCCTT TCACTCTGAGCAGTTACAGAAGATCCTATGAGGCCTTTC TTTCCCAGTGTCAGGTGGACATTCCCACCTGGGGAATCA AACATCCTCTGGGGGTGCTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAAGCAGGACAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCCACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|AF205186.1|AF205186 Porcine reproductive and respiratory syndrome virus strain PL ORF2b and envelope glycoprotein GP2 genes, complete cds	AF205186.1	158	443	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTGCGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAAGCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|AF205188.1|AF205188 Porcine reproductive and respiratory syndrome virus strain GH-6 ORF2b and envelope glycoprotein GP2 genes, complete cds	AF205188.1	158	443	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAAGCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|AF290975.1|AF290975 Porcine reproductive and respiratory syndrome virus envelope glycoprotein 2a (ORF2a) and envelope protein 2b (ORF2b) genes, complete cds	AF290975.1	158	443	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAAGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAGATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAAGCAGGACAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|AF299404.1|AF299404 Porcine reproductive and respiratory syndrome virus isolate pig22-7o ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299404.1	525	810	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCTTGATAGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAATCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACACTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|AF299405.1|AF299405 Porcine reproductive and respiratory syndrome virus isolate pig22-84bo ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299405.1	366	651	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACCCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCTTGATAGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAATCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACACTGTCCCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|AF299406.1|AF299406 Porcine reproductive and respiratory syndrome virus isolate pig27-7o ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299406.1	525	810	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCTTGATAGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAATCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACACTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|AF299407.1|AF299407 Porcine reproductive and respiratory syndrome virus isolate pig27-84bo ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299407.1	378	663	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCTTGATAGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAATCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACACTGTCCCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|AF299408.1|AF299408 Porcine reproductive and respiratory syndrome virus isolate pig3-150bo ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299408.1	525	810	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACACCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCTTGATAGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAATCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACACTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|AF299409.1|AF299409 Porcine reproductive and respiratory syndrome virus isolate pig3-7o ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299409.1	525	810	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCTTGATAGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAATCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACACTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|EF153486.1| Porcine respiratory and reproductive syndrome virus isolate CC-1, complete genome	EF153486.1	12230	12515	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCTCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA	TAQMAN

>gb|AF299410.1|AF299410 Porcine reproductive and respiratory syndrome virus isolate pig3-84bo ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299410.1	525	810	286	Plus - Minus - Minus	AACATCCTTTGGGGCTGCTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAATCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	
>gb|L39364.1|PPSKS1A Porcine respiratory and reproductive syndrome virus (individual isolate KS1), mRNA sequence	L39364.1	158	443	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCTTGATAGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAAGCAGAACAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACACTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|L39366.1|PPSMN1A Porcine respiratory and reproductive syndrome virus (individual isolate MN1), mRNA sequence	L39366.1	158	443	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAG ACACTCTGAGCAATTACAGAAGATCTTATGAGGCCCTTC TTTCCCAGTGCCAAGTGGACATACCCACCTCGGGGAACTA AACATCCTTTGGGGATGCTTTGGCACCATAAGATATCAA CCCTGATTGATGAAATGGTGTCGCTTCGAATGTACCGCA TCATGGAAAAAGCAGGGCAGGCTGCCTGGAAACAGGTG GTGAACGAGGCTACCCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|L39368.1|PPSNE1A Porcine respiratory and reproductive syndrome virus (individual isolate NE1), mRNA sequence	L39368.1	158	443	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGAGCGTATGTGGCCTTTC TTTCCCAGTGCCGGGTGGACATTCCCACCTGGGGAGCTA AACATCCTTTGGGGATGTTTTGGCACCATGAGGTGTCAA CCCTGATTGATGAGATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAGAGCAGGGCAGACTGCCTGGAAACAGGTG GTGAGTGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|L39369.1|PPSSG1A Porcine respiratory and reproductive syndrome virus (individual isolate SG1), mRNA sequence	L39369.1	158	443	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTGCGCGCTCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCTCAGTGCCAGGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGTTCTGGCACCATAAGGTGTCGA CCCTGATTGATGAAATGGTGTCGCGGCGAATGTACCGC ATCATGGAAAAAGCAGGGCAGGCTGCCTGGAAACAGGT GGTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGA TGTGGTGGCTCATTT	TAQMAN
>gb|U20788.1|PRU20788 Porcine reproductive and respiratory syndrome virus ORF-2, ORF-3, and ORF-4 genes, complete cds	U20788.1	185	470	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTGCGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCCTATGAGGCCTTTC TCTCTCAGTGCCAGGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAAGCAGGACAGGCTGCCTGGAAACAGGTA GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|U34296.1|PRU34296 Porcine reproductive and respiratory syndrome virus strain ISU1894 ORF2, ORF3, ORF4 and putative envelope protein genes, complete cds	U34296.1	158	443	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTT TATCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGTTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGCATGTACCGCA TCATGGAAAAAGCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCCCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|U34297.1|PRU34297 Porcine reproductive and respiratory syndrome virus strain ISU22 ORF2, ORF3, ORF4 and putative envelope protein genes, complete cds	U34297.1	158	443	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTGCGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGTTGTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAAGCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|U34300.1|PRU34300 Porcine reproductive and respiratory syndrome virus strain ISU79 ORF2, ORF3, ORF4 putative envelope protein genes, complete cds	U34300.1	158	443	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGTTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAGATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAAGCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|U87392.3|PRU87392 Porcine reproductive and respiratory syndrome virus strain VR-2332, complete genome	U87392.3	12230	12515	286	Plus - Minus - Minus	ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAAGCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|AF299411.1|AF299411 Porcine reproductive and respiratory syndrome virus isolate pig3-84o ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299411.1	525	810	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCTTGATAGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAATCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACACTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
>gb|AF299412.1|AF299412 Porcine reproductive and respiratory syndrome virus isolate pig31-150bo ORF1b	AF299412.1	525	810	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC	TAQMAN

	polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds						TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACACCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCTTGATAGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAATCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACACTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	
	>gb|AF299413.1|AF299413 Porcine reproductive and respiratory syndrome virus isolate pig31-7o ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299413.1	525	810	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCTTGATAGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAATCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACACTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
	>gb|AF299414.1|AF299414 Porcine reproductive and respiratory syndrome virus isolate pig31-84bo ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299414.1	525	810	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCTTGATAGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAATCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACACTGTCCCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
	>gb|AF299415.1|AF299415 Porcine reproductive and respiratory syndrome virus isolate pig32-56o ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299415.1	455	740	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCTTGATAGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAATCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACACTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
	>gb|AF299416.1|AF299416 Porcine reproductive and respiratory syndrome virus isolate pig32-7o ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299416.1	525	810	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACTCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCTTGATAGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAATCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACACTGTCTCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
	>gb|AF299417.1|AF299417 Porcine reproductive and respiratory syndrome virus isolate pig32-84bo ORF1b polyprotein gene, partial cds; GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, and membrane protein M genes, complete cds; and nucleocapsid protein N gene, partial cds	AF299417.1	366	651	286	Plus - Minus - Minus	PARTIAL ATTGGTTTGCTCCGCGATACTCCGTACGCGCCCTGCCAT TCACCCTGAGCAATTACAGAAGATCTTATGAGGCCTTTC TTTCCCAGTGCCAAGTGGACATTCCCACCTGGGGAACTA AACATCCTTTGGGGATGCTTTGGCACCATAAGGTGTCAA CCTTGATAGATGAAATGGTGTCGCGTCGAATGTACCGCA TCATGGAAAAATCAGGGCAGGCTGCCTGGAAACAGGTG GTGAGCGAGGCTACACTGTCCCGCATTAGTAGTTTGGAT GTGGTGGCTCATTT	TAQMAN
[redacted]								
sig_candidate_1807709	>gb|AF095479.1|AF095479 Porcine reproductive and respiratory syndrome virus strain 17704A nucleocapsid protein gene, complete cds	AF095479.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTT AATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGG GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCA TCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTC AGC	TAQMAN
	>gb|AF095480.1|AF095480 Porcine reproductive and respiratory syndrome virus strain 17738B nucleocapsid protein gene, complete cds	AF095480.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTT AATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGG GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCA TCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTC AGC	TAQMAN
160 total sequence hits	>gb|AF095481.1|AF095481 Porcine reproductive and respiratory syndrome virus strain 17835 nucleocapsid protein gene, complete cds	AF095481.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTT AATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGG GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCA TCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTC AGC	TAQMAN
	>gb|L39364.1|PPSKS1A Porcine respiratory and reproductive syndrome virus (individual isolate KS1), mRNA sequence	L39364.1	3027	3184	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTT AATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGG GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCA TCATACTGTGCGCTTGATCCGCGTCACAGCATCACCCTC AGC	TAQMAN
	>gb|L39365.1|PPSKY1A Porcine respiratory and reproductive syndrome virus (individual isolate KY1), mRNA sequence	L39365.1	3027	3184	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTCTCGTCAATCCAGACTGCCTTT AATCAAGGCGCTGGAACTTGTACCCTGTCAGATTCAGG GAGAATAAGTTACACTGTGGAGTTTAGTTTGCCAACGCA TCACACTGTGCGCTTGATCCGCGTCACAGCATCACCCTC AGC	TAQMAN
	>gb|L39366.1|PPSMN1A Porcine respiratory and reproductive syndrome virus (individual isolate MN1), mRNA sequence	L39366.1	3027	3184	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTT AATCAAGGCGCTGGGATTTGTATCCTGTCAGATTCAGGG AGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCAT CATACTGTGCGCTTGATCCGCGTCACAGTATCACCCTCA GC	TAQMAN
	>gb|L39367.1|PPSMO1A Porcine respiratory and reproductive syndrome virus (individual isolate MO1), mRNA sequence	L39367.1	3027	3184	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTT AATCAAGGCGCTGGAACTTGTACCCTGTCAGATTCAGG GAGAATAAGTTACACTGTGGAGTTTAGTTTGCCAACGCA TCACACTGTGCGCTTGATCCGCGTCACAGCATCACCCTC AGC	TAQMAN
	>gb|L39368.1|PPSNE1A Porcine respiratory and reproductive syndrome virus (individual isolate NE1), mRNA sequence	L39368.1	3028	3185	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTT AATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGG GAGAATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCA TCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTC AGC	TAQMAN
	>gb|L39369.1|PPSSG1A Porcine respiratory and reproductive syndrome virus (individual isolate SG1), mRNA sequence	L39369.1	3027	3184	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTC AATCAGGGCGCTGGAACTTGTACCCTGTCAGATTCAGG GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCGACGCA TCATACTGTGCGCCTGATCCGCGTTACAGCATCACCCTC AGC	TAQMAN
	>gb|L40898.1|PPSORFS Porcine respiratory and reproductive syndrome virus mRNA, complete ORF's 3-7	L40898.1	2521	2678	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACAGCCTTT AATCAAGGCGCTGGAACTTGCACCCTGTCAGATTCAGG GAGGATAAGTTACGCTGTGGAGTTTAGTTTGCCTACGCA TCATACTGTGCGCCTGATTCGCGTCACAGCATCACCCTC AGC	TAQMAN

>gb|U02095.1|PRU02095 Porcine reproductive and respiratory syndrome virus IAF-exp91 nucleocapsid protein gene, complete cds	U02095.1	218	375	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACAGCCTTTAATCAAGGCGCTGGAACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACGCTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATTCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|U03040.1|PRU03040 Porcine reproductive and respiratory syndrome virus putative envelope and nucleocapsid protein genes, complete cds	U03040.1	1737	1894	158	Plus - Minus - Minus	GAGCGTCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|U18748.1|PRU18748 Porcine reproductive and respiratory syndrome virus ISU-1894 putative matrix protein and putative nucleocapsid protein genes, complete cds	U18748.1	725	882	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCAACGCATCATACTGTGCGCTTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF095482.1|AF095482 Porcine reproductive and respiratory syndrome virus strain 17739 nucleocapsid protein gene, complete cds	AF095482.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF095483.1|AF095483 Porcine reproductive and respiratory syndrome virus strain 17839 nucleocapsid protein gene, complete cds	AF095483.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF095484.1|AF095484 Porcine reproductive and respiratory syndrome virus strain 17875 nucleocapsid protein gene, complete cds	AF095484.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF095485.1|AF095485 Porcine reproductive and respiratory syndrome virus strain 17876 nucleocapsid protein gene, complete cds	AF095485.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF095486.1|AF095486 Porcine reproductive and respiratory syndrome virus strain 18013 nucleocapsid protein gene, complete cds	AF095486.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF095487.1|AF095487 Porcine reproductive and respiratory syndrome virus strain 18027 nucleocapsid protein gene, complete cds	AF095487.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF095488.1|AF095488 Porcine reproductive and respiratory syndrome virus strain 18031 nucleocapsid protein gene, complete cds	AF095488.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF095489.1|AF095489 Porcine reproductive and respiratory syndrome virus strain 18033 nucleocapsid protein gene, complete cds	AF095489.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGAAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF095490.1|AF095490 Porcine reproductive and respiratory syndrome virus strain 18253 nucleocapsid protein gene, complete cds	AF095490.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF095491.1|AF095491 Porcine reproductive and respiratory syndrome virus strain 18338 nucleocapsid protein gene, complete cds	AF095491.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|U18749.1|PRU18749 Porcine reproductive and respiratory syndrome virus ISU-22 putative matrix protein and putative nucleocapsid protein genes, complete cds	U18749.1	725	882	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|U18751.1|PRU18751 Porcine reproductive and respiratory syndrome virus ISU-55 putative matrix protein and putative nucleocapsid protein genes, complete cds	U18751.1	725	882	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACAGCCTTTAATCAAGGCGCTGGAACTTGTACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCGACGCATCATACTGTGCGCCTGATCCGCGTCACAGCGTCACCCTCAGC	TAQMAN
>dbj|AB023782.1| Porcine reproductive and respiratory syndrome virus ORF 2 to 7 genes, partial and complete cds, strain:Kitasato 93-1	none	2739	2896	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCATACTGCCTTCAATCAAGGCGCTGGAACTTGCACCCTGTCAGACTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCAGCATACCGTGCGCCTAATCCGCGTCACAGCGTCACCCTCAGC	TAQMAN
>gb|U18752.1|PRU18752 Porcine reproductive and respiratory syndrome virus ISU-79 putative matrix protein and putative nucleocapsid protein genes, complete cds	U18752.1	725	882	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAAACTGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCTTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>dbj|D45852.1| Porcine respiratory and reproductive syndrome virus genes for glycoprotein, membrane protein, nucleocapsid protein, complete cds	none	1401	1558	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTTAATCAAGGCGCTGGAACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACCGTGCGCCTTATTCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|U64928.1|PRU64928 Porcine reproductive and respiratory syndrome virus strain IAF-Klop envelope glycoprotein (E), matrix protein (M), and nucleocapsid protein (N) genes, complete cds	U64928.1	1312	1469	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTTAATCAAGGCGCTGGAACTTGTACCCTATCAGATTCAGGGAGAATAAGTTACGCTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATTCGCGTCACAGCATCACCCTCAGC	TAQMAN
>emb|AJ223079.1|PRR223079 Porcine respiratory and reproductive syndrome virus ORFs 2 to 7, isolate Danish DK3506-12	AJ223079.1	3027	3184	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATTCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|U64929.1|PRU64929 Porcine reproductive and respiratory syndrome virus strain IAF-BAJ envelope glycoprotein (E), matrix protein (M), and nucleocapsid protein (N) genes, complete cds	U64929.1	1312	1469	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTTAATCAAGGCGCTGGAACTTGCACCCTATCAGATTCAGGGAGAATAAGTTACGCTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATTCGCGTCACAGCATCACCCTCAGC	TAQMAN
>emb|AJ223080.1|PRR223080 Porcine respiratory and reproductive	AJ223080.1	3027	3184	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGG	TAQMAN

syndrome virus ORFs 2 to 7, isolate Danish DK5163-17						GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCA TCATACTGTGCGCCTGATTCGCGTCACAGCATCACCCTC AGC	
>gb|U64930.1|PRU64930 Porcine reproductive and respiratory syndrome virus strain IAF-DESR envelope glycoprotein (E), matrix protein (M), and nucleocapsid protein (N) genes, complete cds	U64930.1	1312	1469	158	Plus - Minus - Minus	GAGCGGCAATTGTGTTTGTCGTCAATCCAGACTGCCTTC AATCAAGGCGCTGGAACTTGCACCCTGTCGGATTCAGG GAGGATAAGTTACGCTGTGGAGTTCAGTTTGCCTACGCA TCATACTGTACGCTTAATTCGCGTCACAGCATCACCCTC AGC	TAQMAN
>emb|AJ223081.1|PRR223081 Porcine respiratory and reproductive syndrome virus ORFs 2 to 7, isolate Danish DK5163-23	AJ223081.1	3027	3184	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTT AATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGG GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCA TCATACTGTGCGCCTGATTCGCGTCACAGCATCACCCTC AGC	TAQMAN
>gb|U64931.1|PRU64931 Porcine reproductive and respiratory syndrome virus strain IAF 93-653 envelope glycoprotein (E), matrix protein (M), and nucleocapsid protein (N) genes, complete cds	U64931.1	1315	1472	158	Plus - Minus - Minus	GAGCGGCAACTGTGTCTGTCGTCAATCCAGACCGCCTTT AATCAAGGCGCTGGAACTTGCACCCTGTCGGATTCAGG GAGGATAAGTTACGCTGTGGAGTTCAGTTTGCCTACGCA TCATACTGTGCGCCTGATTCGCGTTACAGCATCACCCTC AGC	TAQMAN
>emb|AJ223082.1|PRR223082 Porcine respiratory and reproductive syndrome virus ORFs 2 to 7, isolate MLV RespPRRS	AJ223082.1	3027	3184	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTT AATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGG GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCA TCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTC AGC	TAQMAN
>gb|U64932.1|PRU64932 Porcine reproductive and respiratory syndrome virus strain IAF 93-2616 envelope glycoprotein (E), matrix protein (M), and nucleocapsid protein (N) genes, complete cds	U64932.1	1312	1469	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTC AATCAAGGCGCTGGAACTTGCACCCTCTCAGATTCAGG GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCA TCATACTGTGCGCCTGATTCGCGTTACAGCATCACCCTC AGC	TAQMAN
>gb|AF030306.1|AF030306 Porcine reproductive and respiratory syndrome virus envelope proteins, matrix protein, and nucleocapsid genes, complete cds	AF030306.1	3108	3265	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTTTT AATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGG GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCA TCATACTGTGCGCTTGATCCGCGCCACAGCATCACCCTC AGC	TAQMAN
>gb|U64933.1|PRU64933 Porcine reproductive and respiratory syndrome virus strain IAF 94-3182 envelope glycoprotein (E), matrix protein (M), and nucleocapsid protein (N) genes, complete cds	U64933.1	1312	1469	158	Plus - Minus - Minus	GAGCGGCAATTGTGCCTGTCGTCAATCCAGACAGCTTTC AATCAAGGCGCTGGAACTTGCACCCTGTCAGATTCAGG GAGGATAAGTTACGCTGTGGAGTTCAGTTTGCCTACGCA TCAGACTGTGCGCCTGATTCGCGTCACAGCATCACCCTC AGC	TAQMAN
>gb|AF043949.1| Porcine reproductive and respiratory syndrome virus isolate 89-46448 nucleocapsid protein gene, complete cds	AF043949.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTT AATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGG GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCA TCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTC AGC	TAQMAN
>gb|U75443.1|PRU75443 Porcine reproductive and respiratory syndrome virus matrix protein (M) and nucleocapsid protein (N) genes, complete cds	U75443.1	740	897	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTT AATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGG GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCA TCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTC AGC	TAQMAN
>gb|AF043950.1| Porcine reproductive and respiratory syndrome virus isolate 92-11824 nucleocapsid protein gene, complete cds	AF043950.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTT AATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGG GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCA TCATACTGTGCGCTTGATCCGCGTCACAGCATCACCCTC AGC	TAQMAN
>gb|AF095492.1|AF095492 Porcine reproductive and respiratory syndrome virus strain 18680 nucleocapsid protein gene, complete cds	AF095492.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTT AATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGG GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCA TCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTC AGC	TAQMAN
>gb|AF095493.1|AF095493 Porcine reproductive and respiratory syndrome virus strain 18683 nucleocapsid protein gene, complete cds	AF095493.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTT AATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGG GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCA TCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTC AGC	TAQMAN
>gb|AF095494.1|AF095494 Porcine reproductive and respiratory syndrome virus strain 19015 nucleocapsid protein gene, complete cds	AF095494.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTT AATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGG GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCA TCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTC AGC	TAQMAN
>gb|AF095495.1|AF095495 Porcine reproductive and respiratory syndrome virus strain 19020 nucleocapsid protein gene, complete cds	AF095495.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTT AATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGG GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCA TCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTC AGC	TAQMAN
>gb|AF095496.1|AF095496 Porcine reproductive and respiratory syndrome virus strain 19259 nucleocapsid protein gene, complete cds	AF095496.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTT AATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGG GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCA TCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTC AGC	TAQMAN
>gb|AF095497.1|AF095497 Porcine reproductive and respiratory syndrome virus strain 21192 nucleocapsid protein gene, complete cds	AF095497.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTT AATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGG GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCA TCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTC AGC	TAQMAN
>gb|AF095498.1|AF095498 Porcine reproductive and respiratory syndrome virus strain 21317 nucleocapsid protein gene, complete cds	AF095498.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTT AATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGG GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCA TCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTC AGC	TAQMAN
>gb|AF142476.1|AF142476 Porcine reproductive and respiratory syndrome virus nucleocapsid protein gene, complete cds	AF142476.1	226	383	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCGATTCAGACTGCCTTT AACCAGGGCGCAGGAACTTGTACCCTGTCAGATTCAGG GAGGATAAGTTACATCGTGGAGTTTAGTCTGCCGACGC ATCATACTGTGCGCCTGATCCGCGTCACAGCACCACCCT CAGC	TAQMAN
>gb|AF159149.1|AF159149 Porcine reproductive and respiratory syndrome virus isolate MLV RespPRRS/Repro, complete genome	AF159149.1	15075	15232	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTT AATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGG GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCA TCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTC AGC	TAQMAN
>gb|AF176348.2| Porcine reproductive and respiratory syndrome virus isolate PA8 complete genome	AF176348.2	15099	15256	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTT AATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGG GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCA TCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTC AGC	TAQMAN
>gb|U87392.3|PRU87392 Porcine reproductive and respiratory syndrome	U87392.3	15099	15256	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTT AATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGG	TAQMAN

virus strain VR-2332, complete genome						GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	
>gb|AF184212.1|AF184212 Porcine reproductive and respiratory syndrome virus strain SP, complete genome	AF184212.1	15208	15365	158	Plus - Minus - Minus	GAGCGGCAATTGTGCCTGTCGTCAATACAGACTGCCTTTAATCAAGGCGCTGGTACTTGCACCCTGTCCGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCCACGCATCATACTGTGCGCCTGATTCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF299404.1|AF299404 Porcine reproductive and respiratory syndrome virus isolate pig22-7o ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299404.1	3394	3551	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF299405.1|AF299405 Porcine reproductive and respiratory syndrome virus isolate pig22-84bo ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299405.1	3235	3392	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF299406.1|AF299406 Porcine reproductive and respiratory syndrome virus isolate pig27-7o ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299406.1	3394	3551	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF299407.1|AF299407 Porcine reproductive and respiratory syndrome virus isolate pig27-84bo ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299407.1	3247	3404	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF299408.1|AF299408 Porcine reproductive and respiratory syndrome virus isolate pig3-150bo ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299408.1	3394	3551	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF299409.1|AF299409 Porcine reproductive and respiratory syndrome virus isolate pig3-7o ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299409.1	3394	3551	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF299410.1|AF299410 Porcine reproductive and respiratory syndrome virus isolate pig3-84bo ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299410.1	3394	3551	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF299411.1|AF299411 Porcine reproductive and respiratory syndrome virus isolate pig3-84o ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299411.1	3394	3551	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF299412.1|AF299412 Porcine reproductive and respiratory syndrome virus isolate pig31-150bo ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299412.1	3394	3551	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF299413.1|AF299413 Porcine reproductive and respiratory syndrome virus isolate pig31-7o ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299413.1	3394	3551	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN

>gb|AF299414.1|AF299414 Porcine reproductive and respiratory syndrome virus isolate pig31-84bo ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299414.1	3394	3551	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF299415.1|AF299415 Porcine reproductive and respiratory syndrome virus isolate pig32-56o ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299415.1	3324	3481	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF299416.1|AF299416 Porcine reproductive and respiratory syndrome virus isolate pig32-7o ORF1b polyprotein gene, partial cds; and GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AF299416.1	3394	3551	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF299417.1|AF299417 Porcine reproductive and respiratory syndrome virus isolate pig32-84bo ORF1b polyprotein gene, partial cds; GP2 glycosylated envelope protein, GP3 envelope protein, GP4, GP5 glycosylated envelope protein, and membrane protein M genes, complete cds; and nucleocapsid protein N gene, partial cds	AF299417.1	3235	3391	157	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAG	TAQMAN
>gb|AF317692.1|AF317692 Porcine reproductive and respiratory syndrome virus structural protein M gene, partial cds; and structural protein N gene, complete cds	AF317692.1	720	874	155	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTC	TAQMAN
>gb|AF325691.1|AF325691 Porcine reproductive and respiratory syndrome virus isolate NVSL 97-7985 IA 1-4-2, complete genome	AF325691.1	15077	15234	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTTAATCAAGGCGCTGGAACTTGCACCCTGTCGGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCGACGCATCATACTGTGCGCCTGATTCGCGTCACAGCACCACCCTCAGC	TAQMAN
>gb|AY256686.1| Porcine reproductive and respiratory syndrome virus VR-2332 nucleocapsid protein mRNA, complete cds	AY256686.1	409	566	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF331831.1|AF331831 Porcine reproductive and respiratory syndrome virus BJ-4, complete genome	AF331831.1	15097	15254	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AY262352.1| PRRSV HB-2(sh)/2002, complete genome	AY262352.1	15061	15218	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGATCCAGACTGCCTTTAACCAGGGCGCCGGGACATGTTCCCTGTCAGATTCAGGGAGGATAAGTTACGCTGTGGAGTTTAGTTTGCCGACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF396833.1| Porcine reproductive and respiratory syndrome virus isolate NADC-8 (E) GP2 glycoprotein, GP3 glycoprotein, GP4 glycoprotein, GP5 glycosylated envelope protein, membrane protein, and nucleocapsid protein mRNAs, complete cds	AF396833.1	3027	3184	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTCAACCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATTCGCGTTACAGCATCACCCTCAGC	TAQMAN
>gb|AY457635.1| Porcine reproductive and respiratory syndrome virus HN1, complete genome	AY457635.1	15098	15255	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF396836.1| Porcine reproductive and respiratory syndrome virus isolate NADC-9 (E) GP2 glycoprotein, GP3 glycoprotein, GP4 glycoprotein, GP5 glycosylated envelope protein, membrane protein, and nucleocapsid protein mRNAs, complete cds	AF396836.1	3027	3184	158	Plus - Minus - Minus	GAGCGGCAATTATGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AY569972.1| Porcine reproductive and respiratory syndrome virus isolate 1530B envelope glycoprotein GP2, envelope glycoprotein GP3, envelope glycoprotein GP4, envelope glycoprotein GP5, matrix protein, and nucleocapsid protein genes, complete cds	AY569972.1	3027	3184	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AY585241.1| Porcine reproductive and respiratory syndrome virus strain PL97-1, complete genome	AY585241.1	15099	15256	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AY612613.1| Porcine respiratory and reproductive syndrome virus strain PL97-1/LP1, complete genome	AY612613.1	15099	15256	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AY656994.1| Porcine reproductive and respiratory syndrome virus isolate 17198-6 nucleocapsid (N) gene, complete cds	AY656994.1	211	368	158	Plus - Minus - Minus	ATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN

>gb|AY656995.1| Porcine reproductive and respiratory syndrome virus nucleocapsid (N) gene, complete cds	AY656995.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTTAATCAAGGCGCTGGAACTTGCACCCTGTCGGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCGACGCATCATACTGTGCGCCTGATTCGCGTCACAGCACCACCCTCAGC	TAQMAN
>gb|AY656997.1| Porcine reproductive and respiratory syndrome virus isolate MN-184 nucleocapsid (N) gene, complete cds	AY656997.1	211	368	158	Plus - Minus - Minus	GTCAATCCAGACTGCCTTTAACCAAGGCGCTGGAACTTGTACCCTGTCGGATTCAGGGAGAATAAGTTACGCTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTAATTCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AY656998.1| Porcine reproductive and respiratory syndrome virus isolate SDSU73 nucleocapsid (N) gene, complete cds	AY656998.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTTAATCAAGGCGCTGGAACTTGCACCCTGTCGGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCGACGCATCATACTGTGCGCCTGATTCGCGTCACAGCACCACCCTCAGC	TAQMAN
>gb|AF396837.1| Porcine reproductive and respiratory syndrome virus isolate NADC-9 (251) GP2 glycoprotein, GP3 glycoprotein, GP4 glycoprotein, GP5 glycosylated envelope protein, membrane protein, and nucleocapsid protein mRNAs, complete cds	AF396837.1	3027	3184	158	Plus - Minus - Minus	GAGCGGCAATTATGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF396838.1| Porcine reproductive and respiratory syndrome virus isolate NADC-9 (252p) GP2 glycoprotein, GP3 glycoprotein, GP4 glycoprotein, GP5 glycosylated envelope protein, membrane protein, and nucleocapsid protein mRNAs, complete cds	AF396838.1	3027	3184	158	Plus - Minus - Minus	GAGCGGCAATTATGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF396839.1| Porcine reproductive and respiratory syndrome virus isolate NVSL-14 (E) GP2 glycoprotein, GP3 glycoprotein, GP4 glycoprotein, GP5 glycosylated envelope protein, membrane protein, and nucleocapsid protein mRNAs, complete cds	AF396839.1	3027	3184	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCGATTCAGACTGCCTTTAATCAAGGCGCTGGAACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCGACGCATCATACTGTGCGCCTGATTCGTGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF396840.1| Porcine reproductive and respiratory syndrome virus isolate NVSL-14 (251) GP2 glycoprotein, GP3 glycoprotein, GP4 glycoprotein, GP5 glycosylated envelope protein, membrane protein, and nucleocapsid protein mRNAs, complete cds	AF396840.1	3027	3184	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCGATTCAGACTGCCTTTAATCAAGGCGCTGGAACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCGACGCATCATACTGTGCGCCTGATTCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF396841.1| Porcine reproductive and respiratory syndrome virus isolate NVSL-14 (252p) GP2 glycoprotein, GP3 glycoprotein, GP4 glycoprotein, GP5 glycosylated envelope protein, membrane protein, and nucleocapsid protein mRNAs, complete cds	AF396841.1	3027	3184	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCGATTCAGACTGCCTTTAATCAAGGCGCTGGAACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCGACGCATCATACTGTGCGCCTGATTCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF494042.1| Porcine reproductive and respiratory syndrome virus isolate P129, complete genome	AF494042.1	15083	15240	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCGATCCAGACTGCCTTTAACCAGGGCGCTGGAACTTGTACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCGACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AY032626.1| Porcine reproductive and respiratory syndrome virus strain CH-1a, complete genome	AY032626.1	15100	15257	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCGATCCAGACTGCCTTTAACCAGGGCGCCGGAACTTGTACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCGACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AY684124.1| Porcine reproductive and respiratory syndrome virus N nucleocapsid protein gene, partial cds	AY684124.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTATTCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AY150312.1| PRRSV HB-1(sh)/2002, complete genome	AY150312.1	15099	15256	158	Plus - Minus - Minus	GAGCGGCAATTGTGCCTGTCGTCGATCCAGACTGCCTTTAATCAGGGCGCTGGAACTTGTGCCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCGACGCATCATACTGTGCGTCTGATCCGCGCCACAGCATCACCCTCAGC	TAQMAN
>gb|AY745499.1| Porcine reproductive and respiratory syndrome virus isolate 01NP1 nucleocapsid protein mRNA, complete cds	AY745499.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AY150564.1| Porcine reproductive and respiratory syndrome virus isolate VR-2332, complete genome	AY150564.1	15100	15257	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AY773277.1| Porcine reproductive and respiratory syndrome virus strain FJ-2 nucleocapsid protein mRNA, complete cds	AY773277.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGCCAATCCAGACTGCCTTCAATCAAGGCGCTGGGACTTGTATCCTGTCAGATTCAGGAAGGATAAGTTACACTGTGGAGTTTAGTTTGCCCACGCATCATACTGTGCGCTTGATTCGCGTTACAGCATCACCCTCAGC	TAQMAN
>gb|AY209205.1| Porcine reproductive and respiratory syndrome virus isolate 11aE nucleocapsid gene, complete cds	AY209205.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTTAATCAAGGTGCTGGAACTTGCACCCTGTCGGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTCTGCCTACGCATCATACTGTGCGCTTGATTCGCGTTACAGCATCACCCTCAGC	TAQMAN
>gb|AY881994.1| Porcine reproductive and respiratory syndrome virus strain FJ-1 GP2 envelope glycoprotein, unknown protein, GP3 envelope protein, GP4 envelope glycoprotein, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	AY881994.1	3027	3184	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTCAATCAAGGCGCTGGGACTTGTATCCTGTCAGATTCAGGAAGGATAAGTTACACTGTGGAGTTTAGTTTGCCCACGCATCATACTGTGCGCTTGATTCGCGTTACAGCATCACCCTCAGC	TAQMAN
>gb|AY885248.1| Porcine reproductive and respiratory syndrome virus GP2 envelope protein, GP3 envelope glycoprotein, GP4 envelope protein, GP5 envelope protein, GP6 envelope glycoprotein, and GP7	AY885248.1	3099	3256	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCGCCCTCAGC	TAQMAN

envelope glycoprotein genes, complete cds							
>gb|AY947883.1| Porcine reproductive and respiratory syndrome virus isolate 57258/1 nucleocapsid protein gene, partial cds	AY947883.1	211	365	155	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGCACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTACGCCTGATCCGTGTCACAGCATCACCCTC	TAQMAN
>gb|AY947885.1| Porcine reproductive and respiratory syndrome virus isolate 58395 nucleocapsid protein gene, partial cds	AY947885.1	192	346	155	Plus - Minus - Minus	GAGCGGCATTTGTGTCTGTCATCAATCCAGACCGCCTTTAATCAAGGTGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTC	TAQMAN
>gb|AY947886.1| Porcine reproductive and respiratory syndrome virus isolate 58847 nucleocapsid protein gene, partial cds	AY947886.1	211	365	155	Plus - Minus - Minus	GAGCGGCAACTGTGTCTGTCGTCGATCCAGACTGCCTTTAATCAAGGCGCTGGGACCTGCACCCTGTCAGATTCGGGGAGGTTAAGTTACGCTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTC	TAQMAN
>gb|AY947887.1| Porcine reproductive and respiratory syndrome virus isolate 59946 nucleocapsid protein gene, partial cds	AY947887.1	211	365	155	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGCACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTACGCCTGATCCGTGTCACAGCATCACCCTC	TAQMAN
>gb|AY947888.1| Porcine reproductive and respiratory syndrome virus isolate 317-b nucleocapsid protein gene, partial cds	AY947888.1	176	330	155	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTC	TAQMAN
>gb|AF043951.1| Porcine reproductive and respiratory syndrome virus isolate 93-14620 nucleocapsid protein gene, complete cds	AF043951.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTCAATCAAGGCGCTGGAACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCGACGCATCATACTGTGCGCCTGATTCGCGTCACAGCGTCACCCTCAGC	TAQMAN
>gb|AF043952.1| Porcine reproductive and respiratory syndrome virus isolate 93-6351 nucleocapsid protein gene, complete cds	AF043952.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCGATCCAGACTGCCTTTAATCAAGGCGCTGGAACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCGACGCATCATACTGTGCGCCTGATTCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF043954.1| Porcine reproductive and respiratory syndrome virus isolate 93-27687 nucleocapsid protein gene, complete cds	AF043954.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF043955.1| Porcine reproductive and respiratory syndrome virus isolate 91-46907 nucleocapsid protein gene, complete cds	AF043955.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTCAATCAAGGCGCTGGAACTTGTACCCTGTCAGATTCAGGGAGAATAAGTTACACTGTGGAGTTTAGTTTGCCAACGCATCACACTGTGCGCTTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF043956.1| Porcine reproductive and respiratory syndrome virus isolate 92-01205 nucleocapsid protein gene, complete cds	AF043956.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTTAATCAAGGCGCTGGAACTTGTACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCAACGCATCACACTGTGCGCTTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF043957.1| Porcine reproductive and respiratory syndrome virus isolate 93-44927 nucleocapsid protein gene, complete cds	AF043957.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTTAATCAAGGCGCTGGAACTTGTACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCAACGCATCACACTGTGCGCTTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF043958.1| Porcine reproductive and respiratory syndrome virus isolate 94-18310 nucleocapsid protein gene, complete cds	AF043958.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTATCGTCGATCCAGACTGCCTTTAATCAAGGGCGCTGGAACTTGTACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCGACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF043959.1| Porcine reproductive and respiratory syndrome virus isolate 95-13536 nucleocapsid protein gene, complete cds	AF043959.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGGCTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF043960.1| Porcine reproductive and respiratory syndrome virus isolate 95-15299 nucleocapsid protein gene, complete cds	AF043960.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AF043961.1| Porcine reproductive and respiratory syndrome virus isolate 95-33010 nucleocapsid protein gene, complete cds	AF043961.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCGATCCAGACTGCCTTTAACCAGGGGCGCTGGAACTTGTACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCGACGCATCATACTGTGCGCCTGATTCGCGCCACAGCATCACCCTCAGC	TAQMAN
>gb|AY209209.1| Porcine reproductive and respiratory syndrome virus isolate 15dP nucleocapsid gene, complete cds	AY209209.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACAGCCTTTAATCAAGGCGCTGGAACTTGTATCCTGTCAGATTCAGGGAGGATAAGTTATACTGTGGAGTTTAGTTTGCCGACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AY209212.1| Porcine reproductive and respiratory syndrome virus isolate 18D nucleocapsid gene, complete cds	AY209212.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AY209213.1| Porcine reproductive and respiratory syndrome virus isolate 19aPP1 nucleocapsid gene, complete cds	AY209213.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AY209214.1| Porcine reproductive and respiratory syndrome virus isolate 19aPP2 nucleocapsid gene, complete cds	AY209214.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AY209215.1| Porcine reproductive and respiratory syndrome virus isolate 20bP nucleocapsid gene, complete cds	AY209215.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCTTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AY209217.1| Porcine reproductive and respiratory syndrome virus isolate 22aD nucleocapsid gene, complete cds	AY209217.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCTTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
>gb|AY209218.1| Porcine reproductive and respiratory syndrome virus isolate 23bD nucleocapsid gene, complete cds	AY209218.1	211	368	158	Plus - Minus - Minus	GAGCGACAATTGTGTCTGTCGTCAATCCAGACTGCCTTTAATCAAGGCGCTGGAACTTGTGCCCTGTCAGACTCAGGGAGAATAAGTTACACTGTGGAGTTTAGTCTGCCTACGCATCATACTGTGCGCCTAATTCGCGTCACAGCATCACCTTCAGC	TAQMAN
>gb|DQ056373.1| Porcine respiratory and reproductive syndrome virus strain	DQ056373.1	15099	15256	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGG	TAQMAN

01NP1.2, complete genome						GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCA TCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTC AGC	
>gb|AY209220.1| Porcine reproductive and respiratory syndrome virus isolate 25 nucleocapsid gene, complete cds	AY209220.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTC AATCAAGGCGCTGGAACTTGTACCCTATCAGATTCAGG GAGAATAAGTTACACTGTGGAGTTTAGTCTGCCGACGC ATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCT CAGC	TAQMAN
>gb|DQ176019.1| Porcine respiratory and reproductive syndrome virus isolate MN184A, complete genome	DQ176019.1	14707	14864	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTT AACCAAGGCGCTGGAACTTGTACCCTGTCGGATTCAGG GAGAATAAGTTACGCTGTGGAGTTTAGTTTGCCTACGCA TCATACTGTGCGCCTAATTCGCGTCACAGCATCACCCTC AGC	TAQMAN
>gb|AY209227.1| Porcine reproductive and respiratory syndrome virus isolate 30D nucleocapsid gene, complete cds	AY209227.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTT AATCAAGGTGCTGGAACTTGTACCCTGTCTGATTCAGGG AGAATAAGTTACACTGTGGAGTTTAGTTTGCCGACGCAT CATACTGTGCGTCTGATTCGCGTCACAGCATCACCTTCA GC	TAQMAN
>gb|DQ176020.1| Porcine respiratory and reproductive syndrome virus isolate MN184B, complete genome	DQ176020.1	14707	14864	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTT AACCAAGGCGCTGGAACTTGTACCCTGTCGGATTCAGG GAGAATAAGTTACACTGCGGAGTTTAGTTTGCCTACGCA TCATACTGTGCGCCTAATTCGCGTCACAGCATCACCCTC AGC	TAQMAN
>gb|AY256685.1| Porcine reproductive and respiratory syndrome virus VR-2332 nonfunctional NSP1 and nonfunctional unglycosylated membrane protein, complete sequence; and nucleocapsid protein mRNA, complete cds	AY256685.1	1651	1808	158	Plus - Minus - Minus	GTCACAGCATCACCCTCAGC	TAQMAN
>gb|DQ176021.1| Porcine respiratory and reproductive syndrome virus clone VR-2332 V7, complete genome	DQ176021.1	15099	15256	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTT AATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGG GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCA TCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTC AGC	TAQMAN
>gb|DQ217415.1| Porcine respiratory and reproductive syndrome virus strain VR-2332 clone pVR-V7, complete genome	DQ217415.1	15085	15242	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTT AATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGG GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCA TCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTC AGC	TAQMAN
>gb|DQ246451.1| Porcine respiratory and reproductive syndrome virus strain FJ04A GP2 glycoprotein, GP3 glycoprotein, GP4 glycoprotein, GP5 glycosylated envelope protein, membrane protein M, and nucleocapsid protein N genes, complete cds	DQ246451.1	3027	3184	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTT AATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGG GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCA TCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTC AGC	TAQMAN
>gb|DQ355796.1| Porcine respiratory and reproductive syndrome virus isolate R98 GP3 protein, GP4 protein, GP5 protein, M protein, and N protein mRNA, complete cds	DQ355796.1	2404	2561	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTT AATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGG GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCCACGCA TCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTC AGC	TAQMAN
>gb|DQ379481.1| Porcine respiratory and reproductive syndrome virus isolate SCQ ORF7 mRNA, complete cds	DQ379481.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTT AATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGG GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCA TCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTC AGC	TAQMAN
>gb|DQ459471.1| Porcine respiratory and reproductive syndrome virus isolate S1, complete genome	DQ459471.1	15099	15256	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTT AATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGG GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCA TCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTC AGC	TAQMAN
>gb|DQ473474.1| Porcine respiratory and reproductive syndrome virus isolate LMY, complete genome	DQ473474.1	15099	15256	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTT AATCAAGGCGCTGGGACTTGCACCCTATCAGATTCAGG GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCA TCATACTGTGCGCCTGATCCGTGTCACAGCATCACCTTC AGC	TAQMAN
>gb|AF043962.1| Porcine reproductive and respiratory syndrome virus isolate S-P vaccine nucleocapsid protein gene, complete cds	AF043962.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATACAGACTGCCTTT AATCAAGGCGCTGGTACTTGCACCCTGTCGGATTCAGGG AGGATAAGTTACACTGTGGAGTTTAGTTTGCCCACGCAT CATACTGTGCGCCTGATTCGCGTCACAGCATCACCCTCA GC	TAQMAN
>gb|AF043964.1| Porcine reproductive and respiratory syndrome virus isolate 93-22326 nucleocapsid protein gene, complete cds	AF043964.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTT AACCAAGGCGCTGGAACTTGTATCCTATCAGATTCAGG GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCGACGCA TCATACTGTGCGCCTGATCCGCGTCACAGCACCACCCTC AGC	TAQMAN
>gb|AF043965.1| Porcine reproductive and respiratory syndrome virus isolate 92-22332 nucleocapsid protein gene, complete cds	AF043965.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTT AATCAAGGCGCTGGAACTTGTATCCTGTCAGATTCAGGT AGGATAAGTTACACTGTGGAGTTTAGTTTGCCGACGCAT CATACTGTGCGCCTGATTCGCGTCACAGCACCACCCTCA GC	TAQMAN
>gb|AF043966.1| Porcine reproductive and respiratory syndrome virus isolate 89-46489 nucleocapsid protein gene, complete cds	AF043966.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTT AATCAAGGCGCTGGAACTTGTACCCTGTCAGATTCAGG GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCGACGCA TCATACTGTGCGCCTGATCCGCGTCACAGCACCACCCTC AGC	TAQMAN
>gb|AF043967.1| Porcine reproductive and respiratory syndrome virus isolate 89-47361 nucleocapsid protein gene, complete cds	AF043967.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTT AATCAAGGCGCTGGAACTTGTACCCTGTCAGATTCAGG GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCGACGCA TCATACTGTGCGCCTGATCCGCGTCACAGCACCACCCTC AGC	TAQMAN
>gb|AF043968.1| Porcine reproductive and respiratory syndrome virus isolate 89-47463 nucleocapsid protein gene, complete cds	AF043968.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTT AATCAAGGCGCTGGAACTTGTACCCTGTCAGATTCAGG GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCGACGCA TCATACTGTGCGCCTGATCCGCGTCACAGCACCACCCTC AGC	TAQMAN
>gb|AF043971.1| Porcine reproductive and respiratory syndrome virus isolate 92-6725 nucleocapsid protein gene, complete cds	AF043971.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTT AATCAAGGCGCTGGGACTTGTACCCTGTCAGATTCAGG GAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCA TCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCCC AGC	TAQMAN
>gb|AF043972.1| Porcine reproductive and respiratory syndrome virus isolate IA-D21 nucleocapsid protein gene, complete cds	AF043972.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCGATCCAGACTGCCTTT AATCAAGGCGCTGGAACTTGTACCCTGTCAGATTCAGG GAGAATAAGTTACACTGTGGAGTTTAGTTTGCCGACGCA TCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCCC AGC	TAQMAN

	>gb|AF043973.1| Porcine reproductive and respiratory syndrome virus isolate 28523 nucleocapsid protein gene, complete cds	AF043973.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTTAATCAAGGCGCTGGAACTTGTATCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCGACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCTTCAGC	TAQMAN
	>gb|AF043974.1| Porcine reproductive and respiratory syndrome virus isolate ISU-P nucleocapsid protein gene, complete cds	AF043974.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCGGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCTTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
	>gb|DQ779791.1| Porcine respiratory and reproductive syndrome virus strain Prime Pac, complete genome	DQ779791.1	15208	15365	158	Plus - Minus - Minus	GAGCGGCAATTGTGCCTGTCGTCAATACAGACTGCCTTTAATCAAGGCGCTGGTACTTGCACCCTGTCCGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCCACGCATCATACTGTGCGCCTGATTCGCGTCACAGCATCACCCTCAGC	TAQMAN
	>gb|EF014225.1| Porcine respiratory and reproductive syndrome virus strain JX1 GP5, membrane protein, and nucleocapsid protein genes, complete cds	EF014225.1	1312	1469	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCGATCCAGACTGCCTTCAATCAGGGCGCTGGAACTTGTGCCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCGACGCAACACACTGTGCGTCTGATCCGCGCCACAGCATCACCCTCAGC	TAQMAN
	>gb|EF075945.1| Porcine respiratory and reproductive syndrome virus strain HUB1, complete genome	EF075945.1	15009	15166	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCGATCCAGACTGCCTTCAATCAGGGCGCTGGAACTTGTGCCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCGACGCAACACACTGTGCGTCTGATCCGCGCCACAGCATCACCCTCAGC	TAQMAN
	>gb|EF112445.1| Porcine respiratory and reproductive syndrome virus strain JXA1, complete genome	EF112445.1	15009	15166	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCGATCCAGACTGCCTTCAATCAGGGCGCTGGAACTTGTGCCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCGACGCAACATACTGTGCGTCTGATCCGCGCCACAGCATCACCCTCAGC	TAQMAN
	>gb|EF112446.1| Porcine respiratory and reproductive syndrome virus strain HUB2, complete genome	EF112446.1	15009	15166	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCGATCCAGACTGCCTTCAATCAGGGCGCTGGAACTTGTGCCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCGACGCAACACACTGTGCGTCTGATCCGCGCCACAGCATCACCCTCAGC	TAQMAN
	>gb|EF112447.1| Porcine respiratory and reproductive syndrome virus strain HEB1, complete genome	EF112447.1	15009	15166	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCGATCCAGACTGCCTTCAATCAGGGCGCTGGAACTTGTGCCCTGTCAGACTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCGACGCAACATACTGTGCGTCTGATCCGCGCCACAGCATCACCCTCAGC	TAQMAN
	>gb|EF121950.1| Porcine respiratory and reproductive syndrome virus strain JX1 nucleocapsid protein gene, partial cds	EF121950.1	166	323	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTCAATCAGGGCGCTGGAACTTGTGCCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCGACGCAACACACTGTGCGTCTGATCCGCGCCACAGCATCACCCTCAGC	TAQMAN
	>gb|EF153486.1| Porcine respiratory and reproductive syndrome virus isolate CC-1, complete genome	EF153486.1	15099	15256	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
	>gb|L39361.1|PPSIA1A Porcine respiratory and reproductive syndrome virus (individual isolate IA1), mRNA sequence	L39361.1	3027	3184	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTTAATCAAGGCGCTGGAACTTGTATCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCGACGCATCATACCGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
	>gb|L39362.1|PPSIA6A Porcine respiratory and reproductive syndrome virus (individual isolate IA6), mRNA sequence	L39362.1	3027	3184	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACTGCCTTCAATCAAGGCGCTGTACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCGACGCATCATACTGTGCGCCTGATTCGCGTCACAGCACCACCCTCAGC	TAQMAN
	>gb|AF046869.1|AF046869 Porcine reproductive and respiratory syndrome virus isolate 16244B, 2/18/97(Nebraska)pass.3, complete genome	AF046869.1	15099	15256	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
	>gb|AF054970.1| Porcine reproductive and respiratory syndrome virus nucleocapsid protein gene, complete cds	AF054970.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
	>gb|AF066068.1|PRRSVORF2 Porcine reproductive and respiratory syndrome virus major membrane glycoprotein, membrane protein, and nucleocapsid protein genes, complete cds	AF066068.1	1312	1469	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
	>gb|AF066183.4| Porcine reproductive and respiratory syndrome virus RespPRRS MLV, complete genome	AF066183.4	15100	15257	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
	>gb|AF066384.1|AF066384 Porcine reproductive and respiratory syndrome virus RNA-dependent RNA polymerase gene, partial cds; and GP2 envelope protein, GP3 envelope protein, GP4 envelope protein, GP5 envelope protein, M matrix protein, and N nucleocapsid protein genes, complete cds	AF066384.1	6478	6635	158	Plus - Minus - Minus	GAGCGGCAATTGTGCCTGTCGTCAATACAGACTGCCTTTAATCAAGGCGCTGGTACTTGCACCCTGTCCGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCCACGCATCATACTGTGCGCCTGATTCGCGTCACAGCATCACCCTCAGC	TAQMAN
	>gb|AF090173.1|AF090173 Porcine reproductive and respiratory syndrome virus glycoprotein, membrane protein, and nucleocapsid protein genes, complete cds	AF090173.1	1331	1488	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
	>gb|AF095478.1|AF095478 Porcine reproductive and respiratory syndrome virus strain Vac nucleocapsid protein gene, complete cds	AF095478.1	211	368	158	Plus - Minus - Minus	GAGCGGCAATTGTGTCTGTCGTCAATCCAGACCGCCTTTAATCAAGGCGCTGGGACTTGCACCCTGTCAGATTCAGGGAGGATAAGTTACACTGTGGAGTTTAGTTTGCCTACGCATCATACTGTGCGCCTGATCCGCGTCACAGCATCACCCTCAGC	TAQMAN
[redacted]								
sig_candidate_1810342	>gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome	AY366525.1	284	446	163	Plus - Plus - Minus	CCAAGTCTTTTGCACACGGTGTGTCAGTGCGCGGGCTCTTCTCTCTCCAGAGCTTCAGGACACTGACCTCGGTGCGGTTGGATTGTTTACAGGCCTAGGGATAAGCTACACTGGAAAGTCCCTATCGGCATCCCCCAGGCGGAATGTACTCCATCCGGGTGC	TAQMAN
	>gb|AY375474.1| Porcine reproductive and respiratory syndrome	AY375474.1	63	225	163	Plus - Plus - Minus	CCAAGTCTTTTGCACACGGTGTCTCAGTGCGCGGCCTCTTCTCTCTCCGGAACTTCAGGACACTGACCTCGGTGTAGT	TAQMAN

	virus ORF1 gene, complete cds						TGGATTGTTTTACAAGCCTAAGGACAAGATTCACTGGAA AGTTCCTATCGGCATTCCTCAGGTGGAGTGTACTCCATC CGGGTGC	
6 total sequence hits	>gb|AY588319.1| PRRSV LV4.2.1, complete genome	AY588319.1	284	446	163	Plus - Plus - Minus	CCAAGTCTTTTGCACACGGTGTCTCAGTGCGCGGTCTCT TCTCTCTCCAGAGCTTCAGGACACTGACCTCGGTGCAGT TGGCTTGTTTTACAAGCCTAGGGACAAGCTTCACTGGAA AGTCCCTATCGGCATCCCTCAGGTGGAATGTACTCCATC CGGGTGC	TAQMAN
	>gb|DQ489311.1| Porcine reproductive and respiratory syndrome virus strain SD01-08, complete genome	DQ489311.1	284	446	163	Plus - Plus - Minus	CCAAGTCTTTTGCACACGGTGTCTCAGTGCGCGGCCTCT TCTCTCTCCGGAACTTCAGGACACTGACCTCGGTGTAGT TGGATTGTTTTACAAGCCTAAGGACAAGATTCACTGGAA AGTTCCTATCGGCATTCCTCAGGTGGAGTGTACTCCATC CGGGTGC	TAQMAN
	>gb|DQ864705.1| Porcine respiratory and reproductive syndrome virus strain 01CB1, complete genome	DQ864705.1	207	369	163	Plus - Plus - Minus	CCAAGTCTTTTGCACACGGTGTCTCAGTGCGCGGTCTCT TCTCTCTCCAGAGCTTCAGGACACTGACCTCGGTGCAGT TGGCTTGTTTTACAAGCCTAGGGACAAGCTTCACTGGAA AGTCCCTATCGGCATCCCTCAGGTGGAATGTACTCCATC CGGGTGC	TAQMAN
	>gb|M96262.2|LEYPOLYENV Lelystad virus, complete genome	M96262.2	284	446	163	Plus - Plus - Minus	CCAAGTCTTTTGCACACGGTGTCTCAGTGCGCGGTCTCT TCTCTCTCCAGAGCTTCAGGACACTGACCTCGGTGCAGT TGGCTTGTTTTACAAGCCTAGGGACAAGCTTCACTGGAA AGTCCCTATCGGCATCCCTCAGGTGGAATGTACTCCATC CGGGTGC	TAQMAN
[redacted]								
sig_candidate_1810344	>gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome	AY366525.1	993	1132	140	Plus - Plus - Minus	ACGTCTCATTCTTGCGGTCATCTCGTCCGAAACTCCAAC GTGTTTGATGGCAAGTGCTGGCTCACCTGCTTTTTGGGC CAGTCGGTCGAAGTGCGCTGCCATGAAGAACATCTAGC CAACGCCTTCGGTTACCAAACCAA	TAQMAN
	>gb|AY588319.1| PRRSV LV4.2.1, complete genome	AY588319.1	993	1132	140	Plus - Plus - Minus	ACGTCTCATTCTTGCGGTCACCTTGTCCAGAACCCCGAC GTGTTTGATGGCAAGTGCTGGCTCTCCTGCTTTTTGGGC CAGTCGGTCGAAGTGCGCTGCCATGAGGAACATCTAGC TGACGCCTTCGGTTACCAAACCAA	TAQMAN
4 total sequence hits	>gb|DQ864705.1| Porcine respiratory and reproductive syndrome virus strain 01CB1, complete genome	DQ864705.1	916	1055	140	Plus - Plus - Minus	ACGTCTCATTCTTGCGGTCATCTTGTCCAAAACCCCGAC GTGTTTGATGGCAAGTGCTGGCTCTCCTGCTTTTTGGGC CAGTCGGTCGAAGTGCGCTGCCATGAGGAACATCTAGC TGACGCCTTCGGTTACCAAACCAA	TAQMAN
	>gb|M96262.2|LEYPOLYENV Lelystad virus, complete genome	M96262.2	993	1132	140	Plus - Plus - Minus	ACGTCTCATTCTTGCGGTCACCTTGTCCAGAACCCCGAC GTGTTTGATGGCAAGTGCTGGCTCTCCTGCTTTTTGGGC CAGTCGGTCGAAGTGCGCTGCCATGAGGAACATCTAGC TGACGCCTTCGGTTACCAAACCAA	TAQMAN
[redacted]								
sig_candidate_1810346	>gb|M96262.2|LEYPOLYENV Lelystad virus, complete genome	M96262.2	1553	1667	115	Plus - Plus - Minus	TGACTTCACGTCCCCTCTGACTCAGTACAACAGACCAGA GGATGATTGGGCTTCTGATTATGATCTTGTTCAGGCGAT TCAATGTCTACGACTGCCTGCTACCGTGGTTCGGAAT	TAQMAN
	>gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome	AY366525.1	1553	1667	115	Plus - Plus - Minus	TGACTTCACGTCCCCTCTGACTCAGTACAACAGACCAGA GGATGATTGGGCTTCAGATTATGATCTTGCTCAGGCGAT TCAATGTCTACAACTACCTGCTACCGTGGTTCGGAAT	TAQMAN
7 total sequence hits	>gb|AY383632.1| Porcine reproductive and respiratory syndrome virus strain SDPRRS 01-07 polyprotein gene, partial cds	AY383632.1	177	291	115	Plus - Plus - Minus	TGACTTCACGTCCCCTCTGACTCAGTACAACAGACCAGA GGATGATTGGGCTTCTGATTTTGATCTTATTCAGGCGAT TCAATGTCTACAACTGCCTGCTACCGTGGTTCGGAAT	TAQMAN
	>gb|AY383633.1| Porcine reproductive and respiratory syndrome virus strain SDPRRS 02-10 polyprotein gene, partial cds	AY383633.1	177	289	113	Plus - Plus - Minus	TGACTTTACGTCCCCTCTGACTCAGTACAATAGACCAGA GGATGATTGGGCTTCTGATTATGATCTTGCACAGGCGAT TCAATATTTACAGCTGCCTGCTACCGTGGTTCGGA	TAQMAN
	>gb|AY383634.1| Porcine reproductive and respiratory syndrome virus strain SDPRRS 02-11 polyprotein gene, partial cds	AY383634.1	177	291	115	Plus - Plus - Minus	TGACTTCACGTCCCCTCTGGCCCCGCACAACAGACCAGA GGACGATTGGGCTTCTGATTATGACCTTGTTCAGGCGAT TCAATGTCTACAACTGCCTGCTACCGTGGTTCGGAAT	TAQMAN
	>gb|AY588319.1| PRRSV LV4.2.1, complete genome	AY588319.1	1553	1667	115	Plus - Plus - Minus	TGACTTCACGTCCCCTCTGACTCAGTACAACAGACCAGA GGATGATTGGGCTTCTGATTATGATCTTGTTCAGGCGAT TCAATGTCTACGACTGCCTGCTACCGTGGTTCGGAAT	TAQMAN
	>gb|DQ864705.1| Porcine respiratory and reproductive syndrome virus strain 01CB1, complete genome	DQ864705.1	1476	1590	115	Plus - Plus - Minus	TGACTTCACGTCCCCTCTGACTCAGTACAACAGACCAGA GGATGATTGGGCTTCTGATTATGATCTTGTTCAGGCGAT TCAATGTCTACAACTGCCTGCTACCGTGGTTCGGAAT	TAQMAN
[redacted]								
sig_candidate_1810347	>gb|M96262.2|LEYPOLYENV Lelystad virus, complete genome	M96262.2	1745	1908	164	Plus - Minus - Minus	TCCTCGCTCCCTTTCTCGTGAATGTGTGGTTGGCGTTTGC TCTGAAGGCTGTGTCGCACCGCCTTATCCAGCAGACGGG CTACCTAAACGTGCACTCGAGGCCTTGGCGTCTGCTTAC AGACTACCCTCCGATTGTGTTAGCTCTGGTATTGCTGAC TTTCTTG	TAQMAN
	>gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome	AY366525.1	1745	1908	164	Plus - Minus - Minus	TCCTCGCTCCCTTTCTCGTGAATGTGTGGTTGGCGTTTGT TCTGAAGGCTGCGTCGCGCCGCCTTACCCAGCGGATGG GCTTCCTAAGCGTGCACTCGAGGCCTTGGCGTCTGCTTA CAGACTACCCTCCGATTGTGTTTGCTCTGGTATTGCTGA CTTTCTTG	TAQMAN
7 total sequence hits	>gb|AY383632.1| Porcine reproductive and respiratory syndrome virus strain SDPRRS 01-07 polyprotein gene, partial cds	AY383632.1	369	532	164	Plus - Minus - Minus	TCCTCGCTCCCTTTCTCGTGAATGTGTGGTTGGCGTTTGC TCTGAAGGCTGTGTCGCACCGCCTTATCCAGCGGACGGG CTACCTAAACGTGCCCTTGAGGCCTTGGCGTCTGCTTAT AGACTGCCCTCCGATTGTGTTAGCTCTGGTATTGCTGAC TTTCTTG	TAQMAN
	>gb|AY383634.1| Porcine reproductive and respiratory syndrome virus strain SDPRRS 02-11 polyprotein gene, partial cds	AY383634.1	369	532	164	Plus - Minus - Minus	TCCTCGCTCCCTTTCTCGTGAGTGCGTGGTTGGTGTTTGC TCTGAAGGCTGTGTCGCGCCGCCTTATCCAGCGGACGGA CTTCCTAAACGTGCACTCGAGGCCTTGGCGTCTGCTTAC AGACTACCTTCCGATTGTGTTAGCTCTGGTATTGCTGAC TTTCTTG	TAQMAN
	>gb|AY588319.1| PRRSV LV4.2.1, complete genome	AY588319.1	1745	1908	164	Plus - Minus - Minus	TCCTCGCTCCCTTTCTCGTGAATGTGTGGTTGGCGTTTGC TCTGAAGGCTGTGTCGCACCGCCTTATCCAGCAGACGGG CTACCTAAACGTGCACTCGAGGCCTTGGCGTCTGCTTAC AGACTACCCTCCGATTGTGTTAGCTCTGGTATTGCTGAC TTTCTTG	TAQMAN
	>gb|AY749409.1| Porcine reproductive and respiratory syndrome virus strain SDPRRS 04-48 nonstructural protein nsp2 gene, partial cds	AY749409.1	369	532	164	Plus - Minus - Minus	TCCTCGCTCCCTTTCTCGTGAATGTGTGGTTGGCGTTTGC TCTGAAGGCTGTGTTGCACCGCCTTATCCAGCGGACGGG CTTCCTAAACGCGCACTCGAGGCCCTGGCGTCTGCCTAC AGACTACCCTCAGATTGTGTTAGCTCTGGTATTGCTGAC TTTCTTG	TAQMAN
	>gb|DQ864705.1| Porcine respiratory and reproductive syndrome virus strain 01CB1, complete genome	DQ864705.1	1668	1831	164	Plus - Minus - Minus	TCCTCGCTCCCTTTCTCGTGAATGTGTGGTTGGCGTTTGC TCTGAAGGCTGTGTCGCACCGCCTTATCCAGCAGACGGG CTACCTAAACGTGCACTCGAGGCCTTGGCGTCTGCTTAC AGACTACCCTCCGATTGTGTTAGCTCTGGTATTGCTGAC TTTCTTG	TAQMAN
[redacted]								

sig_candidate_1810348|	>gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome	AY366525.1	2935	3134	200	Plus - Plus - Minus	ATCCTTTCGAATTTGCCGAACTCAAGCGCCCGCGTTTCT CCGCACAAGCCTTAATTGACCGAGGCGGTCCGCTTGCCG ATGTCCATGCGAAAATAAAGAACCGGGTGTATGAACGG TGCCTCCAAGCTTGTGAGCCCGGTAGTCGTGCAACCCCA GCCACCAAGGAGTGGCTCGACAAGATGTGGGATAGGGT GGACATG	TAQMAN
13 total sequence hits	>gb|AY383632.1| Porcine reproductive and respiratory syndrome virus strain SDPRRS 01-07 polyprotein gene, partial cds	AY383632.1	1614	1809	196	Plus - Plus - Minus	TTTCGAATTTGCCGAACTCAAGCGCCCGCGTTTTCCGC ACAAGCCTTAATTGACCGAGGCGGCCCTCTTGCTGATGT CCACGCAAAAATAAAGAACCAGGTATATGAACGGTGCC TCCAAGCTTGTGAGCCCGGTAGTCGTGCAACCCCAGCCA CCAGGGAGTGGCTCGACAAAATGTGGGAAAGGGTGGAC ATG	TAQMAN
	>gb|AY383634.1| Porcine reproductive and respiratory syndrome virus strain SDPRRS 02-11 polyprotein gene, partial cds	AY383634.1	1592	1791	200	Plus - Plus - Minus	ATCCTTTCGAATTTGCCGAACTCAAACGCCCGCGTTTCT CCGCACAAGCCTTAATTGACCGGGGTGGTCCACTTGCCG ATGTCCATGCGAAAATAAAGAACCGGGTGTATGAACAG TGCCTCAAAGCTTGTGAGCCCGGTAGTCGTGCAACCCCA GCCACCAAGGAGTGGCTCGACAAAATGTGGGATAGGGT GGACATG	TAQMAN
	>gb|AY749383.1| Porcine reproductive and respiratory syndrome virus strain MN-03-08_EU nonstructural protein nsp2 gene, partial cds	AY749383.1	1561	1758	198	Plus - Plus - Minus	CCTTTCGAATTTGCCGAACTCAAGCGCCCGCGTTTCTCC GCACAAGCCTTAATTGACCGAGGCGGCCCGCTTGCCGA TGTCCATGCAAAAATAAAGCATCGGGTGTACGAACAGT GCCTTCAAGCTTGTGAGCCTGGTAGCCGTGCAACCCCAG CCACCAAGAAGTGGCTCGACAAAATGTGGGATAGGGTG GACATG	TAQMAN
	>gb|AY383636.1| Porcine reproductive and respiratory syndrome virus strain SDPRRS 03-15 polyprotein gene, partial cds	AY383636.1	1561	1758	198	Plus - Plus - Minus	CCTTTCGAATTTGCCGAACTCAAGCGCCCGCGTTTCTCC GCACAAGCCTTAATTGACCGAGGCGGCCCGCTTGCCGA TGTCCATGCAAAAATAAAGCACCGGGTGTATGAACAAT GCCTTCAAGCTTGTGAGCCTGGTAGCCGTGCAACCCCAG CCACCAAGAAGTGGCTCGACAAAATGTGGGACAGGGTG GACATG	TAQMAN
	>gb|AY749395.1| Porcine reproductive and respiratory syndrome virus strain MN-04-09_EU nonstructural protein nsp2 gene, partial cds	AY749395.1	1561	1758	198	Plus - Plus - Minus	CCTTTCGAATTTGCCGAACTCAAGCGCCCGCGTTTCTCC GCACAAGCCTTAATTGACCGAGGCGGCCCGCTTGCCGA TGTCCATGCAAAAATAAAGCATCGGGTGTACGAACAGT GCCTTCAAGCTTGTGAGCCTGGTAGCCGTGCAACCCCAG CCACCAAGAAGTGGCTCGACAAAATGTGGGATAGGGTG GACATG	TAQMAN
	>gb|AY383637.1| Porcine reproductive and respiratory syndrome virus strain SDPRRS 03-16 polyprotein gene, partial cds	AY383637.1	1561	1758	198	Plus - Plus - Minus	CCTTTCGAATTTGCCGAACTCAAGCGCCCGCGTTTCTCC GCACAAGCCTTAATTGACCGAGGCGGCCCGCTTGCCGA TGTCCATGCAAAAATAAAGCACCGGGTGTATGAACAAT GCCTTCAAGCTTGTGAGCCTGGTAGCCGTGCAACCCCAG CCACCAAGAAGTGGCTCGACAAAATGTGGGACAGGGTG GACATG	TAQMAN
	>gb|DQ864705.1| Porcine respiratory and reproductive syndrome virus strain 01CB1, complete genome	DQ864705.1	2909	3108	200	Plus - Plus - Minus	ATCCTTTCGGATTTGCCGAACTCAAGCGCCCGCGTTTCT CCGCACAAGCCTTAATTGACCGAGGCGGTCCACTTGCCG ATGTCCATGCAAAAATAAAGAACCGGGTATATGAACAG TGCCTCCAAGCTTGTGAGCCCGGTAGTCGTGCAACCCCA GCCACCAGGGAGTGGCTCGACAAAATGTGGGATAGGGT GGACATG	TAQMAN
	>gb|AY383638.1| Porcine reproductive and respiratory syndrome virus strain SDPRRS 03-17 polyprotein gene, partial cds	AY383638.1	1561	1758	198	Plus - Plus - Minus	CCTTTCGAATTTGCCGAACTCAAGCGCCCGCGTTTCTCC GCACAAGCCTTAATTGACCGAGGCGGCCCGCTTGCCGA TGTCCATGCAAAAATAAAGCACCGGGTGTATGAACAAT GCCTTCAAGCTTGTGAGCCTGGTAGCCGTGCAACCCCAG CCACCAAGAAGTGGCTCGACAAAATGTGGGACAGGGTG GACATG	TAQMAN
	>gb|M96262.2|LEYPOLYENV Lelystad virus, complete genome	M96262.2	2986	3185	200	Plus - Plus - Minus	ATCCTTTCGAATTTGCCGAACTCAAGCGCCCGCGTTTCT CCGCACAAGCCTTAATTGACCGAGGCGGTCCACTTGCCG ATGTCCATGCAAAAATAAAGAACCGGGTATATGAACAG TGCCTCCAAGCTTGTGAGCCCGGTAGTCGTGCAACCCCA GCCACCAGGGAGTGGCTCGACAAAATGTGGGATAGGGT GGACATG	TAQMAN
	>gb|AY588319.1| PRRSV LV4.2.1, complete genome	AY588319.1	2986	3185	200	Plus - Plus - Minus	CCTTTCGAATTTGCCGAACTCAAGCGCCCGCGTTTCTCC GCACAAGCCTTAATTGACCGAGGCGGCCCGCTTGCCGA TGTCCATGCAAAAATAAAGCACCGGGTGTATGAACAAT GCCTTCAAGCTTGTGAGCCTGGTAGCCGTGCAACCCCAG CCACCAAGAAGTGGCTCGACAAAATGTGGGACAGGGTG GACATG	TAQMAN
	>gb|AY749375.1| Porcine reproductive and respiratory syndrome virus strain MN-03-01_EU nonstructural protein nsp2 gene, partial cds	AY749375.1	1561	1758	198	Plus - Plus - Minus	CCTTTCGAATTTGCCGAACTCAAGCGCCCGCGTTTCTCC GCACAAGCCTTAATTGACCGAGGCGGCCCGCTTGCCGA TGTCCATGCAAAAATAAAGCACCGGGTGTATGAACAAT GCCTTCAAGCTTGTGAGCCTGGTAGCCGTGCAACCCCAG CCACCAAGAAGTGGCTCGACAAAATGTGGGACAGGGTG GACATG	TAQMAN
	>gb|AY749379.1| Porcine reproductive and respiratory syndrome virus strain MN-03-05_EU nonstructural protein nsp2 gene, partial cds	AY749379.1	1561	1758	198	Plus - Plus - Minus	CCTTTCGAATTTGCCGAACTCAAGCGCCCGCGTTTCTCC GCACAAGCCTTAATTGACCGAGGCGGCCCGCTTGCCGA TGTCCATGCAAAAATAAAGCATCGGGTGTACGAACAGT GCCTTCAAGCTTGTGAGCCTGGTAGCCGTGCAACCCCAG CCACCAAGAAGTGGCTCGACAAAATGTGGGATAGGGTG GACATG	TAQMAN
[redacted]								
sig_candidate_1810351	>gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome	AY366525.1	3773	3913	141	Plus - Plus - Minus	TTCTTGTGACCACGATTCGCCGGAATGTCATGCTGAGCT TTTGGCTCTTGAGCAGCGCCAACTTTGGGAACCTGTGCG CGGCCTTGTGGTCGGCCCCTCGGGCCTCTTATGTGTCAT TCTTGGCAAGTTACTCGGTGGGTC	TAQMAN
6 total sequence hits	>gb|AY375474.1| Porcine reproductive and respiratory syndrome virus ORF1 gene, complete cds	AY375474.1	3552	3692	141	Plus - Plus - Minus	TTCTTGTGACCACGATTCGCCGGAATGTCATGCTGAGCT TTTGGCTCTTGAGCAGCGCCAACTTTGGGAACCTGTGCG CGGCCTTGTGGTTGGCCCCTCAGGTCTCTTATGTGTCAT CCTTGGCAAGTTACTCGGTGGGTC	TAQMAN
	>gb|AY588319.1| PRRSV LV4.2.1, complete genome	AY588319.1	3824	3964	141	Plus - Plus - Minus	TTCTTGTGACCACGATTCGCCGGAGTGTCATGCTGAGCT TTTGGCTCTTGAGCAGCGCCAACTTTGGGAACCTGTGCG CGGCCTTGTGGTCGGCCCCTCAGGCCTCTTATGTGTCAT TCTTGGCAAGTTACTCGGTGGGTC	TAQMAN
	>gb|DQ489311.1| Porcine reproductive and respiratory syndrome virus strain SD01-08, complete genome	DQ489311.1	3773	3913	141	Plus - Plus - Minus	TTCTTGTGACCACGATTCGCCGGAATGTCATGCTGAGCT TTTGGCTCTTGAGCAGCGCCAACTTTGGGAACCTGTGCG CGGCCTTGTGGTTGGCCCCTCAGGTCTCTTATGTGTCAT CCTTGGCAAGTTACTCGGTGGGTC	TAQMAN
	>gb|DQ864705.1| Porcine respiratory and reproductive syndrome virus strain 01CB1, complete genome	DQ864705.1	3747	3887	141	Plus - Plus - Minus	TTCTTGTGACCACGATTCGCCGGAGTGTCAAGCTGAGCT TTTGGCTCTTGAGCAGCGCCAACTTTGGGAACCTGTGCG CGGCCTTGTGGTCGGCCCCTCAGGCCTCTTATGTGTCAT TCTTGGCAAGTTACTCGGTGGGTC	TAQMAN
	>gb|M96262.2|LEYPOLYENV Lelystad virus, complete genome	M96262.2	3824	3964	141	Plus - Plus - Minus	TTCTTGTGACCACGATTCGCCGGAGTGTCATGCTGAGCT TTTGGCTCTTGAGCAGCGCCAACTTTGGGAACCTGTGCG CGGCCTTGTGGTCGGCCCCTCAGGCCTCTTATGTGTCAT TCTTGGCAAGTTACTCGGTGGGTC	TAQMAN
[redacted]								

sig_candidate_1810355	>gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome	AY366525.1	5160	5288	129	Plus - Plus - Minus	GAAGGCACTTATATGGCCGCCGTCCGGAGAGCTGCTTTA ACTGGGCGAACTTTAATCTTCACCCCGTCTGCAGTTGGA TCCCTTCTCGAAGGTGCCTTCAGGACTCAAAAACCCTGC CTTAACACCGTG	TAQMAN
	>gb|AY375474.1| Porcine reproductive and respiratory syndrome virus ORF1 gene, complete cds	AY375474.1	4939	5067	129	Plus - Plus - Minus	GAAGGCACTTATATGGCCGCCGTCCGGAGAGCTGCTTTA ACTGGACGAACTTTGATCTTCACCCCGTCTGCGGTCGGA TCCCTTCTTGAAGGTGCTTTCAGGACTCATAAACCCTGC CTTAACACCGTG	TAQMAN
6 total sequence hits	>gb|AY588319.1| PRRSV LV4.2.1, complete genome	AY588319.1	5211	5339	129	Plus - Plus - Minus	GAAGGCACTTATATGGCCGCCGTCCGGAGAGCTGCTTTA ACTGGGCGAACTTTAATCTTCACCCCGTCTGCAGTTGGA TCCCTTCTCGAAGGTGCTTTCAGGACTCATAAACCCTGC CTTAACACCGTG	TAQMAN
	>gb|DQ489311.1| Porcine reproductive and respiratory syndrome virus strain SD01-08, complete genome	DQ489311.1	5160	5288	129	Plus - Plus - Minus	GAAGGCACTTATATGGCCGCCGTCCGGAGAGCTGCTTTA ACTGGACGAACTTTGATCTTCACCCCGTCTGCGGTCGGA TCCCTTCTTGAAGGTGCTTTCAGGACTCATAAACCCTGC CTTAACACCGTG	TAQMAN
	>gb|DQ864705.1| Porcine respiratory and reproductive syndrome virus strain 01CB1, complete genome	DQ864705.1	5134	5262	129	Plus - Plus - Minus	GAAGGCACTTATATGGCCGCCGTCCGGAGAGCTGCTTTA ACTGGGCGAACTTTAATCTTCACCCCGTCTGCAGTTGGA TCCCTTCTCGAAGGTGCTTTCAGGACTCATAAACCCTGC CTTAACACCGTG	TAQMAN
	>gb|M96262.2|LEYPOLYENV Lelystad virus, complete genome	M96262.2	5211	5339	129	Plus - Plus - Minus	GAAGGCACTTATATGGCCGCCGTCCGGAGAGCTGCTTTA ACTGGGCGAACTTTAATCTTCACCCCGTCTGCAGTTGGA TCCCTTCTCGAAGGTGCTTTCAGGACTCATAAACCCTGC CTTAACACCGTG	TAQMAN
sig_candidate_1810360	>gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome	AY366525.1	6769	6879	111	Plus - Plus - Minus	GCGGCTCCAAATTCAGTGTTTGCACTGTTGTGTCCAACA CACCCGTGGATGCCTTAACCGGCATCCCACTCCAGACAC CAACCCCTCTTTTTGAGAATGGTCCGCGTCATC	TAQMAN
	>gb|AY588319.1| PRRSV LV4.2.1, complete genome	AY588319.1	6820	6930	111	Plus - Plus - Minus	GCGGCTCCAAATTCAGTGTTTGTACTGTCGTGTCCAACA CACCCGTGGACGCCTTAACCGGCATCCCACTCCAGACAC CAACCCCTCTTTTTGAGAATGGTCCGCGTCATC	TAQMAN
4 total sequence hits	>gb|DQ864705.1| Porcine respiratory and reproductive syndrome virus strain 01CB1, complete genome	DQ864705.1	6743	6853	111	Plus - Plus - Minus	GCGGCTCCAAATTCAGTGTTTGTACTGTCGTGTCCAACA CACCCGTGGACGCCTTGACCGGCATCCCACTCCAGACAC CAACCCCTCTTTTTGAGAATGGTCCGCGTCATC	TAQMAN
	>gb|M96262.2|LEYPOLYENV Lelystad virus, complete genome	M96262.2	6820	6930	111	Plus - Plus - Minus	GCGGCTCCAAATTCAGTGTTTGTACTGTCGTGTCCAACA CACCCGTGGACGCCTTAACCGGCATCCCACTCCAGACAC CAACCCCTCTTTTTGAGAATGGTCCGCGTCATC	TAQMAN
sig_candidate_1810362	>gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome	AY366525.1	7517	7602	86	Plus - Minus - Minus	CACGCTGTTGTGGCAAACTTATGTTCGGGTGTCATCTTG ATGAGACCTCACCCACCGTCCCTTGTTGACGTTCTTCTG AAACCCGG	TAQMAN
	>gb|AY375474.1| Porcine reproductive and respiratory syndrome virus ORF1 gene, complete cds	AY375474.1	7296	7381	86	Plus - Minus - Minus	CACGCTGTTGTGGCAAACTTATGTTCTGGTGTCATCTTG ATGAGACCTCACCCACCGTCCCTTGTTGATGTTCTTCTG AAACCCGG	TAQMAN
6 total sequence hits	>gb|AY588319.1| PRRSV LV4.2.1, complete genome	AY588319.1	7568	7653	86	Plus - Minus - Minus	CACGCTGTTGTGGCAAACTTATGCTCCGGTGTCATCTTG ATGAGACCTCACCCACCGTCCCTTGTCGACGTTCTTCTG AAACCCGG	TAQMAN
	>gb|DQ489311.1| Porcine reproductive and respiratory syndrome virus strain SD01-08, complete genome	DQ489311.1	7517	7602	86	Plus - Minus - Minus	CACGCTGTTGTGGCAAACTTATGTTCTGGTGTCATCTTG ATGAGACCTCACCCACCGTCCCTTGTTGATGTTCTTCTG AAACCCGG	TAQMAN
	>gb|DQ864705.1| Porcine respiratory and reproductive syndrome virus strain 01CB1, complete genome	DQ864705.1	7491	7576	86	Plus - Minus - Minus	CACGCTGTTGTGGCAAACTTATGTTCCGGTGTCATCTTG ATGAGACCTCACCCACCGTCCCTTGTTGACGTTCTTCTG AAACCCGG	TAQMAN
	>gb|M96262.2|LEYPOLYENV Lelystad virus, complete genome	M96262.2	7568	7653	86	Plus - Minus - Minus	CACGCTGTTGTGGCAAACTTATGTTCCGGTGTCATCTTG ATGAGACCTCACCCACCGTCCCTTGTCGACGTTCTTCTG AAACCCGG	TAQMAN
sig_candidate_1810365	>gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome	AY366525.1	8154	8320	167	Plus - Minus - Minus	TTGGCCATATTGGTAAGGCGCCGCCATTGTTCCTTCCAT CAACCTATCCCGCCAAAAACTCTATGGCAGGGATCAAT GGCCAGAGGTTTCCAACAAAGGACGTTCAGAGCATACC TGAAATTGATGAAATGTGTGCCCGCGCCGTCAAGGAGA ATTGGCAAACTGTG	TAQMAN
	>gb|AY375474.1| Porcine reproductive and respiratory syndrome virus ORF1 gene, complete cds	AY375474.1	7933	8099	167	Plus - Minus - Minus	TTGGCCATATTGGTAAGGCACCGCCATTGTTCCTCCCAT CAACCTATCCCGCTAAAAATTCTATGGCAGGGATCAATG GCCAGAGGTTCCCAACAAAGGACGTCCAGAGCATACCT GAAATTGACGAAATGTGTGCCCGCGCCGTCAAGGAGAA TTGGCAAACTGTG	TAQMAN
6 total sequence hits	>gb|AY588319.1| PRRSV LV4.2.1, complete genome	AY588319.1	8205	8371	167	Plus - Minus - Minus	TTGGCCATATTGGTAAGGCGCCGCCATTGTTCCTCCCAT CAACCTATCCCGCCAAGAACTCTATGGCAGGGATCAAT GGCCAGAGGTTCCCAACAAAGGACGTTCAGAGCATACC TGAAATTGATGAAATGTGTGCCCGCGCTGTCAAGGAGA ATTGGCAAACTGTG	TAQMAN
	>gb|DQ489311.1| Porcine reproductive and respiratory syndrome virus strain SD01-08, complete genome	DQ489311.1	8154	8320	167	Plus - Minus - Minus	TTGGCCATATTGGTAAGGCACCGCCATTGTTCCTCCCGT CAACCTATCCCGCTAAAAATTCTATGGCAGGGATCAATG GCCAGAGGTTCCCAACAAAGGACGTCCAGAGCATACCT GAAATTGACGAAATGTGTGCCCGCGCCGTCAAGGAGAA TTGGCAAACTGTG	TAQMAN
	>gb|DQ864705.1| Porcine respiratory and reproductive syndrome virus strain 01CB1, complete genome	DQ864705.1	8128	8294	167	Plus - Minus - Minus	TTGGCCATATTGGTAAGGCGCCGCCATTGTTCCTCCCAT CAACCTATCCCGCCAAGAACTCTATGGCAGGGATCAAT GGCCAGAGGTTCCCAACAAAGGACGTTCAGAGCATACC TGAAATTGATGAAATGTGTGCCCGCGCCGTCAAGGAGA ATTGGCAAACTGTG	TAQMAN
	>gb|M96262.2|LEYPOLYENV Lelystad virus, complete genome	M96262.2	8205	8371	167	Plus - Minus - Minus	TTGGCCATATTGGTAAGGCGCCGCCATTGTTCCTCCCAT CAACCTATCCCGCCAAGAACTCTATGGCAGGGATCAAT GGCCAGAGGTTCCCAACAAAGGACGTTCAGAGCATACC TGAAATTGATGAAATGTGTGCCCGCGCTGTCAAGGAGA ATTGGCAAACTGTG	TAQMAN
sig_candidate_1810367	>gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome	AY366525.1	8558	8680	123	Plus - Minus - Minus	CCCGCCATTGTAAGATGGTTCGTCGCCAACCTCCTGTAT GAACTTGCAGGATGTGAAGAGTACTTGCCTAGCTATGTG CTTAATTGCTGCCATGACCTTGTGGCAACACAGGATGGT GCCTTC	TAQMAN
	>gb|AY588319.1| PRRSV LV4.2.1, complete genome	AY588319.1	8609	8731	123	Plus - Minus - Minus	CCCGCCATTGTAAGATGGTTTGTTGCTAACCTCCTGTAT GAACTTGCAGGATGTGAAGAGTACTTGCCTAGCTATGTG CTTAATTGCTGCCATGACCTCGTGGCAACACAGGATGGT GCCTTC	TAQMAN
4 total sequence hits	>gb|DQ864705.1| Porcine respiratory and reproductive syndrome virus strain 01CB1, complete genome	DQ864705.1	8532	8654	123	Plus - Minus - Minus	CCCGCCATTGTAAGATGGTTTGTTGCCAACCTCCTGTAT GAACTTGCAGGATGTGAAGAGTACTTGCCTAGCTATGTG CTTAATTGCTGCCATGACCTCGTGGCAACACAGGATGGT GCCTTC	TAQMAN

	>gb|M96262.2|LEYPOLYENV Lelystad virus, complete genome	M96262.2	8609	8731	123	Plus - Minus - Minus	CCCGCCATTGTAAGATGGTTTGTTGCCAACCTCCTGTATGAACTTGCAGGATGTGAAGAGTACTTGCCTAGCTATGTGCTTAATTGCTGCCATGACCTCGTGGCAACACAGGATGGTGCCTTC	TAQMAN
sig_candidate_1810368	>gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome	AY366525.1	8713	8902	190	Plus - Plus - Minus	CGTCACCAGTGTGTCCAACACCGTATATTCACTGGTGATTTATGCCCAGCACATGGTGTTGTCGGCCTTGAAAATGGGTCATGAAATCGGTCTCAAGTTCCTCGAGGAACAGCTCAAATTTGAGGACCTCCTCGAAATTCAGCCTATGCTGGTATACTCTGATGACCTTGTCTTGTACGCTGAAAGACCC	TAQMAN
6 total sequence hits	>gb|AY375474.1| Porcine reproductive and respiratory syndrome virus ORF1 gene, complete cds	AY375474.1	8492	8677	186	Plus - Plus - Minus	CGTCACCAGTGTGTCTAACACCGTATATTCACTGATAATCTATGCCCAGCACATGGTGTTGTCGGCCTTAAAAATGGGTCATGAAATCGGTCTCAAGTTCCTCGAGGAACAGCTCAAATTCGAGGACCTCCTCGAAATCCAGCCTATGTTGGTCTATTCTGATGACCTTGTCTTGTACGCTGAAAG	TAQMAN
	>gb|AY588319.1| PRRSV LV4.2.1, complete genome	AY588319.1	8764	8953	190	Plus - Plus - Minus	CGTCACCAGTGTGTCCAACACCGTATATTCACTGGTAATTTATGCCCAGCACATGGTATTGTCGGCCTTGAAAATGGGTCATGAAATTGGTCTTAAGTTCCTCGAGGAACAGCTCAAGTTCGAGGACCTCCTTGAAATTCAGCCTATGTTGGTATACTCTGATGATCTTGTCTTGTACGCTGAAAGACCC	TAQMAN
	>gb|DQ489311.1| Porcine reproductive and respiratory syndrome virus strain SD01-08, complete genome	DQ489311.1	8713	8898	186	Plus - Plus - Minus	CGTCACCAGTGTGTCTAACACCGTATATTCACTGATAATCTATGCCCAGCACATGGTGTTGTCGGCCTTAAAAATGGGTCATGAAATCGGTCTCAAGTTCCTCGAGGAACAGCTCAAATTCGAGGACCTCCTCGAAATCCAGCCTATGTTGGTCTATTCTGATGACCTTGTCTTGTACGCTGAAAG	TAQMAN
	>gb|DQ864705.1| Porcine respiratory and reproductive syndrome virus strain 01CB1, complete genome	DQ864705.1	8687	8876	190	Plus - Plus - Minus	CGTCACCAGTGTGTCCAACACCGTATATTCACTGGTAATTTATGCCCAGCACATGGTATTGTCGGCCTTGAAAATGGGTCATGAAATTGGTCTTAAGTTCCTCGAGGAACAGCTCAAATTCGAGGACCTCCTTGAAATTCAGCCTATGTTGGTATACTCTGATGACCTTGTCTTGTACGCTGAAAGACCC	TAQMAN
	>gb|M96262.2|LEYPOLYENV Lelystad virus, complete genome	M96262.2	8764	8953	190	Plus - Plus - Minus	CGTCACCAGTGTGTCCAACACCGTATATTCACTGGTAATTTATGCCCAGCACATGGTATTGTCGGCCTTGAAAATGGGTCATGAAATTGGTCTTAAGTTCCTCGAGGAACAGCTCAAGTTCGAGGACCTCCTTGAAATTCAGCCTATGTTGGTATACTCTGATGATCTTGTCTTGTACGCTGAAAGACCC	TAQMAN
sig_candidate_1810369	>gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome	AY366525.1	8951	9119	169	Plus - Plus - Minus	GGTTTCAGAACGGACCCAAAGAAGACAGTCATAACTGATAAACCCAGCTTCCTCGGCTGCAGAATTGAGGCAGGGCGACAGCTGGTTCCCAATCGCGACCGCATCCTGGCTGCTCTCGCATACCACATGAAGGCGCAGAACGCCTCAGAGTATTATGCGTCTGCTGCCG	TAQMAN
6 total sequence hits	>gb|AY375474.1| Porcine reproductive and respiratory syndrome virus ORF1 gene, complete cds	AY375474.1	8730	8898	169	Plus - Plus - Minus	GGTTTCAGAACGGACCCAAAGAAGACAGTCATAACTGATAAACCCAGCTTCCTCGGCTGCAGAATTGAGGCGGGGCGACAGCTGGTCCCTAATCGCGACCGCATCCTGGCTGCTCTCGCATATCACATGAAGGCGCAGAACGCCTCAGAGTATTATGCGTCTGCTGCCG	TAQMAN
	>gb|AY588319.1| PRRSV LV4.2.1, complete genome	AY588319.1	9002	9170	169	Plus - Plus - Minus	GGTTTCAGAACGGACCCAAAGAAAACCGTCATAACTGATAAACCCAGCTTCCTCGGCTGCAGAATTGAGGCAGGGCGACAGCTAGTCCCCAATCGCGACCGCATCCTGGCTGCTCTTGCATATCACATGAAGGCGCAGAACGCCTCAGAGTATTATGCGTCTGCTGCCG	TAQMAN
	>gb|DQ489311.1| Porcine reproductive and respiratory syndrome virus strain SD01-08, complete genome	DQ489311.1	8951	9119	169	Plus - Plus - Minus	GGTTTCAGAACGGACCCAAAGAAGACAGTCATAACTGATAAACCCAGCTTCCTCGGCTGCAGAATTGAGGCGGGGCGACAGCTGGTCCCTAATCGCGACCGCATCCTGGCTGCTCTCGCATATCACATGAAGGCGCAGAACGCCTCAGAGTATTATGCGTCTGCTGCCG	TAQMAN
	>gb|DQ864705.1| Porcine respiratory and reproductive syndrome virus strain 01CB1, complete genome	DQ864705.1	8925	9093	169	Plus - Plus - Minus	GGTTTCAGAACGGACCCAAAGAAAACCGTCATAACTGATAAACCCAGCTTCCTCGGCTGCAGAATTGAGGCAGGGCGACAGCTAGTCCCCAATCGCGACCGCATCCTGGCTGCTCTTGCATATCACATGAAGGCGCAGAACGCCTCAGAGTATTATGCGTCTGCTGCCG	TAQMAN
	>gb|M96262.2|LEYPOLYENV Lelystad virus, complete genome	M96262.2	9002	9170	169	Plus - Plus - Minus	GGTTTCAGAACGGACCCAAAGAAAACCGTCATAACTGATAAACCCAGCTTCCTCGGCTGCAGAATTGAGGCAGGGCGACAGCTAGTCCCCAATCGCGACCGCATCCTGGCTGCTCTTGCATATCACATGAAGGCGCAGAACGCCTCAGAGTATTATGCGTCTGCTGCCG	TAQMAN
sig_candidate_1810374	>gb|M96262.2|LEYPOLYENV Lelystad virus, complete genome	M96262.2	10358	10518	161	Plus - Plus - Minus	TTGCATCTACCATCGCCAAAGTCCCTAAATAAATCCCGAGCACTTGTAGCCATCACTCGGGCAAGACACGGGTTGTTCATTTATGACCCTCATAACCAGCTCCAGGAGTTTTTCAACTTAACCCCTGAGCGCACTGATTGTAACCTTGTGTTCAGCCGTGG	TAQMAN
	>gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome	AY366525.1	10307	10467	161	Plus - Plus - Minus	TTGCATCTACCATCGCCAAAATCTCTAAACAAATCCCGAGCACTTGTGGCCATCACTCGGGCAAGACACGGGTTGTTCATTTATGACCCTCATAATCAGCTTCAGGAGTTTTTCAACCTAACCCCTGAGCGTACTGATTGCAACCTTGTGTTCAGCCGTGG	TAQMAN
7 total sequence hits	>gb|AY375474.1| Porcine reproductive and respiratory syndrome virus ORF1 gene, complete cds	AY375474.1	10086	10246	161	Plus - Plus - Minus	TTGCATCTACCATCACCAAAGTCCCTAAATAAATCCCGAGCACTTGTAGCCATCACTCGGGCAAGACACGGGTTGTTCATTTATGACCCTCACAACCAGCTCCAGGAGTTTTTCAACCTGATCCCTGAGCGCACTGATTGCAACCTTGTGTTCAGCCGTGG	TAQMAN
	>gb|AY588319.1| PRRSV LV4.2.1, complete genome	AY588319.1	10358	10518	161	Plus - Plus - Minus	TTGCATCTACCATCGCCAAAGTCCCTAAATAAATCCCGAGCACTTGTAGCCATCACTCGGGCAAGACACGGGTTGTTCATTTATGACCCTCATAACCAGCTCCAGGAGTTTTTCAACTTAACCCCTGAGCGCACTGATTGTAACCTTGTGTTCAGCCGTGG	TAQMAN
	>gb|DQ489311.1| Porcine reproductive and respiratory syndrome virus strain SD01-08, complete genome	DQ489311.1	10307	10467	161	Plus - Plus - Minus	TTGCATCTACCATCACCAAAGTCCCTAAATAAATCCCGAGCACTTGTAGCCATCACTCGGGCAAGACACGGGTTGTTCATTTATGACCCTCACAACCAGCTCCAGGAGTTTTTCAACCTGATCCCTGAGCGCACTGATTGCAACCTTGTGTTCAGCCGTGG	TAQMAN
	>gb|DQ864705.1| Porcine respiratory and reproductive syndrome virus strain 01CB1, complete genome	DQ864705.1	10281	10441	161	Plus - Plus - Minus	TTGCATCTACCATCGCCAAAGTCCCTAAATAAATCCCGAGCACTTGTAGCCATCACTCGGGCAAGACACGGGTTGTTCATTTATGACCCTCATAACCAGCTCCAGGAGTTTTTCAACTTAACCCCTGAGCGCACTGATTGTAACCTTGTGTTCAGCCGTGG	TAQMAN
	>gb|L04493.1|PRWPOLGLYM Porcine reproductive and respiratory syndrome virus (PRRSV) RNA polymerase, 3' end; complete ORF3, ORF4, ORF5; glycoprotein gene, membrane protein gene, nucleocapsid protein gene, complete cds's	L04493.1	176	336	161	Plus - Plus - Minus	TTGCATCTACCATCGCCAAAGTCCCTAAATAAATCCCGAGCACTTGTAGCCATCACTCGGGCAAGACACGGGTTGTTCATTTATGACCCTCATAACCAGCTCCAGGAGTTTTTCAACTTAACCCCTGAGCGCACTGATTGTAACCTTGTGTTCAGCCGTGG	TAQMAN

sig_candidate_1810375	>gb|M96262.2|LEYPOLYENV Lelystad virus, complete genome	M96262.2	10540	10682	143	Plus - Plus - Minus	TGCGGATAATGCAGTCACAACTGTAGCGAAGGCCCTTG AGACAGGTCCATCTCGATTTCGAGTATCAGACCCGAGGT GCAAGTCTCTCTTAGCCGCTTGTTCGGCCAGTCTGGAAG GGAGCTGTATGCCACTACCGCAAGTGG	TAQMAN
	>gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome	AY366525.1	10489	10631	143	Plus - Plus - Minus	TGCGGATAATGCAGTCACAACTGTGGCGAAGGCCCTAG AAACAGGTCCATCTCGATTTCGAGTGTCAGACCCGAGGT GCAAGTCTCTCTTAGCCGCTTGTTCGGCCAGTCTGGAGG GGAGCTGTATGCCACTACCGCAAGTGG	TAQMAN
7 total sequence hits	>gb|AY375474.1| Porcine reproductive and respiratory syndrome virus ORF1 gene, complete cds	AY375474.1	10268	10410	143	Plus - Plus - Minus	TGCGGACAATGCAGTCACAACTGTAGCGAAGGCCCTAG GGACAGGTCCATCTCGATTTCGAGTATCAGACCCGAGGT GCAAGTCTCTCTTAGCTGCTTGTTCGGCCAGTCTGGAGG GGAGCTGTATGCCACTACCACAAGTGG	TAQMAN
	>gb|AY588319.1| PRRSV LV4.2.1, complete genome	AY588319.1	10540	10682	143	Plus - Plus - Minus	TGCGGATAATGCAGTCACAACTGTAGCGAAGGCCCTTG AGACAGGTCCATCTCGATTTCGAGTATCAGACCCGAGGT GCAAGTCTCTCTTAGCCGCTTGTTCGGCCAGTCTGGAAG GGAGCTGTATGCCACTACCGCAAGTGG	TAQMAN
	>gb|DQ489311.1| Porcine reproductive and respiratory syndrome virus strain SD01-08, complete genome	DQ489311.1	10489	10631	143	Plus - Plus - Minus	TGCGGACAATGCAGTCACAACTGTAGCGAAGGCCCTAG GGACAGGTCCATCTCGATTTCGAGTATCAGACCCGAGGT GCAAGTCTCTCTTAGCTGCTTGTTCGGCCAGTCTGGAGG GGAGCTGTATGCCACTACCACAAGTGG	TAQMAN
	>gb|DQ864705.1| Porcine respiratory and reproductive syndrome virus strain 01CB1, complete genome	DQ864705.1	10463	10605	143	Plus - Plus - Minus	TGCGGATAATGCAGTCACAACTGTAGCGAAGGCCCTAG AGACAGGTCCATCTCGATTTCGAGTATCAGACCCGAGGT GCAAGTCTCTCTTAGCCGCTTGTTCGGCCAGTCTGGAAG GGAGCTGTATGCCACTACCGCAAGTGG	TAQMAN
	>gb|L04493.1|PRWPOLGLYM Porcine reproductive and respiratory syndrome virus (PRRSV) RNA polymerase, 3' end; complete ORF3, ORF4, ORF5; glycoprotein gene, membrane protein gene, nucleocapsid protein gene, complete cds's	L04493.1	358	500	143	Plus - Plus - Minus	TGCGGATAATGCAGTCACAACTGTAGCGAAGGCCCTAG AGACAGGTCCATCTCGATTTCGAGTATCAGACCCGAGGT GCAAGTCTCTCTTAGCCGCTTGTTCGGCCAGTCTGGAAG GGAGCTGTATGCCACTACCGCAAGTGG	TAQMAN
[redacted]								
sig_candidate_1810382	>emb|AJ223078.1|PRR223078 Porcine respiratory and reproductive syndrome virus ORFs 2 to 7, isolate Danish DK111-92	AJ223078.1	379	521	143	Plus - Minus - Minus	GTGGTTGGTGAGGCCACTCTCACAAAGCTGTCAGACCTT GACATAGTTGCTCATTTCCAACACCTGGCTGCAGTGGAA GCGGATTCTTGCCGCTTTCTCAGCTCACGACTTGTGATG CTAAAAAATCTCGCCGTTGGCAATGT	TAQMAN
	>emb|X92942.1|PRRSENVNP Porcine reproductive and respiratory syndrome virus envelope protein and nucleoprotein genes	X92942.1	443	585	143	Plus - Minus - Minus	GTGGTTAGTGAGGCCACTCTTACAAAGCTGTCAGGACTT GATATAGTTACTCATTTCCAACACCTGGCCGCAGTGGAG GCGGATTCTTGCCGCTTTCTCAGCTCACGACTTGTGATG CTAAAAAATCTTGCCGTTGGCAATGT	TAQMAN
12 total sequence hits	>gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome	AY366525.1	12123	12265	143	Plus - Minus - Minus	GTGGTTGGTGAGGCCACTCTCACGAAGCTTTCAGGGCTC GACATAGTTACCCATTTCCAACACCTGGCCGCAGTGGAG GCGGATTCTTGTCGCTTTCTCAGCTCACGACTCGTGATG CTAAAGAATCTTGCCGTTGGCAATGT	TAQMAN
	>gb|DQ864705.1| Porcine respiratory and reproductive syndrome virus strain 01CB1, complete genome	DQ864705.1	12097	12239	143	Plus - Minus - Minus	GTGGTTGGTGAGGCCACTCTCACGAAGCTGTCAGGGCTC GATATAGTTACTCATTTCCAATACCTGGCCGCAGTGGAG GCGGATTCTTGCCGCTTTCTCAGCTCACGACTCGTGATG CTAAAAAATCTTGCCGTTGGCAATGT	TAQMAN
	>gb|AY395079.1| Porcine reproductive and respiratory syndrome virus isolate SD-01-07 glycoprotein GP2, protein E, glycoprotein GP3, glycoprotein GP4, glycoprotein GP5, membrane protein M, and nucleocapsid protein N genes, complete cds	AY395079.1	379	521	143	Plus - Minus - Minus	GTGGTTGGTGAGGCCACTCTCACGAAACTGTCAAGGCTC GATATAGTTACTCATTTTCAACACCTGGCCGCAGTGGAG GCGGATTCTTGTCGCTTTCTCAGCTCACGACTAGTAATG CTAAAAAATCTTGCCGTTGGCAATGT	TAQMAN
	>gb|L04493.1|PRWPOLGLYM Porcine reproductive and respiratory syndrome virus (PRRSV) RNA polymerase, 3' end; complete ORF3, ORF4, ORF5; glycoprotein gene, membrane protein gene, nucleocapsid protein gene, complete cds's	L04493.1	1992	2134	143	Plus - Minus - Minus	GTGGTTGGTGAGGCCACTCTCACGAAGCTGTCAGGGCTC GATATAGTTACTCATTTCCAACACCTGGCCGCAGTGGAG GCGGATTCTTGCCGCTTTCTCAGCTCACGACTCGTGATG CTAAAAAATCTTGCCGTTGGCAATGT	TAQMAN
	>gb|AY395080.1| Porcine reproductive and respiratory syndrome virus isolate SD-01-08 glycoprotein GP2, protein E, glycoprotein GP3, glycoprotein GP4, glycoprotein GP5, membrane protein M, and nucleocapsid protein N genes, complete cds	AY395080.1	379	521	143	Plus - Minus - Minus	GTGGTCGGTGAGGCCACTCTCACGAAGCTATCAAAGCTT GATATAGTTACTCATTTCCAACATCTGGCCGCAGTGGAG GCGGATTCTTGCCGCTTTCTCAGCTCACGACTCGTGATG CTAAAAAATCTTGCCGTTGGCAATGT	TAQMAN
	>gb|M96262.2|LEYPOLYENV Lelystad virus, complete genome	M96262.2	12174	12316	143	Plus - Minus - Minus	GTGGTTGGTGAGGCCACTCTCACGAAGCTGTCAGGGCTC GATATAGTTACTCATTTCCAACACCTGGCCGCAGTGGAG GCGGATTCTTGCCGCTTTCTCAGCTCACGACTCGTGATG CTAAAAAATCTTGCCGTTGGCAATGT	TAQMAN
	>gb|AY395081.1| Porcine reproductive and respiratory syndrome virus isolate SD-02-10 glycoprotein GP2, protein E, glycoprotein GP3, glycoprotein GP4, glycoprotein GP5, membrane protein M, and nucleocapsid protein N genes, complete cds	AY395081.1	379	521	143	Plus - Minus - Minus	GTGGTCGGTGAGGCCACTCTTACGAAGCTGTCAAAGCTT GATATAGTTACTCATTTCCAACATCTGGCCGCAGTGGAG GCGGATTCTTGCCGCTTTCTCAGCTCACGACTCGTGATG CTAAAAAATCTTGCCGTTGGCAATGT	TAQMAN
	>gb|AY588319.1| PRRSV LV4.2.1, complete genome	AY588319.1	12174	12316	143	Plus - Minus - Minus	GTGGTTGGTGAGGCCACTCTCACGAAGCTGTCAGGGCTC GATATAGTCACTCATTTCCAACACCTGGCCGCAGTGGAG GCGGATTCTTGCCGCTTTCTCAGCTCACGACTCGTGATG CTAAAAAATCTTGCCGTTGGCAATGT	TAQMAN
	>gb|DQ009659.1| Porcine respiratory and reproductive syndrome virus strain v3 envelope glycoprotein gene, partial cds	DQ009659.1	381	521	141	Plus - Minus - Minus	GGTTGGTGAGGCCACTCTCACGAAGCTGTCAAGGCTCG ATATAGTTACTCATTTCCAATACCTGGCCGCAGTAGAGG CGGATTCTTGCCGCTTTCTCAGCTCACGACTCGTGATGC TAAAAAATCTTGCCGTTGGCAATGT	TAQMAN
	>gb|DQ489311.1| Porcine reproductive and respiratory syndrome virus strain SD01-08, complete genome	DQ489311.1	12123	12265	143	Plus - Minus - Minus	GTGGTCGGTGAGGCCACTCTCACGAAGCTATCAAAGCTT GATATAGTTACTCATTTCCAACATCTGGCCGCAGTAGAG GCGGATTCTTGCCGCTTTCTCAGCTCACGACTCGTGATG CTAAAAAATCTTGCCGTTGGCAATGT	TAQMAN
[redacted]								
sig_candidate_1810383	>gb|AY749407.1| Porcine reproductive and respiratory syndrome virus strain SDPRRS 03-17 GP3 protein gene, complete cds	AY749407.1	10	138	129	Plus - Minus - Minus	CAGTGTGCACGCTTCCATTTTTTCCTCTGTGGCTTCATCT ATCACCTTGTTTGTAGTGCTTTGGCTTCGAATTCCAGCTC TACGCTATGTTTTTGGTTTCCACTGGCCCACGGCAACAC ATCATTCGAG	TAQMAN
	>gb|AY749410.1| Porcine reproductive and respiratory syndrome virus strain SDPRRS 04-48 GP3 protein gene, complete cds	AY749410.1	10	138	129	Plus - Minus - Minus	CAGTGTGCATGCTTCCATTTTTTCCTCTGTGGCTTCATCT GTTACCTTGTTCATAGTGCTTTGGCTGCGAATTCCAGCT CTACGCTATGTTTTTGGTTTCCATTGGCCCATGGCAACA CATCATTCGAG	TAQMAN
51 total sequence hits	>gb|DQ009654.1| Porcine respiratory and reproductive syndrome virus strain v1 envelope glycoprotein gene, partial	DQ009654.1	10	138	129	Plus - Minus - Minus	CAGTGGGCACGCTTCCATTTTTTCCTCTGTGGCTTCATCT GTTACCTTGTTCATAGTGTTTTGGCTTCGAATTCCAGCCG TACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACAC	TAQMAN

cds						ATCATTCGAG	
>gb|AF171696.1|AF171696 Porcine reproductive and respiratory syndrome virus predicted ORF3/ORF4 fusion protein gene, partial cds	AF171696.1	49	177	129	Plus - Minus - Minus	CAGTGTGCACGCCTCCATTTTTTCCTCTGTGGCCTCGTCA GTTACCTTGTTCATAGTTCTTTGGCTTCGAATTCCAGTTC TACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACAC ATCATTCGAG	TAQMAN
>gb|AF303361.1|AF303361 Porcine reproductive and respiratory syndrome virus strain 32413-99DK GP3 gene, complete cds	AF303361.1	10	138	129	Plus - Minus - Minus	CAGTGTGCACGCCTCCATTTTCTCCTCTGTGGCTTCGTCA GTTACCTTGTTCGTAGTTCTTTGGCTTCGAATTCCAGTTC TACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACAC GTCATTCGAG	TAQMAN
>gb|AF303367.1|AF303367 Porcine reproductive and respiratory syndrome virus strain 974-98IT GP3 gene, complete cds	AF303367.1	10	138	129	Plus - Minus - Minus	CAGTGTGCACGCATCCATTTTCTCCTCTGTGGCTTCATCG GTCACCTTGTTCGTGGTGCTTTGGCTTCGAATTCCAACTC TACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACAC ATCATTCGAG	TAQMAN
>gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome	AY366525.1	12362	12490	129	Plus - Minus - Minus	CAGTGTGCACGCTTCCATTTTTTCCTCTGTGGCTTCATCT GTTACTTTGTTCACAGTGCTTTGGCTTCGAATTCCAGCTC TACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACAC ATCATTCGAG	TAQMAN
>gb|DQ009655.1| Porcine respiratory and reproductive syndrome virus strain v3 envelope glycoprotein gene, partial cds	DQ009655.1	14	138	125	Plus - Minus - Minus	GTGCACGCTTCCATTTTTTCCTCTGTGGCTTCATCTGTTA CCTTGTTCATAGTGCTTTGGCTTCGAATTCCAGCTCTACG CTATGTTTTTGGTTTCCATTGGCCCACGGCAACACATCA TTCGAG	TAQMAN
>gb|AY395080.1| Porcine reproductive and respiratory syndrome virus isolate SD-01-08 glycoprotein GP2, protein E, glycoprotein GP3, glycoprotein GP4, glycoprotein GP5, membrane protein M, and nucleocapsid protein N genes, complete cds	AY395080.1	618	746	129	Plus - Minus - Minus	CAGTGTGCATGCTTCCATTTTTTCCTCTGTGGCTTCATCT GTTACCTTGTTCATAGTGCTTTGGCTGCGAATTCCAGCT CTACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACA CATCATTCGAG	TAQMAN
>gb|DQ489311.1| Porcine reproductive and respiratory syndrome virus strain SD01-08, complete genome	DQ489311.1	12362	12490	129	Plus - Minus - Minus	CAGTGTGCATGCTTCCATTTTTTCCTCTGTGGCTTCATCT GTTACCTTGTTCATAGTGCTTTGGCTGCGAATTCCAGCT CTACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACA CATCATTCGAG	TAQMAN
>gb|AY395081.1| Porcine reproductive and respiratory syndrome virus isolate SD-02-10 glycoprotein GP2, protein E, glycoprotein GP3, glycoprotein GP4, glycoprotein GP5, membrane protein M, and nucleocapsid protein N genes, complete cds	AY395081.1	618	746	129	Plus - Minus - Minus	CAGTGTGCATGCTTCCATTTTTTCCTCTGTGGCTTCATCT GTTACCTTGTTCATAGTGCTTTGGCTGCGAATTCCAGCT CTACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACA CGTCATTCGAG	TAQMAN
>gb|DQ864705.1| Porcine respiratory and reproductive syndrome virus strain 01CB1, complete genome	DQ864705.1	12340	12464	125	Plus - Minus - Minus	GTGCACGCTTCCATTTTTTCCTCTGTGGCTTCATCTGTTA CCTTGTTCATAGTGCTTTGGCTTCGAATTCCAGCTCTACG CTATGTTTTTGGTTTCCATTGGCCCACGGCAACACATCA TTCGAG	TAQMAN
>gb|AY588319.1| PRRSV LV4.2.1, complete genome	AY588319.1	12413	12541	129	Plus - Minus - Minus	CAGTGTGCACGCTTCCATTTTTTCCTCTGTGGCTTCATCT GTTACCTTGTTCATAGTGCTTTGGCTTCGAATTCCAGCTC TACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACAC ATCATTCGAG	TAQMAN
>gb|L04493.1|PRWPOLGLYM Porcine reproductive and respiratory syndrome virus (PRRSV) RNA polymerase, 3' end; complete ORF3, ORF4, ORF5; glycoprotein gene, membrane protein gene, nucleocapsid protein gene, complete cds's	L04493.1	2235	2359	125	Plus - Minus - Minus	GTGCACGCTTCCATTTTTTCCTCTGTGGCTTCATCTGTTA CCTTGTTCATAGTGCTTTGGCTTCGAATTCCAGCTCTACG CTATGTTTTTGGTTTCCATTGGCCCACGGCAACACATCA TTCGAG	TAQMAN
>gb|AY749376.1| Porcine reproductive and respiratory syndrome virus strain MN-03-01_EU GP3 protein gene, complete cds	AY749376.1	10	138	129	Plus - Minus - Minus	CAGTGTGCACGCTTCCATTTTTTCCTCTGTGGCTTCATCT ATCACCTTGTTTGTAGTGCTTTGGCTTCGAATTCCAGCTC TACGCTATGTTTTTGGTTTCCACTGGCCCACGGCAACAC ATCATTCGAG	TAQMAN
>gb|L77913.1|PPSBE1ORFA Porcine respiratory and reproductive syndrome virus (isolate Be1) envelope protein (gp3) RNA, complete cds	L77913.1	10	138	129	Plus - Minus - Minus	CAGTGTGCACGCTTCCATTTTTTCCTCTGTGGCTTCATCT GTTACCTTGTTCATAGTGCTTTGGCTTCGAATTCCAGCTC TACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACAC ATCATTCGAG	TAQMAN
>gb|AY749380.1| Porcine reproductive and respiratory syndrome virus strain MN-03-05_EU GP3 protein gene, complete cds	AY749380.1	14	138	125	Plus - Minus - Minus	GTGCACGCTTCCATTTTTTCCTCTGTGGCTTCGTCTGTCA CCTTGTTCATAGTACTTTGGCTTCGAATTCCAGCTCTACG TTATGTTTTTGGTTTCCATTGGCCCACGGCAACACATCA TTCGAG	TAQMAN
>gb|L77915.1|PPSH3ORFA Porcine respiratory and reproductive syndrome virus (isolate H3) envelope protein (gp3) RNA, complete cds	L77915.1	10	138	129	Plus - Minus - Minus	CAGTGTGCACGCTTCCATTTTTTCCTCTGTGGCTTCATCT GTTACCTTGTTCATAGTGCTTTGGCTTCGAATTCCAGCTC TACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACAC ATCATTCGAG	TAQMAN
>gb|AY749384.1| Porcine reproductive and respiratory syndrome virus strain MN-03-08_EU GP3 protein gene, complete cds	AY749384.1	10	138	129	Plus - Minus - Minus	CAGTGTGCACGCTTCCATTTTTTCCTCTGTGGCTTCATCT ATCACCTTGTTTGTAGTGCTTTGGCTTCGAATTCCAGCTC TACGCTATGTTTTTGGTTTCCACTGGCCCACGGCAACAC ATCATTCGAG	TAQMAN
>gb|L77917.1|PPSHA1ORFA Porcine respiratory and reproductive syndrome virus (isolate Ha1) envelope protein (gp3) RNA, complete cds	L77917.1	10	138	129	Plus - Minus - Minus	CAGTGTGCACGCTTCCATTTTTTCCTCTGTGGTTTCATCT GTTACCTTGTTCATAGTGCTTTGGCTTCGAATTCCAGCTC TACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACAC ATCATTCGAG	TAQMAN
>gb|L77919.1|PPSL2ORFA Porcine respiratory and reproductive syndrome virus (isolate L2) envelope protein (gp3) RNA, complete cds	L77919.1	10	138	129	Plus - Minus - Minus	CAGTGTGCACGCTTCCATTTTTTCCTCTGTGGCTTCATCT GTTACCTTGTTCATAGTGCTTTGGCTTCGAATTCCAGCTC TACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACAC ATCATTCGAG	TAQMAN
>gb|L77921.1|PPSLE1ORFA Porcine respiratory and reproductive syndrome virus (isolate Le1) envelope protein (gp3) RNA, complete cds	L77921.1	10	138	129	Plus - Minus - Minus	CAGTGTGCACGCTTCCATTTTTTCCTCTGTGGCTTCATCT GTTACCTTGTTCATAGTGCTTTGGCTTCGAATTCCAGCTT TACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACAC ATCATTCGAG	TAQMAN
>gb|L77923.1|PPSNO1ORFA Porcine respiratory and reproductive syndrome virus (isolate No1) envelope protein (gp3) RNA, complete cds	L77923.1	10	138	129	Plus - Minus - Minus	CAGTGTGCACGCTTCCATTTTTTCCTCTGTGGCTTCATCT GTTACCTTGTTCATAGTGCTTTGGCTTCGAATTCCAGCTC TACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACAC ATCATTCGAG	TAQMAN
>emb|AJ223078.1|PRR223078 Porcine respiratory and reproductive syndrome virus ORFs 2 to 7, isolate Danish DK111-92	AJ223078.1	618	746	129	Plus - Minus - Minus	CAGTGTGCACGCCTCCATTTTTTCCTCTGTGGCCTCGTCA GTTACCTTGTTCATAGTTCTTTGGCTTCGAATTCCAGTTC TACGCTATGTTTTTGGTTTCCACTGGCCCACGGCAACAC ATCATTCGAG	TAQMAN
>emb|X92942.1|PRRSENVNP Porcine reproductive and respiratory syndrome virus envelope protein and nucleoprotein genes	X92942.1	682	810	129	Plus - Minus - Minus	CAGTGTGCATGCTTCCATTTTTTCCTCTGTAGCTTCATCT GTTACCTTGTTCATAGTGCTTTGGCTTCGAATTCCAATTC TACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACAC ATCATTCGAG	TAQMAN
>gb|AF171671.1|AF171671 Porcine reproductive and respiratory syndrome virus isolate 111_92 envelope glycoprotein gene, complete cds	AF171671.1	10	138	129	Plus - Minus - Minus	CAGTGTGCACGCCTCCATTTTTTCCTCTGTGGCCTCGTCA GTTACCTTGTTCATAGTTCTTTGGCTTCGAATTCCAGTTC TACGCTATGTTTTTGGTTTCCACTGGCCCACGGCAACAC ATCATTCGAG	TAQMAN
>gb|AF171686.1|AF171686 Porcine reproductive and respiratory syndrome virus isolate 31690 envelope glycoprotein gene, complete cds	AF171686.1	10	138	129	Plus - Minus - Minus	CAGTGTGCACGCCTCCATTTTTTCCTCTGTGGCTTCGTCA GTTACCTTGTTCATAGTACTTTGGCTTCGAATTCCAGTTC TACGCTATGTTTTTGGTTTCCATTGGCCCACGGTAACAC ATCATTCGAG	TAQMAN

>gb|AF171672.1|AF171672 Porcine reproductive and respiratory syndrome virus isolate 12654 envelope glycoprotein gene, complete cds	AF171672.1	10	138	129	Plus - Minus - Minus	TCATTCGAG	TAQMAN
>gb|AF171687.1|AF171687 Porcine reproductive and respiratory syndrome virus isolate 32_10 envelope glycoprotein gene, complete cds	AF171687.1	10	138	129	Plus - Minus - Minus	CAGTGTGCACGCCTCCATTTTTTCCTCTGTGGCCTCGTCA GTTACCTTGTTCATAGTTCTTTGGCTTCGAATTCCAGTTC TACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACAC ATCATTCGAG	TAQMAN
>gb|AF171673.1|AF171673 Porcine reproductive and respiratory syndrome virus isolate 12985 envelope glycoprotein gene, complete cds	AF171673.1	10	138	129	Plus - Minus - Minus	CAGTGTGCACGCCTCCATTTTTTCCTCTGTGGCCTCGTCA GTTACCTTGTTCATAGTTCTTTGGCTTCGAATTCCAGTTC TACGCTATGCTTTTGGTTTCCATTGGCCCACGGCAACAC ATCATTCGAG	TAQMAN
>gb|AF171688.1|AF171688 Porcine reproductive and respiratory syndrome virus isolate 34_92 envelope glycoprotein gene, complete cds	AF171688.1	10	138	129	Plus - Minus - Minus	CAGTGTGCACGCCTCCATTTTTTCCTCTGTGGCCTCGTCA GTTACCTTGTTCATAGTTCTTTGGCTTCGAATTCCAGTTC TACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACAC ATCATTCGAG	TAQMAN
>gb|AF171674.1|AF171674 Porcine reproductive and respiratory syndrome virus isolate 13759 envelope glycoprotein gene, complete cds	AF171674.1	10	138	129	Plus - Minus - Minus	CAGTGTGCACGCCTCCATTTTTTCCTCTGTGGCCTCGTCA GTTACCTTGTTCATAGTTCTTTGGCTTCGAATTCCAGTTC TACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACAC ATCATTCGAG	TAQMAN
>gb|AF171689.1|AF171689 Porcine reproductive and respiratory syndrome virus isolate 38_8 envelope glycoprotein gene, complete cds	AF171689.1	10	138	129	Plus - Minus - Minus	CAGTGTGCACGCCTCCATTTTTTCCTCTGTGGCTTCGTCA GTTACCTTGTTCATAGTTCTTTGGCTTCGAATTCCAGTTC TACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACAC ATCATTCGAG	TAQMAN
>gb|AF171677.1|AF171677 Porcine reproductive and respiratory syndrome virus isolate 18009_4 envelope glycoprotein gene, complete cds	AF171677.1	10	138	129	Plus - Minus - Minus	CAGTGTGCACGCCTCCATTTTTTCCTCTGTGGCTTCGTCA GTTACCTTGTTCATAGTTCTTTGGCTTCGAATTCCAGTAC TACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACAC ATCATTCGAG	TAQMAN
>gb|AF171690.1|AF171690 Porcine reproductive and respiratory syndrome virus isolate 48_92 envelope glycoprotein gene, complete cds	AF171690.1	10	138	129	Plus - Minus - Minus	CAGTGTGCACGCCTCCATTTTTTCCTCTGTAGCTTCGTCA GTTACCTTGTTCATAGTTCTTTGGCTTCGAATTCCAGTTC TACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACAC ATCATTCGAG	TAQMAN
>gb|AF171678.1|AF171678 Porcine reproductive and respiratory syndrome virus isolate 20567 envelope glycoprotein gene, complete cds	AF171678.1	10	134	125	Plus - Minus - Minus	CAGTGTGCACGCCTCCATTTTTTCCTCTGTGGCCTCGTCA GTTACCTTGTTCATAGTTCTTTGGCTTCGAATTCCAGTTC TACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACAC ATCATT	TAQMAN
>gb|AY749388.1| Porcine reproductive and respiratory syndrome virus strain MN-03-10_EU GP3 protein gene, complete cds	AY749388.1	10	134	125	Plus - Minus - Minus	CAGTGTGCACGCTTCCATTTTTTCCTCTGTGGCTTCATCT GTTGCCTTGTTCATAGTGCTTTGGCTTCGAATTCCAGCTC TACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACAC ATCATT	TAQMAN
>gb|AF171691.1|AF171691 Porcine reproductive and respiratory syndrome virus isolate 54_228A envelope glycoprotein gene, complete cds	AF171691.1	10	134	125	Plus - Minus - Minus	CAGTGTGCACGCTTCCATTTTTTCCTCTGTGGCTTCATCT GTTACCTTGTTCATAGTGCTTTGGCTTCGAATTCCAGCTC TACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACAC ATCATT	TAQMAN
>gb|AF171685.1|AF171685 Porcine reproductive and respiratory syndrome virus isolate 31540 envelope glycoprotein gene, complete cds	AF171685.1	10	138	129	Plus - Minus - Minus	CAGTGTGCACGCCTCCATTTTTTCCTCTGTGGCTTCGTCT GTTACCTTGTTTGTAGTTCTTTGGCTTCGAATTCCAGTTC TACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACAC GTCATTCGAG	TAQMAN
>gb|AY749392.1| Porcine reproductive and respiratory syndrome virus strain MN-04-06_EU GP3 protein gene, complete cds	AY749392.1	10	138	129	Plus - Minus - Minus	CAGTGTGCATGCTTCCATTTTTTCATCTGTGGCTTCATCT GTTACCTTGTTCATAGTGCTTTGGCTGCGAATTCCAGCT CTACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACA CGTCATTCGAG	TAQMAN
>gb|AF171692.1|AF171692 Porcine reproductive and respiratory syndrome virus isolate 5767_6 envelope glycoprotein gene, complete cds	AF171692.1	10	134	125	Plus - Minus - Minus	CAGTGTGCACGCTTCCATTTTTTCCTCTGTGGCTTCATCT GTTACCTTGTTCATAGTGCTTTGGCTTCGAATTCCAGCTC TACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACAC ATCATT	TAQMAN
>gb|AY749396.1| Porcine reproductive and respiratory syndrome virus strain MN-04-09_EU GP3 protein gene, complete cds	AY749396.1	10	138	129	Plus - Minus - Minus	CAGTGTGCATGCTTCCATTTTTTCCTCTGTGGCTTCATCT GTTACCTTGTTCATAGTGCTTTGGCTGCGAATTCCAGCT CTACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACA CGTCATTCGAG	TAQMAN
>gb|AF171693.1|AF171693 Porcine reproductive and respiratory syndrome virus isolate 6501 envelope glycoprotein gene, complete cds	AF171693.1	10	138	129	Plus - Minus - Minus	CAGTGTGCACGCCTCCATTTTTTCCTCTGTGGCCTCGTCA GTTACCTTGTTCATAGTTCTTTGGCTTCGAATTCCAGTTC TACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACAC ATCATTCGAG	TAQMAN
>gb|AY749399.1| Porcine reproductive and respiratory syndrome virus strain SDPRRS 02-11 GP3 protein gene, complete cds	AY749399.1	10	138	129	Plus - Minus - Minus	CAGTGTACACGCTTCCATTTTTTCCTCTGTGGCTTCATCT GTTACCTTGTTCATAGTGCTTTGGCTTCGAATTCCAGCTC TACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACAC ATCATTCGAG	TAQMAN
>gb|L77925.1|PPSNY4ORFA Porcine respiratory and reproductive syndrome virus (isolate NY4) envelope protein (gp3) RNA, complete cds	L77925.1	10	138	129	Plus - Minus - Minus	CAGTGTGCATGCTTCCATTTTTTCCTCTGTGGCTTCATCT ATTACCTTGTTCATAGTGCTTTGGCTTCGAATTCCAGCTC TACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACAC ATCATTCGAG	TAQMAN
>gb|AF171694.1|AF171694 Porcine reproductive and respiratory syndrome virus isolate 6504 envelope glycoprotein gene, complete cds	AF171694.1	10	138	129	Plus - Minus - Minus	CAGTGTGCACGCCTCCATTTTTTCCTCTGTGGCCTCGTCA GTTACCTTGTTCATAGTTCTTTGGCTTCGAATTCCAGTTC TACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACAC ATCATTCGAG	TAQMAN
>gb|AY749401.1| Porcine reproductive and respiratory syndrome virus strain SDPRRS 03-12 GP3 protein gene, complete cds	AY749401.1	10	138	129	Plus - Minus - Minus	CAGTGTGCATGCTTCCATTTTTTCCTCTGTGGCTTCATCT GTTACCTTGTTCATAGTACTTTGGCTGCGAATTCCAGCT CTACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACA CATCGTTCGAG	TAQMAN
>gb|M96262.2|LEYPOLYENV Lelystad virus, complete genome	M96262.2	12413	12541	129	Plus - Minus - Minus	CAGTGTGCACGCTTCCATTTTTTCCTCTGTGGCTTCATCT GTTACCTTGTTCATAGTGCTTTGGCTTCGAATTCCAGCTC TACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACAC ATCATTCGAG	TAQMAN
>gb|AF171695.1|AF171695 Porcine reproductive and respiratory syndrome virus isolate 6617 envelope glycoprotein gene, complete cds	AF171695.1	10	138	129	Plus - Minus - Minus	CAGTGTGCACGCCTCCATTTTTTCCTCTGTGGCCTCGTCA GTTACCTTGTTCATAGTTCTTTGGCTTCGAATTCCAGTTC TACGCTATGTTTTTGGTTTCCATTGGCCCACGGCAACAC ATCATTCGAG	TAQMAN
>gb|AY749403.1| Porcine reproductive and respiratory syndrome virus strain SDPRRS 03-15 GP3 protein gene, complete cds	AY749403.1	10	138	129	Plus - Minus - Minus	CAGTGTGCACGCTTCCATTTTTTCCTCTGTGGCTTCATCT ATCACCTTGTTTGTAGTGCTTTGGCTTCGAATTCCAGCTC TACGCTATGTTTTTGGTTTCCACTGGCCCACGGCAACAC ATCATTCGAG	TAQMAN
>gb|AY749405.1| Porcine reproductive and respiratory syndrome virus strain SDPRRS 03-16 GP3 protein gene, complete cds	AY749405.1	10	138	129	Plus - Minus - Minus	CAGTGTGCACGCTTCCATTTTTTCCTCTGTGGCTTCATCT ATCACCTTGTTTGTAGTGCTTTGGCTTCGAATTCCAGCTC TACGCTATGTTTTTGGTTTCCACTGGCCCACGGCAACAC ATCATTCGAG	TAQMAN
[redacted]							
sig_candidate_1810384	>gb|AF171691.1|AF171691 Porcine reproductive and respiratory syndrome virus isolate 54_228A envelope glycoprotein gene, complete cds	AF171691.1	270	444	175	Plus - Plus - Minus	GTTAATGCCCATCCCGTCCGGGTACGACAACCTCAAACT TGAGGGTTATTATGCTTGGCTGGCTTTTTTGTCCTTTTCC TACGCGGCCCAATTCCATCCGGAGTTGTTCGGGATAGGG AATGTGTCGCGCGTCTTCGTGGACAAGCAACACCAGTTC ATTTGTGCCGAGCATGAT	TAQMAN
	>gb|AF171692.1|AF171692 Porcine reproductive and respiratory syndrome virus isolate 5767_6 envelope glycoprotein gene, complete cds	AF171692.1	270	444	175	Plus - Plus - Minus	GTTAATGTCCATCCCGTCCGGGTACGACAACCTCAAACT TGAGGGTTATTATGCTTGGCTGGCTTTTTTGTCCTTTTCC TACGCGGCCCAATTCCATCCGGAGTTGTTCGGGATAGGG AATGTGTCGCGCGTCTTCGTGGACAAGCGACACCAGTTC ATTTGTGCCGAGCATGAT	TAQMAN

23 total sequence hits	>gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome	AY366525.1	12622	12796	175	Plus - Plus - Minus	GTTAATGTCCATCCCGTCCGGGTACGACAACCTCAAACTTGAGGGGTTATTATGCTTGGCTGGCTTTTTTGTCTTTTTCCTACGCGGCCCAATTCCATCCAGAGTTGTTCGGGATCGGAAATGTGTCGCGCGTCTTCGTGGACAAGTGGCACCAGTTCATTTGTGCCGAGCATGAT	TAQMAN
	>gb|AY395080.1| Porcine reproductive and respiratory syndrome virus isolate SD-01-08 glycoprotein GP2, protein E, glycoprotein GP3, glycoprotein GP4, glycoprotein GP5, membrane protein M, and nucleocapsid protein N genes, complete cds	AY395080.1	878	1052	175	Plus - Plus - Minus	GTTAATGTCCATCCCGTCCGGGTACGACAACCTCAAACTTGAGGGATATTATGCTTGGCTGGCTTTTTTGTCCTTTTCCTACGCGGCCCAATTCCATCCGGAGCTGTTCGGGATAGGGAATGTGTCGCGCGTCTTCGTGGACAAGCGACACCAGTTTATTTGTGCCGAGCATGAT	TAQMAN
	>gb|AY749401.1| Porcine reproductive and respiratory syndrome virus strain SDPRRS 03-12 GP3 protein gene, complete cds	AY749401.1	270	443	174	Plus - Plus - Minus	GTTAATGTCCATCCCGTCCGGGTACGACAACCTCAAACTTGAGGGATATTATGCTTGGCTGGCTTTTTTGTCCTTTTCCTACGCGGCCCAATTCCATCCGGAGCTGTTCGGGATAGGGAATGTGTCGCGCGTCTTCGTGGACAAGCGACACCAGTTTATTTGTGCCGAGCATGA	TAQMAN
	>gb|AY395081.1| Porcine reproductive and respiratory syndrome virus isolate SD-02-10 glycoprotein GP2, protein E, glycoprotein GP3, glycoprotein GP4, glycoprotein GP5, membrane protein M, and nucleocapsid protein N genes, complete cds	AY395081.1	882	1052	171	Plus - Plus - Minus	ATGTCCATCCCGTCCGGGTACGACAACCTCAAACTTGAGGGATATTATGCTTGGCTGGCTTTTTTGTCCTTTTCCTACGCGGCCCAATTCCACCCGGAACTGTTCGGGATAGGGAATGTGTCGCGCGTCTTCGTGGACAAGCGACACCAGTTCATTTGTGCCGAGCATGAT	TAQMAN
	>gb|AY749410.1| Porcine reproductive and respiratory syndrome virus strain SDPRRS 04-48 GP3 protein gene, complete cds	AY749410.1	270	443	174	Plus - Plus - Minus	GTTAATGTCCATCCCGTCTGGGTACGACAACCTCAAACTTGAGGGGTATTATGCTTGGCTGGCTTTTTTGTCCTTTTCCTACGCGGCCCAATTCCATCCGGAGCTGTTCGGGATAGGGAATGTGTCGCGCGTCTTCGTGGACAAGCAACACCAGTTTATTTGTGCCGAGCATGA	TAQMAN
	>gb|L77921.1|PPSLE1ORFA Porcine respiratory and reproductive syndrome virus (isolate Le1) envelope protein (gp3) RNA, complete cds	L77921.1	270	444	175	Plus - Plus - Minus	GTTAATGTCCATCCCGTCCGGGTACGACAACCTCAAACTTGAGGGTTATTATGCTTGGCTGGCTTTTTTGTCCTTTTCCTACGCGGCCCAATTCCATCCGGAGTTGTTCGGGATAGGGAATGTGTCGCGCGTCTTCGTGGACAAGCGACACCAGTTCATTTGTGCCGAGCATGAT	TAQMAN
	>gb|AY588319.1| PRRSV LV4.2.1, complete genome	AY588319.1	12673	12847	175	Plus - Plus - Minus	GTTAATGTCCATCCCGTCCGGGTACGACAACCTCAAACTTGAGGGTTATTATGCTTGGCTGGCTTTTTTGTCCTTTTCCTACGCGGCCCAATTCCATCCGGAGTTGTTCGGGATAGGGAATGTGTCGCGCGTCTTCGTGGACAAGCGACACCAGTTCATTTGTGCCGAGCATGAT	TAQMAN
	>gb|DQ009655.1| Porcine respiratory and reproductive syndrome virus strain v3 envelope glycoprotein gene, partial cds	DQ009655.1	270	444	175	Plus - Plus - Minus	GTTAATGCCCATCCCGTCCGGGTACGACAACCTCAAACTTGAGGGTTATTATGCTTGGCTGGCTTTTTTGTCCTTTTCCTACGCGGCCCAATTCCATCCGGAGTTGTTCGGGATAGGGAATGTGTCGCGCGTCTTCGTGGACAAGCGACACCAGTTCATTTGTGCCGAGCATGAT	TAQMAN
	>gb|L77923.1|PPSNO1ORFA Porcine respiratory and reproductive syndrome virus (isolate No1) envelope protein (gp3) RNA, complete cds	L77923.1	270	444	175	Plus - Plus - Minus	GTTAATGTCCATCCCGTCCGGGTATGACAACCTCAAACTTGAGGGTTATTATGCTTGGCTGGCTTTTTTGTCCTTTTCCTACGCGGCCCAATTCCATCCGGAGTTGTTCGGGATAGGGAATGTGTCGCGCGTCTTCGTGGACAAGCGACACCAGTTCATTTGTGCCGAGCATGAT	TAQMAN
	>gb|AY749380.1| Porcine reproductive and respiratory syndrome virus strain MN-03-05_EU GP3 protein gene, complete cds	AY749380.1	273	444	172	Plus - Plus - Minus	AATGTCCATCCCGTCCGGGTACGACAACCTCAAACTTGAGGGTTATTATGCTTGGCTAGCCTTTTTGTCCTTTTCCTACGCGGCCCAATTTCATCCGGAGTTGTTCGGGATAGGGAATGTGTCACGCGTCTTCGTAGACAAGCAACACCAGTTTATTTGTGCCGAGCATGAT	TAQMAN
	>gb|DQ489311.1| Porcine reproductive and respiratory syndrome virus strain SD01-08, complete genome	DQ489311.1	12622	12794	173	Plus - Plus - Minus	GTTAATGTCCATCCCGTCCGGGTACGACAACCTCAAACTTGAGGGATATTATGCTTGGCTGGCTTTTTTGTCCTTTTCCTACGCGGCCCAATTCCATCCGGAGCTGTTCGGGATAGGGAATGTGTCGCGCGTCTTCGTGGACAAGCGACACCAGTTTATTTGTGCCGAGCATG	TAQMAN
	>gb|L77925.1|PPSNY4ORFA Porcine respiratory and reproductive syndrome virus (isolate NY4) envelope protein (gp3) RNA, complete cds	L77925.1	270	444	175	Plus - Plus - Minus	GTTAATGTCCATCCCGTCCGGGTACGACAACCTCAAACTTGAGGGTTATTATGCTTGGCTGGCTTTTTTGTCCTTTTCCTACGCGGCCCAATTCCATCCGGAGTTATTCGGGATAGGGAATGTGTCACGCGTCTTCGTGGACAAGCAACACCAGTTCATTTGTGCCGAGCATGAT	TAQMAN
	>gb|AY749392.1| Porcine reproductive and respiratory syndrome virus strain MN-04-06_EU GP3 protein gene, complete cds	AY749392.1	274	444	171	Plus - Plus - Minus	ATGTCCATCCCGTCCGGGTACGACAACCTCAAACTTGAGGGATATTATGCTTGGCTGGCTTTTTTGTCCTTTTCTTACGCGGCCCAATTCCATCCGGAGCTGTTCGGGATAGGGAATGTGTCGCGCGTCTTCGTGGACAAGCGACACCAGTTCATTTGTGCCGAGCATGAT	TAQMAN
	>gb|DQ864705.1| Porcine respiratory and reproductive syndrome virus strain 01CB1, complete genome	DQ864705.1	12596	12770	175	Plus - Plus - Minus	GTTAATGTCCATCCCGTCCGGGTACGACAACCTCAAACTTGAGGGTTATTATGCTTGGCTGGCTTTTTTGTCCTTTTCCTACGCGGCCCAATTCCATCCGGAGTTGTTCGGGATAGGGAATGTGTCGCGCGTCTTCGTGGACAAGCGACACCAGTTCATTTGTGCCGAGCATGAT	TAQMAN
	>gb|M96262.2|LEYPOLYENV Lelystad virus, complete genome	M96262.2	12673	12847	175	Plus - Plus - Minus	GTTAATGTCCATCCCGTCCGGGTACGACAACCTCAAACTTGAGGGTTATTATGCTTGGCTGGCTTTTTTGTCCTTTTCCTACGCGGCCCAATTCCATCCGGAGTTGTTCGGGATAGGGAATGTGTCGCGCGTCTTCGTGGACAAGCGACACCAGTTCATTTGTGCCGAGCATGAT	TAQMAN
	>gb|AY749396.1| Porcine reproductive and respiratory syndrome virus strain MN-04-09_EU GP3 protein gene, complete cds	AY749396.1	274	444	171	Plus - Plus - Minus	ATGTCCATCCCGTCCGGGTACGACAACCTCAAACTTGAGGGATATTATGCTTGGCTGGCTTTTTTGTCCTTTTCCTATGCGGCCCAATTCCATCCGGAGCTGTTCGGGATAGGGAATGTGTCGCGCGTCTTCGTGGACAAGCGACACCAGTTCATTGTGCCGAGCATGAT	TAQMAN
	>gb|L04493.1|PRWPOLGLYM Porcine reproductive and respiratory syndrome virus (PRRSV) RNA polymerase, 3' end; complete ORF3, ORF4, ORF5; glycoprotein gene, membrane protein gene, nucleocapsid protein gene, complete cds's	L04493.1	2491	2665	175	Plus - Plus - Minus	GTTAATGTCCATCCCGTCCGGGTACGACAACCTCAAACTTGAGGGTTATTATGCTTGGCTGGCTTTTTTGTCCTTTTCCTACGCGGCCCAATTCCATCCGGAGTTGTTCGGGATAGGGAATGTGTCGCGCGTCTTCGTGGACAAGCGACACCAGTTCATTTGTGCCGAGCATGAT	TAQMAN
	>gb|L77913.1|PPSBE1ORFA Porcine respiratory and reproductive syndrome virus (isolate Be1) envelope protein (gp3) RNA, complete cds	L77913.1	270	444	175	Plus - Plus - Minus	GTTAATGTCCATCCCGTCCGGGTACGACAACAGCAAACTTGAGGGTTATTATGCTTGGCTGGCTTTTTTGTCCTTTTCCTACGCGGCCCAATTCCATCCCGAGTTGTTCGGGATAGGGAATGTGTCGCGCGTCTTCGTGGACAAGCGACACCAGTTCATTTGTGCCGAGCATGAT	TAQMAN
	>gb|L77915.1|PPSH3ORFA Porcine respiratory and reproductive syndrome virus (isolate H3) envelope protein (gp3) RNA, complete cds	L77915.1	270	444	175	Plus - Plus - Minus	GTTAATGTCCATCCCGTCCGGGTACGACAACCTCAAACTTGAGGGTTATTATGCTTGGCTGGCTTTTTTGTCCTTTTCCTACGCGGCCCAATTCCATCCGGAGTTGTTCGGGATAGGGAATGTGTCGCGCGTCTTCGTGGACAAGCGACACCAGTTCATTTGTGCCGAGCATGAT	TAQMAN
	>gb|L77917.1|PPSHA1ORFA Porcine respiratory and reproductive syndrome virus (isolate Ha1) envelope protein (gp3) RNA, complete cds	L77917.1	273	444	172	Plus - Plus - Minus	AATGTCCATCCCGTCCGGGTACGACAACCTCAAACTTGAGGGTTATTATGCTTGGCTGGCTTTTTTGTCCTTCTCCTACGCGGCCCAATTCCATCCAGAGTTGTTCGGGATAGGGAATGTGTCACGCGTCTTCGTGGACAAGCGACACCAGTTCATTTGTGCCGAGCATGAT	TAQMAN

	>gb|L77919.1|PPSL2ORFA Porcine respiratory and reproductive syndrome virus (isolate L2) envelope protein (gp3) RNA, complete cds	L77919.1	270	444	175	Plus - Plus - Minus	GTTAATGTCCATCCCGTCCGGGTACGACAACCTTAAACTTGAGGGTTATTATGCTTGGCTGGCTTTTTTGTCCTTTTCCTACGCGGCCCAATTCCATCCAGAGTTGTTCGGGATAGGGAATGTGTCGCGCGTCTTCGTGGACAAGCGACACCAGTTCATTTGTGCCGAGCATGAT	TAQMAN
sig_candidate_1810386	>emb|AJ223078.1|PRR223078 Porcine respiratory and reproductive syndrome virus ORFs 2 to 7, isolate Danish DK111-92	AJ223078.1	1456	1536	81	Plus - Minus - Minus	GCTTTCTGCGTGCCTTTTCTACGCCTCAGAAATGAGCGAGAAAGGTTTTAAAGTTATCTTTGGGAATGTCTCTGGTGT	TAQMAN
	>emb|X92942.1|PRRSENVNP Porcine reproductive and respiratory syndrome virus envelope protein and nucleoprotein genes	X92942.1	1520	1600	81	Plus - Minus - Minus	GCTTTCTGCGTGCCTTTTCTACGCTTCAGAAATGAGCGAAAAAGGCTTCAAAGTTATCTTTGGGAACGTCTCTGGCGT	TAQMAN
26 total sequence hits	>gb|AF315699.1|AF315699 Porcine reproductive and respiratory syndrome virus isolate Recomb-A envelope glycoprotein gene, partial cds	AF315699.1	116	196	81	Plus - Minus - Minus	GCTTTCTGCGTGCCTTTTCTACGCCTCAGAAATGAGCGAGAAAGGTTTTAAAGTTATCTTTGGGAATGTCTCTGGTGT	TAQMAN
	>gb|AF315700.1|AF315700 Porcine reproductive and respiratory syndrome virus isolate Recomb-B envelope glycoprotein gene, partial cds	AF315700.1	116	196	81	Plus - Minus - Minus	GCTTTCTGCGTGCCTTTTCTACGCCTCAGAAATGAGCGAGAAAGGTTTTAAAGTTATCTTTGGGAATGTCTCTGGTGT	TAQMAN
	>gb|AF315706.1|AF315706 Porcine reproductive and respiratory syndrome virus isolate Recomb-H envelope glycoprotein gene, partial cds	AF315706.1	116	196	81	Plus - Minus - Minus	GCTTTCTGCGTGCCTTTTCTACGCCTCAGAAATGAGCGAGAAAGGTTTTAAAGTTATCTTTGGGAATGTCTCTGGTGT	TAQMAN
	>gb|AF315701.1|AF315701 Porcine reproductive and respiratory syndrome virus isolate Recomb-C envelope glycoprotein gene, partial cds	AF315701.1	116	196	81	Plus - Minus - Minus	GCTTTCTGCGTGCCTTTTCTACGCCTCAGAAATGAGCGAGAAAGGTTTTAAAGTTATCTTTGGGAATGTCTCTGGTGT	TAQMAN
	>gb|AF315707.1|AF315707 Porcine reproductive and respiratory syndrome virus isolate Recomb-J envelope glycoprotein gene, partial cds	AF315707.1	116	196	81	Plus - Minus - Minus	GCTTTCTGCGTGCCTTTTCTACGCCTCAGAAATGAGCGAGAAAGGTTTTAAAGTTATCTTTGGGAATGTCTCTGGTGT	TAQMAN
	>gb|AY395081.1| Porcine reproductive and respiratory syndrome virus isolate SD-02-10 glycoprotein GP2, protein E, glycoprotein GP3, glycoprotein GP4, glycoprotein GP5, membrane protein M, and nucleocapsid protein N genes, complete cds	AY395081.1	1456	1536	81	Plus - Minus - Minus	GCTTTCTGCGTGCCTTTTCTACGCTTCAGAAATGAGCGAGAAAGGCTTTAAAGTTATTTTTGGGAATGTCTCTGGCGT	TAQMAN
	>gb|AF315702.1|AF315702 Porcine reproductive and respiratory syndrome virus isolate Recomb-D envelope glycoprotein gene, partial cds	AF315702.1	116	196	81	Plus - Minus - Minus	GCTTTCTGCGTGCCTTTTCTACGCCTCAGAAATGAGCGAGAAAGGTTTTAAAGTTATCTTTGGGAATGTCTCTGGTGT	TAQMAN
	>gb|AF315708.1|AF315708 Porcine reproductive and respiratory syndrome virus isolate Recomb-K envelope glycoprotein gene, partial cds	AF315708.1	116	196	81	Plus - Minus - Minus	GCTTTCTGCGTGCCTTTTCTACGCCTCAGAAATGAGCGAGAAAGGTTTTAAAGTTATCTTTGGGAATGTCTCTGGTGT	TAQMAN
	>gb|AY588319.1| PRRSV LV4.2.1, complete genome	AY588319.1	13251	13331	81	Plus - Minus - Minus	GCTTTCTGCGTGCCTTTTCTACGCCTCAGAAATGAGCGAGAAAGGCTTCAAAGTCATCTTTGGGAATGTCTCTGGCGT	TAQMAN
	>gb|AF315703.1|AF315703 Porcine reproductive and respiratory syndrome virus isolate Recomb-E envelope glycoprotein gene, partial cds	AF315703.1	116	196	81	Plus - Minus - Minus	GCTTTCTGCGTGCCTTTTCTACGCCTCAGAAATGAGCGAGAAAGGTTTTAAAGTTATCTTTGGGAATGTCTCTGGTGT	TAQMAN
	>gb|AF315709.1|AF315709 Porcine reproductive and respiratory syndrome virus isolate Recomb-L envelope glycoprotein gene, partial cds	AF315709.1	116	196	81	Plus - Minus - Minus	GCTTTCTGCGTGCCTTTTCTACGCCTCAAAAATGAGCGAGAAAGGTTTTAAAGTCATCTTTGGGAATGTCTCTGGCGT	TAQMAN
	>gb|DQ064785.1| Porcine respiratory and reproductive syndrome virus strain CReSA-VP21 glycoprotein 4 (GP4) gene, complete cds	DQ064785.1	306	386	81	Plus - Minus - Minus	GCTTTCTGCGTGCCTTTTCTACGCTTCAGAAATGAGCGAAAAGGCTTCAAAGTTATCTTTGGGAACGTCTCTGGCGT	TAQMAN
	>gb|AF315704.1|AF315704 Porcine reproductive and respiratory syndrome virus isolate Recomb-F nonfunctional envelope glycoprotein gene, partial sequence	AF315704.1	116	196	81	Plus - Minus - Minus	GCTTTCTGCGTGCCTTTTCTACGCCTCAGAAATGAGCGAGAAAGGTTTTAAAGTTATCTTTGGGAATGTCTCTGGTGT	TAQMAN
	>gb|AF315710.1|AF315710 Porcine reproductive and respiratory syndrome virus isolate Recomb-M envelope glycoprotein gene, partial cds	AF315710.1	116	196	81	Plus - Minus - Minus	GCTTTCTGCGTGCCTTTTCTACGCCTCAGAAATGAGCGAGAAAGGCTTCAAAGTCATCTTTGGGAATGTCTCTGGCGT	TAQMAN
	>gb|DQ489311.1| Porcine reproductive and respiratory syndrome virus strain SD01-08, complete genome	DQ489311.1	13200	13280	81	Plus - Minus - Minus	GCTTTCTGCGTGCCTTTTCTACGCCTCGGAAATGAGCGAGAAAGGCTTCAAAGTTATCTTTGGGAATGTCTCTGGCGT	TAQMAN
	>gb|AF315705.1|AF315705 Porcine reproductive and respiratory syndrome virus isolate Recomb-G envelope glycoprotein gene, partial cds	AF315705.1	116	196	81	Plus - Minus - Minus	GCTTTCTGCGTGCCTTTTCTACGCCTCAGAAATGAGCGAGAAAGGTTTTAAAGTTATCTTTGGGAATGTCTCTGGTGT	TAQMAN
	>gb|AF315711.1|AF315711 Porcine reproductive and respiratory syndrome virus isolate Recomb-N envelope glycoprotein gene, partial cds	AF315711.1	116	196	81	Plus - Minus - Minus	GCTTTCTGCGTGCCTTTTCTACGCCTCAGAAATGAGCGAGAAAGGCTTCAAAGTCATCTTTGGGAATGTCTCTGGCGT	TAQMAN
	>gb|DQ864705.1| Porcine respiratory and reproductive syndrome virus strain 01CB1, complete genome	DQ864705.1	13174	13254	81	Plus - Minus - Minus	GCTTTCTGCGTGCCTTTTCCACGCCTCAGAAATGAGCGAGAAAGGCTTCAAAGTTATCTTTGGGAATGTCTCTGGCGT	TAQMAN
	>gb|AY034879.1| Porcine reproductive and respiratory syndrome virus strain 111/92 structural protein gp3, structural protein gp4, and structural protein gp5 genes, partial cds	AY034879.1	116	196	81	Plus - Minus - Minus	GCTTTCTGCGTGCCTTTTCTACGCCTCAGAAATGAGCGAGAAAGGTTTTAAAGTTATCTTTGGGAATGTCTCTGGTGT	TAQMAN
	>gb|L04493.1|PRWPOLGLYM Porcine reproductive and respiratory syndrome virus (PRRSV) RNA polymerase, 3' end; complete ORF3, ORF4, ORF5; glycoprotein gene, membrane protein gene, nucleocapsid protein gene, complete cds's	L04493.1	3069	3149	81	Plus - Minus - Minus	GCTTTCTGCGTGCCTTTTCTACGCCTCAGAAATGAGCGAGAAAGGCTTCAAAGTTATCTTTGGGAATGTCTCTGGCGT	TAQMAN
	>gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome	AY366525.1	13200	13280	81	Plus - Minus - Minus	GCTTTCTGCGTGCCTTTTCTACGCTTCAGAAATGAGCGAGAAAGGCTTCAAAGTCATCTTTGGGAATGTCTCTGGCGT	TAQMAN
	>gb|M96262.2|LEYPOLYENV Lelystad virus, complete genome	M96262.2	13251	13331	81	Plus - Minus - Minus	GCTTTCTGCGTGCCTTTTCTACGCCTCAGAAATGAGCGAGAAAGGCTTCAAAGTCATCTTTGGGAATGTCTCTGGCGT	TAQMAN

	>gb|AY395079.1| Porcine reproductive and respiratory syndrome virus isolate SD-01-07 glycoprotein GP2, protein E, glycoprotein GP3, glycoprotein GP4, glycoprotein GP5, membrane protein M, and nucleocapsid protein N genes, complete cds	AY395079.1	1456	1536	81	Plus - Minus - Minus	GCTTTCAGCGTGCCTTTTCTACGCCTCGGAAATGAGCGAGAAGGGCTTCAAAGTTGTCTTTGGGAATGTCTCTGGCGTTGT	TAQMAN
	>gb|AY395080.1| Porcine reproductive and respiratory syndrome virus isolate SD-01-08 glycoprotein GP2, protein E, glycoprotein GP3, glycoprotein GP4, glycoprotein GP5, membrane protein M, and nucleocapsid protein N genes, complete cds	AY395080.1	1456	1536	81	Plus - Minus - Minus	GCTTTCTGCGTGCCTTTTCTACGCCTCGGAAATGAGCGAGAAAGGCTTTAAAGTTATCTTTGGGAATGTCTCTGGCGTTGT	TAQMAN
[redacted]								
sig_candidate_1810387	>gb|AY035927.1| Porcine reproductive and respiratory syndrome virus strain 1751/93 major envelope glycoprotein GP5 gene, complete cds	AY035927.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCACATCCTCTCACTGGGTTTTCTCACAACAAGTCATTTTTTTGACGCGCTCGGTCTCGGAGCTGTATCCACTGCAGGATTTGTTGGCGGGGCGGTATGTTCTCAGCAGCGTCTACGGCGCTTGTGCTTTCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAAA	TAQMAN
137 total sequence hits	>gb|AY035936.1| Porcine reproductive and respiratory syndrome virus strain 65/2/91 major envelope glycoprotein GP5 gene, complete cds	AY035936.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTCTCACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGCTCGGTCTCGGCGCTGTGTCCACTACAGGATTTGTTGGCGGGCGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCTTTCGCAGCGCTCGTATGTTTTGTCATCCGTGCTGTTAAAA	TAQMAN
	>gb|AY035938.1| Porcine reproductive and respiratory syndrome virus strain H2-D768 major envelope glycoprotein GP5 gene, complete cds	AY035938.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTCTCACTGGGTTTTCTCACAACAAGTCATTTTTTTGACGCGCTCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGCGGGCGGTATGTACTCTGCAGCGTCTACGGCGCTTGTGCTTTCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
	>gb|AY035939.1| Porcine reproductive and respiratory syndrome virus strain L1-D767 major envelope glycoprotein GP5 gene, complete cds	AY035939.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTCTCACTGGGTTTTCTCACAACAAGTCATTTTTTTGACGCGCTCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGCGGGCGGTATGTACTCTGCAGCGTCTACGGCGCTTGTGCTTTCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
	>gb|AY035940.1| Porcine reproductive and respiratory syndrome virus strain NY3-D769 major envelope glycoprotein GP5 gene, complete cds	AY035940.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGCTGCCACTCATATCCTCTCACTGGGTTTTCTCACAACAAGTCATTTTTTTGACGCGCCTCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTCGGCGGGCGGTATGTACTCAGCAGCGTCTACGGCGCTTGTGCTTTCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAAA	TAQMAN
	>gb|AY297122.1| Porcine reproductive and respiratory syndrome virus strain 02CB12 GP5 envelope protein (ORF5) gene, partial cds	AY297122.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGTTTTACCCGGTTGCCACTCATATCCTCTCACTGGGCTTTCTCACAACAAGCCATTTTTTTGACGCGCTCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGCGGGCGGTATGTACTCTGCAGCGTCTACGGCGCTTGTGCTTTCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
	>gb|AY297123.1| Porcine reproductive and respiratory syndrome virus strain 02SB2 truncated GP5 envelope protein (ORF5) gene, complete cds	AY297123.1	199	393	195	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCAGTTGCCACTCATATCCTCTCACTGGGTTTTCTCACAACAAGCCATTTTTTGACGCGCTCGGTCTCGGCGTTGTATCCACTGCAGGACTTGTTGGCGGGCGGTACGTACTCTGCAGCGTCTACGGCGCTTGTGCTTTCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
	>gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome	AY366525.1	13641	13836	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTCGTCACTCATATCCTCTCACTGGGTTTTCTCACGACAAGTCATTTTTTTGACGCGCTCGGTCTCGGCGCTGTGTCCACCGCAGGATTTATTGACGGGCGGTATGTGCTCAGCAGCATCTACGGCGCTTGTGCTTTCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
	>gb|AY395074.1| Porcine reproductive and respiratory syndrome virus isolate SD-03-12 major envelope glycoprotein GP5 mRNA, complete cds	AY395074.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGTCACTCATATCCTCTCACTGGGTTTTCTCACGACAAGTCATTTTTTTGACGCGCTCGGTCTCGGCGCTGTATCCACTGCAGGGTTTGTCGGAGGGCGGTATGTGCTTAGCAGCGTCTACGGCGCTTGTGCTTTCGCAGCGTTCGTATGCTTCGTCATCCGTGCTGCTAAAA	TAQMAN
	>gb|AY395080.1| Porcine reproductive and respiratory syndrome virus isolate SD-01-08 glycoprotein GP2, protein E, glycoprotein GP3, glycoprotein GP4, glycoprotein GP5, membrane protein M, and nucleocapsid protein N genes, complete cds	AY395080.1	1897	2092	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGTCACTCATATCCTCTCACTGGGTTTTCTCACGACAAGTCATTTTTTTGACGCGCTCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTCGGAGGGCGGTATGTACTTAGCAGCGTCTACGGCGCTTGTGCTTTCGCAGCGTTCGTATGCTTCGTCATCCGTGCTGCTAAAA	TAQMAN
	>gb|AY395081.1| Porcine reproductive and respiratory syndrome virus isolate SD-02-10 glycoprotein GP2, protein E, glycoprotein GP3, glycoprotein GP4, glycoprotein GP5, membrane protein M, and nucleocapsid protein N genes, complete cds	AY395081.1	1897	2092	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGTCACTCATATCCTCTCACTGGGTTTTCTCACGACGAGTCATTTCTTTGACGCGCTCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTCGGAAGGCGGTATGTACTTAGCAGCGTCTACGGCGCTTGTGCTTTCGCAGCGTTCGTATGCTTTGTCATCCGTGTTGCTAAAA	TAQMAN
	>gb|AY422798.1| Porcine reproductive and respiratory syndrome virus isolate MN-02-02_EU GP5 protein (ORF5) gene, complete cds	AY422798.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGCTGTCACTCATATCCTCTCACTGGGTTTTCTCACGACAAGTCATTTTTTTGACGCGCTCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTCGGAGGGCGGTATGTACTTAGCAGCGTCTACGGCGCTTGTGCTTTCGCAGCGTTCGTATGCTTTGTCATCCGTGCTGCTAAAA	TAQMAN
	>gb|AY422799.1| Porcine reproductive and respiratory syndrome virus isolate MN-01-03_EU GP5 protein (ORF5) gene, complete cds	AY422799.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGTCACTCATATCCTCTCACTGGGTTTTCTCACGACAAGTCATTTTTTTGACGCGCTTGGTCTCGGCGCTGTATCCACTGCAGGATTCATCGGAGGGCGGTATGTACTTAGCAGCGTCTACGGTGCTTGTGCTCTCGCAGCGTTCGCATGCTTTGTCATCCGTGCTGCTAAAA	TAQMAN
	>emb|X92942.1|PRRSENVNP Porcine reproductive and respiratory syndrome virus envelope protein and nucleoprotein genes	X92942.1	1958	2153	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTCTCACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGCTCGGTCTCGGCGCTGTATCCACTATAGGATTTGTTGGCGGGCGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCTTTCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGTTAAAA	TAQMAN
	>gb|AF253532.1|AF253532 Porcine reproductive and respiratory syndrome virus isolate V-502 major envelope glycoprotein GP5 gene, complete cds	AF253532.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTCTCACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGCTCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGCGGGCGGTACGTACTCTGCAGCGTCTACGGCGCTTGTGCTTTCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
	>gb|AF253533.1|AF253533 Porcine reproductive and respiratory syndrome virus isolate V-516 major envelope glycoprotein GP5 gene, complete cds	AF253533.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTCTCACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGCTCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGCGGGCGGTACGTACTCTGCAGCGTCTACGGCGCTTGTGCTTTCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
	>gb|AF253534.1|AF253534 Porcine reproductive and respiratory syndrome virus isolate V-548 major envelope	AF253534.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTCTCACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGCTCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGCG	TAQMAN

glycoprotein GP5 gene, complete cds						GGCGGTACGTACTCTGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTCT	
>gb|AF253535.1|AF253535 Porcine reproductive and respiratory syndrome virus isolate V-546 major envelope glycoprotein GP5 gene, complete cds	AF253535.1	199	394	196	Plus - Minus - Minus	CACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGCG GGCGGTACGTACTCTGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTCT	TAQMAN
>gb|AF253536.1|AF253536 Porcine reproductive and respiratory syndrome virus isolate V-547 major envelope glycoprotein GP5 gene, complete cds	AF253536.1	199	394	196	Plus - Minus - Minus	CACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGCG GGCGGTACGTACTCTGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTCT	TAQMAN
>gb|AF378797.1| Porcine reproductive and respiratory syndrome virus strain Arnsberg major envelope glycoprotein GP5 gene, partial cds	AF378797.1	103	298	196	Plus - Minus - Minus	CACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGCG GGCGGTACGTACTCTGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA GAGACCTTTGTGCTTTACCCGGTTGTTACTCATATCCTTT	TAQMAN
>gb|AF378798.1| Porcine reproductive and respiratory syndrome virus strain AS1 major envelope glycoprotein GP5 gene, partial cds	AF378798.1	103	298	196	Plus - Minus - Minus	CACTGGGTTTTCTCACTACAAGCCATTTTTTTGATGTGCT CGGTCTCAGCGCTGTGTCCGCTACAGGGTTTGTTGACAA GCGGTATGTGCTCAGCAGCGTGTACGGCGCTTGTGCCTT CGCAGCGCTCGTATGTTTTGTCATCCGTGCTGCTAAAA GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTCT	TAQMAN
>gb|AF378799.1| Porcine reproductive and respiratory syndrome virus strain H2 major envelope glycoprotein GP5 gene, partial cds	AF378799.1	103	298	196	Plus - Minus - Minus	CACTGGGTTTTCTCACAACAAGTCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGCG GGCGGTATGTACTCTGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA GAGACCTTTGTGCTTTACCCGTTGTCACTCATATCCTCT	TAQMAN
>gb|AY422800.1| Porcine reproductive and respiratory syndrome virus isolate MN-01-04_EU GP5 protein (ORF5) gene, complete cds	AY422800.1	199	394	196	Plus - Minus - Minus	CACTGGGTTTTCTCACGACAAGTCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTCGGAG GGCGGTATGTACTTAGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTATGCTTCGTCATCCGTGCTGCTAAAA GAGACCTTTGTGCTTTACCCAGTTGTCACTCACATCCTCT	TAQMAN
>gb|AY422803.1| Porcine reproductive and respiratory syndrome virus isolate MN-00-07_EU GP5 protein (ORF5) gene, complete cds	AY422803.1	199	394	196	Plus - Minus - Minus	CACTGGGTTTTCTCACAACAAGTCATTTTTTTGATGCGCT CGGTCTCGGCGCTGTGTCCACTGCAGGGTTTGTTGGCGG GCGGTATGTACTTAGCAGCGTCTACGGCGCTTGTGCCTT CGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA GAGACCTTTGTGCTTTACCCAGTTGTCACTCATATCCTCT	TAQMAN
>gb|AY422804.1| Porcine reproductive and respiratory syndrome virus isolate MN-00-08_EU GP5 protein (ORF5) gene, complete cds	AY422804.1	199	394	196	Plus - Minus - Minus	CACTGGGTTTTCTCACGACAAGTCATTTTTTTGATGCGCT CGGTCTCGGCGCTGTATCCACTGCAGGATTTGTCGGAGG GCGGCATGTACTCAGCAGCGTCTACGGCGCCTGTGCTTT CGCAGCGTTCGTATGCTTTGTCATCCGTGCTGCTAAAA GAGACCTTTGTGCTTTACCCGGTAGTCACTCATATCCTC	TAQMAN
>gb|AY422805.1| Porcine reproductive and respiratory syndrome virus isolate MN-00-09_EU GP5 protein (ORF5) gene, complete cds	AY422805.1	199	394	196	Plus - Minus - Minus	TCATTGGGTTTTCTCACGACAAGTCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTCGGAA AGCGGTATGTACTCAGCAGCGTCTACGGCGCTTGTGCCT TCGCAGCGTTCGCATGCTTCATCATCCGTGCTGCTAAAA GAGACCTTTGTGCTTTACCCGGTTGTCACCCATATCCTCT	TAQMAN
>gb|AY422806.1| Porcine reproductive and respiratory syndrome virus isolate MN-00-10_EU GP5 protein (ORF5) gene, complete cds	AY422806.1	199	394	196	Plus - Minus - Minus	CACTGGGTTTTCTCACGACAAGTCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTCGGAG GGCGGTATGTACTCAGCAGCGTCTACGGCGCTTGTGCTT TTGCAGCGTTCGTATGCTTCGTCATCCGTGCTGCTAAAA GAGACCTTTGTGCTTTACCCGGTAGTAACTCATATCCTC	TAQMAN
>gb|AY422807.1| Porcine reproductive and respiratory syndrome virus isolate MN-00-11_EU GP5 protein (ORF5) gene, complete cds	AY422807.1	199	394	196	Plus - Minus - Minus	TCATTGGGTTTTCTCACGACAAGTCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTATCCATTGCAGGATTTGTCGGAA AGCGGTATGTACTTAGCAGCGTCTACGGCGCTTGCGCCT TCGCAGCGTTCGTATGCTTCATCATCCGTGCTGCTAAAA GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTCT	TAQMAN
>gb|AY588319.1| PRRSV LV4.2.1, complete genome	AY588319.1	13692	13887	196	Plus - Minus - Minus	CACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGCG GGCGGTACGTACTCTGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA GAGACCTTTGTGTTTTACCCGGTTGCCACTCATATCCTCT	TAQMAN
>gb|AY615786.1| Porcine respiratory and reproductive syndrome virus strain SE44 envelope glycoprotein (ORF5) gene, complete cds	AY615786.1	199	394	196	Plus - Minus - Minus	CACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGCG GGCGGTATGTACTCTGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA GAGACCTTTGTGTTTTACCCGGTTGCCACTCATATCCTCT	TAQMAN
>gb|AY615787.1| Porcine respiratory and reproductive syndrome virus strain SE53 envelope glycoprotein (ORF5) gene, complete cds	AY615787.1	199	394	196	Plus - Minus - Minus	CACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGCG GGCGGTATGTACTCTGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTCT	TAQMAN
>gb|AY633973.1| Porcine reproductive and respiratory syndrome virus isolate B13 major envelope glycoprotein gene, complete cds	AY633973.1	199	394	196	Plus - Minus - Minus	CACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGCG GGCGGTACGTACTCTGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA GAGACCTTTGTGTTTTACCCGGTTGCCACTCATATCCTCT	TAQMAN
>gb|AY743931.1| Porcine reproductive and respiratory syndrome virus isolate Porcilis GP5 gene, complete cds	AY743931.1	199	394	196	Plus - Minus - Minus	CACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGCG GGCGGTATGTACTCTGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTCT	TAQMAN
>gb|AY749389.1| Porcine reproductive and respiratory syndrome virus strain MN-03-10_EU GP5 protein gene, complete cds	AY749389.1	199	394	196	Plus - Minus - Minus	CACTGGGCTTTCTTACAACAAGTCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTGTCCACTGTAGGATTTGTTGGCG GGCGGTATGTACTCAGCAGTGTCTACGGCGCTTGTGCTT CCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGTTAAAA GAGACCTTTGTGTTTTACCCGGTTGCCACTCATATCCTCT	TAQMAN
>gb|AY875856.1| Porcine respiratory and reproductive syndrome virus envelope glycoprotein gene, complete cds	AY875856.1	199	394	196	Plus - Minus - Minus	CACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGCA GGCGGTATGTACTCAGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTT	TAQMAN
>gb|DQ009639.1| Porcine respiratory and reproductive syndrome virus strain CRESA15 envelope glycoprotein (ORF5) gene, complete cds	DQ009639.1	199	394	196	Plus - Minus - Minus	CACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGC TCGGTCTCGGCGCTGTGTCCACTACAGGATTTGTTGGCG GGCGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCTT TCGCAGCGCTCGTATGTTTTGTCATCCGCGCTGCTAAAA GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTT	TAQMAN
>gb|DQ009640.1| Porcine respiratory and reproductive syndrome virus strain CRESA16 envelope glycoprotein (ORF5) gene, complete cds	DQ009640.1	199	394	196	Plus - Minus - Minus	CACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGC TCGGTCTCGGCGCTGTGTCCACTACAGGATTTGTTGGCG GGCGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCTT TCGCAGCGCTCGTGTGTTTTGTCATCCGCGCTGCTAAAA GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTT	TAQMAN
>gb|DQ009642.1| Porcine respiratory and reproductive syndrome virus strain CRESA19 envelope glycoprotein (ORF5) gene, complete cds	DQ009642.1	199	394	196	Plus - Minus - Minus	CACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGC TCGGTCTCGGCGCTGTGTCCACTACAGGATTTGTTGGCG GGCGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCTT TCGCAGCGCTCGTATGTTTTGTCATCCGCGCTGCTAAAA	TAQMAN
>gb|DQ009644.1| Porcine respiratory and reproductive syndrome virus strain	DQ009644.1	202	394	193	Plus - Minus - Minus	ACCTTTGTGCTTTACCCGGTTGTAACTCATATTCTTTCAC TGGGTTTTCTTACAACAAGCCATTTCTTTGATGCGCTCG	TAQMAN

CRESA21 envelope glycoprotein (ORF5) gene, complete cds						GTCTCGGTGCTGTGTCCATCATCGGGTTTCATGGCCACC GGCATGTACTCGGCAGTATCTATGGTGCTTGTGCTTTCG CAGCGCTCGTGTGCTTTGTCATCCGTGCTGCAAAAA	
>gb|DQ009647.1| Porcine respiratory and reproductive syndrome virus strain CRESA-VP21 envelope glycoprotein (ORF5) gene, complete cds	DQ009647.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGC TCGGTCTCGGCGCTGTGTCCACTACAGGATTTGTTGGCG GGCGGTATGTACTCTGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGCTCGTATGTTTTGTCATCCGCGCTGCTAAAA	TAQMAN
>gb|DQ064787.1| Porcine respiratory and reproductive syndrome virus strain V3 envelope glycoprotein (GP5) gene, complete cds	DQ064787.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGTTTTACCCGGTTGCCACTCATATCCTCT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGCG GGCGGTATGTACTCTGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|DQ064788.1| Porcine respiratory and reproductive syndrome virus strain CReSA-V1 envelope glycoprotein (GP5) gene, complete cds	DQ064788.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGC TCGGTCTCGGCGCTGTGTCCACTACAGGATTTGTTGGCG GGCGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCTT TCGCAGCGCTCGTATGTTTTGTCATCCGCGCTGCTAAAA	TAQMAN
>gb|DQ324668.1| Porcine respiratory and reproductive syndrome virus strain Amervac PRRS major envelope glycoprotein GP5 gene, partial cds	DQ324668.1	172	367	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGC TCGGTCTCGGCGCTGTGTCCACTACAGGATTTGTTGGCG GGCGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCTT TCGCAGCGCTCGTATGTTTTGTCATCCGCGCTGCTAAAA	TAQMAN
>gb|DQ324678.1| Porcine respiratory and reproductive syndrome virus strain Porcilis PRRS major envelope glycoprotein GP5 gene, partial cds	DQ324678.1	172	367	196	Plus - Minus - Minus	GAGACCTTTGTGTTTTACCCGGTTGCCACTCATATCCTCT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGCG GGCGGTATGTACTCTGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|DQ324681.1| Porcine respiratory and reproductive syndrome virus strain Pyrsvac-187 major envelope glycoprotein GP5 gene, partial cds	DQ324681.1	172	367	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGC TCGGTCTCGGCGCTGTGTCCACTACAGGATTTGTTGGCG GGCGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCTT TCGCAGCGCTCGTATGTTTTGTCATCCGCGCTGCTAAAA	TAQMAN
>gb|DQ345725.1| Porcine respiratory and reproductive syndrome virus strain VP-046 bis envelope glycoprotein gene, complete cds	DQ345725.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGC TCGGTCTCGGCGCTGTGTCCACTACAGGATTTGTTGGCG GGCGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCTT TCGCAGCGCTCGTATGTTTTGTCATCCGCGCTGCTAAAA	TAQMAN
>gb|DQ345726.1| Porcine respiratory and reproductive syndrome virus strain All-183 envelope glycoprotein gene, complete cds	DQ345726.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGC TCGGTCTCGGCGCTGTGTCCACTACAGGATTTGTTGGCG GGCGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCTT TCGCAGCGCTCGTATGTTTTGTCATCCGCGCTGCTAAAA	TAQMAN
>gb|DQ345727.1| Porcine respiratory and reproductive syndrome virus strain DV envelope glycoprotein gene, complete cds	DQ345727.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGTTTTACCCGGTTGCCACTCATATCCTCT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGCG GGCGGTATGTACTCTGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|DQ345728.1| Porcine respiratory and reproductive syndrome virus isolate Spain 1/1991 envelope glycoprotein gene, complete cds	DQ345728.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGC TCGGTCTTGGCGCTGTGTCCACTACAGGATTTGTTGGCG GGCGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCTT TCGCAGCGCTCGTATGTTTTGTCATCCGTGCTGCCAAAA	TAQMAN
>gb|DQ366648.1| Porcine respiratory and reproductive syndrome virus strain HU10 major envelope glycoprotein gene, partial cds	DQ366648.1	103	298	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGC TCGGTCTCGGCGCTGTGTCCACTACAGGATTTGTTGGCG GGCGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCCT TCGCAGCGCTCGTATGTTTTGTCATCCGCGCTGCTAAAA	TAQMAN
>gb|DQ345729.1| Porcine respiratory and reproductive syndrome virus isolate Spain 2/1991 envelope glycoprotein gene, complete cds	DQ345729.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGC TCGGTCTCGGCGCTGTGTCCACTACAGGATTTGTTGGCG GGCGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCTT TCGCAGCGCTCGTATGTTTTGTCATCCGCGCTGCTAAAA	TAQMAN
>gb|DQ366649.1| Porcine respiratory and reproductive syndrome virus strain HU11 major envelope glycoprotein gene, partial cds	DQ366649.1	103	298	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGC TCGGTCTCGGCGCTGTGTCCACTACAGGATTTGTTGGCG GGCGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCTT TCGCAGCGCTCGTATGTTTTGTCATCCGCGCTGCTAAAA	TAQMAN
>gb|DQ345730.1| Porcine respiratory and reproductive syndrome virus isolate Spain 3/1992 envelope glycoprotein gene, complete cds	DQ345730.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGC TCGGTCTTGGCGCTGTGTCCATTACAGGATTTGTTGGCG GGCGGTATGTACTCAGCAGCATGTACGGCGCTTGTGCTT TCGCAGCGCTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|DQ366651.1| Porcine respiratory and reproductive syndrome virus strain HU13 major envelope glycoprotein gene, partial cds	DQ366651.1	103	298	196	Plus - Minus - Minus	GAGACCTTTGTGTTTTACCCGGTTGCCACTCATATCCTCT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGCG GGCGGTATGTACTCTGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|DQ345732.1| Porcine respiratory and reproductive syndrome virus isolate Spain 5/1992 envelope glycoprotein gene, complete cds	DQ345732.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGC TCGGTCTCGGCGCTGTGTCCGCTATAGGATTTGTTGGCA GGCGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCCT TCGCAGCGCTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|DQ366652.1| Porcine respiratory and reproductive syndrome virus strain HU14 major envelope glycoprotein gene, partial cds	DQ366652.1	103	298	196	Plus - Minus - Minus	GAGACCTTTGTGTTTTACCCGGTTGCCACTCATATCCTCT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGTG GGCGGTATGTACTCAGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCAAAAA	TAQMAN
>gb|DQ366656.1| Porcine respiratory and reproductive syndrome virus strain HU18 major envelope glycoprotein gene, partial cds	DQ366656.1	103	298	196	Plus - Minus - Minus	GAGACCTTTGTGTTTTACCCGGTTGTCACTCACATCCTCT CACTGGGTTTTCTCACGACGAGCCATTTTCTTGACGCGC TCGGTCTCGGCGTTGTGTCCGCAGCTGGGTTTGTTGGCC AGCGGTATGTACTCAGCAGCGTTTACGGCACTTGTGCTT TCGCAGCGTTCGTGTGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|DQ366657.1| Porcine respiratory and reproductive syndrome virus strain HU19 major envelope glycoprotein gene, partial cds	DQ366657.1	103	298	196	Plus - Minus - Minus	GAGACCTTTGTGTTTTACCCGGTTGCCACTCATATCCTCT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGCA GGCGGTATGTACTCTGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGTTAAAA	TAQMAN
>gb|DQ384983.1| Porcine respiratory and reproductive syndrome virus strain HUN17 major envelope glycoprotein GP5 gene, partial cds	DQ384983.1	94	289	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGC TCGGTCTCGGCGCTGTGTCCACTACAGGATTTGTTGGCG GGCGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCTT TCGCAGCGCTCGTATGTTTTGTCATCCGCGCTGCTAAAA	TAQMAN
>gb|DQ384984.1| Porcine respiratory and reproductive syndrome virus strain HUN18 major envelope glycoprotein GP5 gene, partial cds	DQ384984.1	94	289	196	Plus - Minus - Minus	GAGACCTTTGTGTTTTACCCGGTTGCCACTCATATCCTCT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGCG GGCGGTATGTACTCTGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN

>gb|DQ384985.1| Porcine respiratory and reproductive syndrome virus strain HUN19 major envelope glycoprotein GP5 gene, partial cds	DQ384985.1	94	289	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTTCACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGCTCGGTCTCGGCGCTGTGTCCACTACAGGATTTGTTGGCGGGCGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCTTTCGCAGCGCTCGTATGTTTTGTCATCCGCGCTGCTAAAA	TAQMAN
>gb|DQ384986.1| Porcine respiratory and reproductive syndrome virus strain HUN22 major envelope glycoprotein GP5 gene, partial cds	DQ384986.1	94	289	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTTCACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGCTCGGTCTCGGCGCTGTGTCCACTACAGGATTTGTTGGCGGGCGGTATGTGCTCAGCAGCGTGTACGGCGCTTGTGCTTTCGCAGCGCTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|DQ345733.1| Porcine respiratory and reproductive syndrome virus isolate Spain 6/1992 envelope glycoprotein gene, complete cds	DQ345733.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTTCACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGCTCGGTCTTGGCGCTGTGTCCATTACAGGATTTGTTGGCGGGCGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCTTTCGCAGCGCTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|DQ345734.1| Porcine respiratory and reproductive syndrome virus isolate Spain 7/1992 envelope glycoprotein gene, complete cds	DQ345734.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTCTCACTGGGTTTTCTCACAACAAGTCATTTTTTTGATGCGCTCGGTCTCGGCGCTGTGTCCACTACAGGATTTGTTGGCGGGAGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCTTTCGCAGCGCTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|DQ345735.1| Porcine respiratory and reproductive syndrome virus isolate Spain 8/1992 envelope glycoprotein gene, complete cds	DQ345735.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTCTCACTGGGTTTTCTCACAACAAGTCATTTTTTTGACGCGCTCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGCGGGCGGTATGTACTCTGCAGCGTCTACGGCGCTTGTGCTTTCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|DQ345736.1| Porcine respiratory and reproductive syndrome virus isolate Spain 9/1993 envelope glycoprotein gene, complete cds	DQ345736.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTACTTTACCCGGTTGCCACTCATATCCTTTCACTGGGTTTTCTCACAACAAGCCATTTTCTTGACGCGCTCGGTCTCGGCGCCGTGTCCGCTACAGGATTTGTTGGCGGGCGGTATGTGCTCAGCAGCGTGTACGGCGCTTGTGCTTTTGCAGCGCTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|DQ345737.1| Porcine respiratory and reproductive syndrome virus isolate Spain 10/1993 envelope glycoprotein gene, partial cds	DQ345737.1	173	368	196	Plus - Minus - Minus	GAGACCTTTGTGTTTTACCCGGTTGCCACTCATATCCTCTCACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGCTCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGCGGGCGGTATGTACTCTGCAGCGTCTACGGCGCTTGTGCTTTCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|DQ345738.1| Porcine respiratory and reproductive syndrome virus isolate Spain 11/1994 envelope glycoprotein gene, partial cds	DQ345738.1	173	368	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTTCACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGTGCTCGGTCTTGGCGCTGTGTCCATTACAGGATTTGTTGGCAGGCGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCTTTTGCAGCGCTCGTATGTCTTGTCATCCGTGTTGCTAAAA	TAQMAN
>gb|DQ345740.1| Porcine respiratory and reproductive syndrome virus isolate Spain 13/2000 envelope glycoprotein gene, complete cds	DQ345740.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTTCACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGCTCGGTCTCGGCGCTGTGTCCACTACAGGATTTGTTGGCGGGCGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCTTTCGCAGCGCTCGCATGTTTTGTCATCCGCGCTGCTAAAA	TAQMAN
>gb|DQ384987.1| Porcine respiratory and reproductive syndrome virus strain HUN23 major envelope glycoprotein GP5 gene, partial cds	DQ384987.1	94	289	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTTCACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGCTCGGTCTCGGCGCTGTGTCCACTACAGGATTTGTTGGCGGGCGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCCTTGCAGCGCTCGTATGTTTTGTCATCCGCGCTGCTAAAA	TAQMAN
>gb|DQ345741.1| Porcine respiratory and reproductive syndrome virus isolate Spain 14/2000 envelope glycoprotein gene, complete cds	DQ345741.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGTCACACATGTCCTCTCACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGCCTCGGTCTTGGCGCCGTGTCCACCATAGGATTTGTTGACAAGCGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCTCTCGCAGCGCTCGTATGCTTTGTCATCCGTGCTGCCAAAA	TAQMAN
>gb|DQ384988.1| Porcine respiratory and reproductive syndrome virus strain HUN25 major envelope glycoprotein GP5 gene, partial cds	DQ384988.1	94	289	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTTCACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGCTCGGTCTCGGCGCTGTGTCCACTACAGGATTTGTTGGCGGGCGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCTTTCGCAGCGCTCGTATGTTTTGTCATCCGCGCTGCTAAAA	TAQMAN
>gb|DQ345745.1| Porcine respiratory and reproductive syndrome virus isolate Spain 18/2000 envelope glycoprotein gene, complete cds	DQ345745.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTTCACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGCTCGGTCTCGGCGCTGTGTCCACTACAGGATTTGTTGGCGGGCGATATGTACTCAGCAGCGTGTACGGCGCTTGTGCTTTCGCAGCGCTCGTATGTTTTGTCATCCGCGCTGCTAAAA	TAQMAN
>gb|DQ384989.1| Porcine respiratory and reproductive syndrome virus strain HUN26 major envelope glycoprotein GP5 gene, partial cds	DQ384989.1	94	289	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTTCACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGCTCGGTCTCGGCGCTGTGTCCACTACAGGATTTGTTGGCGGGCGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCTTTCGCAGCGCTCGTATGTTTTGTCATCCGCGCTGCTAAAA	TAQMAN
>gb|DQ345747.1| Porcine respiratory and reproductive syndrome virus isolate Spain 20/2002 envelope glycoprotein gene, complete cds	DQ345747.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTTCACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGCTCGGTCTCGGCGCTGTGTCCACTACAGGATTTGTTGGCAGGCGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCTTTCGCAGCGCTCGTATGTTTTGTCATCCGCGCTGCTAAAA	TAQMAN
>gb|DQ405282.1| Porcine respiratory and reproductive syndrome virus isolate 2005/86AS ORF5 gene, partial cds	DQ405282.1	237	427	191	Plus - Minus - Minus	ACCTTTGTGCTTTACCCGGTTGCCACTCACATCCTGTCACTGGGTTTTCTTACCACAAGTCATTTTTTTGATGCGCTCGGTCTCAGCGCTGTGTCCACTACAGGATTCATCGGTGGGCGGTATGTGCTCAGCAGTGTGTACGGCGCTTGTGCTTTCGCAGCGCTCGTATGTTTTGTCATCCGTGCTGCTAA	TAQMAN
>gb|DQ475238.1| Porcine respiratory and reproductive syndrome virus isolate PRRSV0000614 envelope glycoprotein gene, complete cds	DQ475238.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCAGTTGTCACTCACATCCTCTCACTGGGTTTTCTCACAACAAGTCATTTTTTTGATGCGCTCGGTCTCGGCGCTGTGTCCACTGCAGGGTTTGTTGGCGGGCGTATGTACTTAGCAGCGTCTACGGTGCTTGTGCTCTCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|DQ475472.1| Porcine respiratory and reproductive syndrome virus isolate PRRSV0000902 envelope glycoprotein gene, complete cds	DQ475472.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTACTTTACCCGGTTGTCACTCATATCCTCTCACTGGGTTTTCTCACGACAAGTCATTTTTTTGACGCGCTTGGTCTCGGCGCTGTATCCACTGCAGGATTCATCGGAGGGCGGTATGTACTTAGCAGCGTCTACGGTGCTTGTGCTCTCGCAGCGTTCGCATGCTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|DQ476364.1| Porcine respiratory and reproductive syndrome virus isolate PRRSV0002045 envelope glycoprotein gene, complete cds	DQ476364.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGTTACTCACATCCTCTCCCTGGGTTTCCTCACGACAAGCCATTTTTTTGACGCGCTCGGTCTCGGCGCTGTGTCCACTGCTGGATTTGTTGGCGGGCGGTATGTGCTCAGCAGCGTCTACGGCATTTGTGCTTTTGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|DQ476779.1| Porcine respiratory and reproductive syndrome virus isolate PRRSV0002539 envelope glycoprotein gene, complete cds	DQ476779.1	199	394	196	Plus - Minus - Minus	GAGACTTTTGTGCTTTACCCGGTTGTCACTCATATCATCTCACTGGGTTTTCTCACGACAAGTCATTTTTTTGACGCGCTCGGCCTCGGCGCTGTGTCCACTGCAGGGTTTGTCGGAGAGCGGTATGTACTTAGCAGCGTCTACGGCGCTTGTGCTTTCGCGGCGTTCGTATGCTTCGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|DQ476800.1| Porcine respiratory and reproductive syndrome virus isolate PRRSV0002566 envelope glycoprotein gene, complete cds	DQ476800.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGTCACTCATATCCTCTCACTGGGTTTTCTCACGACAAGTCATTTTTTTGACGCGCTCGGTCTCGGCGCTGTATCCACTGCAGGGTTTGTCGGAGGGCGGTACGTACTTAGCAGCGTCTACGGCGCTTGTGCTTTCGCAGCGTTCGTATGCTTCGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|DQ477659.1| Porcine respiratory and reproductive syndrome virus isolate PRRSV0003620 envelope	DQ477659.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTCGTCACTCATATCCTCTCACTGGGTTTTCTCACGACAAGTCATTTTTTTGACGCGCTCGGTCTCGGCGCTGTGTCCACCGCAGGATTTATTGACG	TAQMAN

glycoprotein gene, complete cds						GGCGGTATGTGCTCAGCAGCATCTACGGCGCTTGTGCTT TCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTT	
>gb|AF378804.1| Porcine reproductive and respiratory syndrome virus strain Lek major envelope glycoprotein GP5 gene, partial cds	AF378804.1	103	298	196	Plus - Minus - Minus	CACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGTGCT CGGTCTCGGCGCTGTGTCCATTACAGGATTTGCTGATGG GCGGCATGTACTCAGCAGCGTGTACGGCGCTTGTGCTTT CGCAGCGCTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|AF378806.1| Porcine reproductive and respiratory syndrome virus strain Kwi major envelope glycoprotein GP5 gene, partial cds	AF378806.1	103	298	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCTACTCATATTCTTT CACTGGGTTTTCTCACAACAAGCCATTTCTTAGACGCGC TCGGTCTCAGCGCTGTGTCCGCTACAGGATTTACAGGTG GGCGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCTT TCGCAGCGCTCGTGTGTTTTGTCATCCGCGCTGCTAAAA	TAQMAN
>gb|AF378808.1| Porcine reproductive and respiratory syndrome virus strain Zbr major envelope glycoprotein GP5 gene, partial cds	AF378808.1	103	298	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCTACTCATATCCTTT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGC TCGGTCTCGGCGCTGTGTCCGCTACAGGATTTGTTGGCG GGCGGTATGTGCTCAGCAGCGTGTACGGCGCTTGTGCTT TCGCAGCGCTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|AF378813.1| Porcine reproductive and respiratory syndrome virus strain Sma major envelope glycoprotein GP5 gene, partial cds	AF378813.1	103	298	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATTCTTT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGC TCGGTCTCGGCGCTGTGTCCACTACAGGATTTGTTGGCG GGCGGTATGTGCTCAGCAGCGTGTATGGCGCTTGTGCTT TCGCAGCGCTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|AF378815.1| Porcine reproductive and respiratory syndrome virus strain Gra major envelope glycoprotein GP5 gene, partial cds	AF378815.1	103	298	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTCT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTGTCCACTACAGGATTTGTTGGCG GGCGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCTT TCGCAGCGCTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|AF378819.1| Porcine reproductive and respiratory syndrome virus strain Porcilis PRRS major envelope glycoprotein GP5 gene, partial cds	AF378819.1	103	298	196	Plus - Minus - Minus	GAGACCTTTGTGTTTTACCCGGTTGCCACTCATATCCTCT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGCG GGCGGTATGTACTCTGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|AF378820.1| Porcine reproductive and respiratory syndrome virus strain Pyrsvac-183 major envelope glycoprotein GP5 gene, partial cds	AF378820.1	103	298	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGC TCGGTCTCGGCGCTGTGTCCACTACAGGATTTGTTGGCG GGCGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCTT TCGCAGCGCTCGTATGTTTTGTCATCCGCGCTGCTAAAA	TAQMAN
>gb|AF486464.1| Porcine reproductive and respiratory syndrome virus isolate PRRSV-BS82 major envelope glycoprotein (ORF5) gene, complete cds	AF486464.1	202	392	191	Plus - Minus - Minus	ACCTTTGTGCTTTACCCAGCTGCCACTCATATCCTCTCAT TGGGTTTTCTTACAACAAGCCATTTTTTTGACGCGCTTG GTCTCGGCGCTGTATCCACTGCAGGGTTTGTTGGCGGGC GATACGTACTTAGCAGCATCTACGGCGCTTGCGCTTTCG CAGCGTTCATATGTTTCGTCATCCGTGCTGCTAA	TAQMAN
>gb|AF495503.1| Porcine respiratory and reproductive syndrome virus strain v1 truncated envelope glycoprotein (ORF5) gene, complete cds	AF495503.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGC TCGGTCTCGGCGCTGTGTCCACTACAGGATTTGTTGGCG GGCGGTATGTACTCAGCAACGTGTACGGCGCTTGTGCTT TCGCAGCGCTCGTATGTTTTGTCATCCGCGCTGCTAAAA	TAQMAN
>gb|AF495510.1| Porcine respiratory and reproductive syndrome virus strain uab4 envelope glycoprotein (ORF5) gene, complete cds	AF495510.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGC TCGGTCTCGGCGCTGTGTCCACTACAGGATTTGTTGGCG GGCGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCTT TCGCAGCGCTCGTATGTTTTGTCATCCGCGCTGCTAAAA	TAQMAN
>gb|DQ345748.1| Porcine respiratory and reproductive syndrome virus isolate Spain 21/2002 envelope glycoprotein gene, complete cds	DQ345748.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCACTCCTC TCACTGGGTTTCTTCACAACAAGCCATTTTTTTGATGCG CTCGGTCTCGGCGCAGTATCCACTGCAGGACTTGTTGAC GGGCGGTACGTACTCAGCAGCGTCTACGGCGCTTGTGCT TTTGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAA A	TAQMAN
>gb|DQ345749.1| Porcine respiratory and reproductive syndrome virus isolate Spain 22/2002 envelope glycoprotein gene, complete cds	DQ345749.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATTCTCT CACTGGGTTTTCTCACAACAAGTCATTTCTTTGATGCGC TCGGTCTTGGCACTGTGTCCACTGCAGGATTTGTTAGCG GGCGGTATGTACTAAGCAGCGTGTACGGCGCTTGTGCTC TCGCAGCGTTCGTATGCTTTGTCATCCGTGCTGCCAAAA	TAQMAN
>gb|DQ345752.1| Porcine respiratory and reproductive syndrome virus isolate Spain 25/2002 envelope glycoprotein gene, complete cds	DQ345752.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGC TCGGTCTCGGCGCTGTGTCCACTACAGGATTTGTTGGCG GGCGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCTT TCGCAGCGCTTGTATGCTTTGTCATCCGCGCTGCTAAAA	TAQMAN
>gb|DQ366640.1| Porcine respiratory and reproductive syndrome virus strain HU02 major envelope glycoprotein gene, partial cds	DQ366640.1	103	298	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACCCACATCTTC TCACTGGGTTTCCTCACAACAAGTCATTTTTTTGACGCG CTCGGTCTCGGCGCTGTATCCGCTGCAGGATTTGTTGGT GGGCGGCATGTACTCAGCAGTGTCTATGGCACTTGTGCT TTTGCAGCGTTTGTATGTTTTGTCATCCGTGCCGCTAAA A	TAQMAN
>gb|DQ366641.1| Porcine respiratory and reproductive syndrome virus strain HU03 major envelope glycoprotein gene, partial cds	DQ366641.1	103	298	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACACCACATCCTC TCACTGGGTTTTCTCACAACAAGTCATTTATTTGACGCG CTTGGTCTCGGCGCTGTGTCTGCTGCAGGATTTGTCGGT CAGCGGTATGTACTCAGCAGTGTCTATGGCACTTGTGCT TTCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAA A	TAQMAN
>gb|DQ366642.1| Porcine respiratory and reproductive syndrome virus strain HU04 major envelope glycoprotein gene, partial cds	DQ366642.1	103	298	196	Plus - Minus - Minus	GAGACCTTTGTGCTCTACCCGGTTGCCACTCATATCCTC TCACTGGGTTTTCTCACGACAAGTCATTTTTTTGATGCGC TCGGTCTCGGCGCTGTATCCACTACAGGATTCATTGGTG GGCGGTATGTACTCAGCAGTGTTTACGGCGCTTGTGCTT TCGCAGCGTTCGTATGTTTTGTCATCCGTACTGCTAAAA	TAQMAN
>gb|DQ366644.1| Porcine respiratory and reproductive syndrome virus strain HU06 major envelope glycoprotein gene, partial cds	DQ366644.1	103	298	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCTGTTGTCACTCACATTCTCT CACTGGGTTTTCTCACAACAAGTCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTATCCGTAACAGGATTTGTTGGTG GGCGGTATGTACTCAGCAGTGTCTATGGCACTTGTGCTT TTGCAGCGTTCGTGTGCTTTGTCATCCGTGCCGCTAAAA	TAQMAN
>gb|DQ477805.1| Porcine respiratory and reproductive syndrome virus isolate PRRSV0003791 envelope glycoprotein gene, complete cds	DQ477805.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTCT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGCG GGCGGTACGTACTCTGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|DQ366645.1| Porcine respiratory and reproductive syndrome virus strain HU07 major envelope glycoprotein gene, partial cds	DQ366645.1	103	298	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGTCACCCACATTCTCT CACTGGGTTTTCTCACAACAAGTCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTATCCGCAGCAGGATTTGTTGGTG GGCGGTATGTGCTCAGCAGTGTCTATGGCACTTGTGCTT TTGCAGCGTTCGTATGTTTTGTCATCCGTGCCGCTAAAA	TAQMAN
>gb|DQ478339.1| Porcine respiratory and reproductive syndrome virus isolate PRRSV0004476 envelope glycoprotein gene, complete cds	DQ478339.1	199	394	196	Plus - Minus - Minus	GAGACTTTTGTGCTTTACCCGGTTGTCACTCATATCATCT CACTGGGTTTTCTCACGACAAGTCATTTTTTTGACGCGC TCGGCCTCGGCGCTGTGTCCACTGCAGGGTTTGTCGGAG AGCGGTATGTACTTAGCAGCATCTACGGCGCTTGTGCTT TCGCGGCGTTCGTATGCTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|DQ366646.1| Porcine respiratory and reproductive syndrome virus strain HU08 major envelope glycoprotein gene, partial cds	DQ366646.1	103	298	196	Plus - Minus - Minus	GAGACCTTTGTACTTTACCCGGTTGTTACTCACATCCTTT CACTGGGTTTTCTCACAACAAGCCACTTTTTTGACACGC TCGGTCTCGGCGCTGTGTCCACAGCAGGATTTGTTGGCC GGCGGTACGTACTTAGCAGCATCTATGGCGCCTGTGCTT	TAQMAN

						TCGCAGCGTTCGTATGTTTTGTCATCCGTGTTGCTAAAA	
>gb|DQ489311.1| Porcine reproductive and respiratory syndrome virus strain SD01-08, complete genome	DQ489311.1	13641	13836	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGTCACTCATATCCTCT CACTGGGTTTTCTCACGACAAGTCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTCGGAG GGCGGTATGTACTTAGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTATGCTTCGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|DQ366647.1| Porcine respiratory and reproductive syndrome virus strain HU09 major envelope glycoprotein gene, partial cds	DQ366647.1	103	298	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGC TCGGTCTCGGCGCTGTGTCCACTACAGGATTTGTTGGCG GGCGGTATGTGCTCAGCAGCGTGTACGGCGCTTGTGCTT TCGCAGCGCTCGTATGTTTTGTCATCCGCGCTGCTAAAA	TAQMAN
>gb|DQ864705.1| Porcine respiratory and reproductive syndrome virus strain 01CB1, complete genome	DQ864705.1	13615	13810	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCAGTTGCCACTCATATCCTCT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGC TCGGTCTCGGCGTTGTATCCACTGCAGGACTTGTTGGCG GGCGGTACGTACTCTGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|L04493.1|PRWPOLGLYM Porcine reproductive and respiratory syndrome virus (PRRSV) RNA polymerase, 3' end; complete ORF3, ORF4, ORF5; glycoprotein gene, membrane protein gene, nucleocapsid protein gene, complete cds's	L04493.1	3510	3705	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCAGTTGCCACTCATATCCTCT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGC TCGGTCTCGGCGTTGTATCCACTGCAGGACTTGTTGGCG GGCGGTACGTACTCTGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|M96262.2|LEYPOLYENV Lelystad virus, complete genome	M96262.2	13692	13887	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTCT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGCG GGCGGTACGTACTCTGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|U40687.1|PRU40687 Porcine reproductive and respiratory syndrome virus viral membrane-associated protein gene, partial cds	U40687.1	173	368	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGC TCGGTCTCGGCGCTGTGTCCACTACGGGATTTGTTGGCG GGCGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCTT TCGCAGCGCTCGTATGTTTTGTCATCCGCGCTGCTAAAA	TAQMAN
>gb|U40689.1|PRU40689 Porcine reproductive and respiratory syndrome virus viral membrane-associated protein gene, partial cds	U40689.1	173	368	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTCGTAACTCATATTCTCT CACTGGGTTTTCTCACAACAAGTCATTTTTTTGATGCGCT CGGTCTCGGCGCTGTGTCCACTACAGGATTTGTTGGCGG ACGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCTTT CGCAGCGCTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|U40690.1|PRU40690 Porcine reproductive and respiratory syndrome virus viral membrane-associated protein gene, partial cds	U40690.1	173	368	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGC TCGGTCTCGGCGCTGTGTCCGCTATAGGGTTTGTTGGCA GGCGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCCT TCGCAGCGCTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|U40691.1|PRU40691 Porcine reproductive and respiratory syndrome virus viral membrane-associated protein gene, partial cds	U40691.1	173	368	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTTT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCGC TCGGTCTTGGCGCTGTGTCCATTACAGGATTTGTTGGCG GGCGGTATGTACTCAGCAGCGTGTACGGCGCTTGTGCTTT CGCAGCGCTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|AF495511.1| Porcine respiratory and reproductive syndrome virus strain uab1 envelope glycoprotein (ORF5) gene, complete cds	AF495511.1	202	394	193	Plus - Minus - Minus	ACCTTTGTGCTTTACCCGGTTGCAACTCACATTCTTTCAC TGGGTTTTCTCACAACAAGCCATTTCTTTGATGCGCTCG GTCTCGGCGCTGTGTCCGTCACCGGGTTTTATGACAAAC GGTATGTGCTTGGCAGTGTCTACGGCGCTTGTGCTTTCG CAGCGCTCGTGTGCTTTGTCATCCGTGCTGCAAAAA	TAQMAN
>gb|AF495516.1| Porcine respiratory and reproductive syndrome virus strain uab10 envelope glycoprotein (ORF5) gene, complete cds	AF495516.1	200	394	195	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTGTCACTCAAATCCTTT CACTGGGCTTTCTTACAACAAGCCATTTTTTTGACGCGC TCGGTCTTGGCGCTGTATCCACTGCAGGATTTGTTGGCG GGCGGTATGTACTCAGCAGCGTATACGGCTCTTGTGCTT TCGCAGCGCTCGTATGTTTTGTCATCCGTGCTGCAAAAA	TAQMAN
>gb|AY035900.1| Porcine reproductive and respiratory syndrome virus strain 92V58 major envelope glycoprotein GP5 gene, complete cds	AY035900.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTCT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGC TCGGTCTCAGCGCTGTATCCACTGCAGGATTTGTTGGCG GGCGGTATGTACTCAGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|AY035901.1| Porcine reproductive and respiratory syndrome virus strain AV30 major envelope glycoprotein GP5 gene, complete cds	AY035901.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATTCTCT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGC TCGGTCTCAGCGCTGTATCCACTGCAGGATTTGTTGGCG GGCGGTATGTACTCAGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|AY035902.1| Porcine reproductive and respiratory syndrome virus strain 12654 major envelope glycoprotein GP5 gene, complete cds	AY035902.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTGGTGACTCATATTATC TCACTGGGTTTTCTTACTACAAGCCATTTTTTTGACGCTC TCGGTCTCGGGGCTGTGTCTGTTACAGGATTTGTTGGC GACGGTATGTGCTCAGTAGTGTGTACGGCGCTTGTGCAC TCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|AY035910.1| Porcine reproductive and respiratory syndrome virus strain 24554/97 major envelope glycoprotein GP5 gene, complete cds	AY035910.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTGGCGACCCATATCATC TCACTGGGTTTTCTTACAACAAGCCATTTCTTTGATGCG CTCGGTCTCGGGGCTGTGTCTGTTACAGGATTTGTTGGC GGACGGTATGTGCTCAGTAGTGTGTACGGCGCTGTTGCA TTCGCAGCGCTCGTATGTTTTGTCATTCGTGCTGCTAAA A	TAQMAN
>gb|AY035911.1| Porcine reproductive and respiratory syndrome virus strain 25434/98 major envelope glycoprotein GP5 gene, complete cds	AY035911.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTGGCGACCCATATCATC TCACTGGGTTTTCTTACAACAAGCCATTTCTTTGATGCG CTCGGTCTCGGGGCTGTGTCTGTTACAGGATTTGTTGGC GGACGGTATGTGCTCAGTAGTGTGTACGGCGCTTGTGCA TTCGCAGCGCTCGTATGTTTTGTCATTCGTGCTGCTAAA A	TAQMAN
>gb|AY035912.1| Porcine reproductive and respiratory syndrome virus strain 28639/98 major envelope glycoprotein GP5 gene, complete cds	AY035912.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTGGCGACCCATATCATC TCACTGGGTTTTCTTACAACAAGCCATTTCTTTGATGCG CTCGGTCTCGGGGCTGTGTCTGTTACAGGATTTGTTGGC GGACGGTATGTGCTCAGTAGTGTGTACGGTGCTTGTGCA TTCGCAGCGCTCGTATGTTTTGTCATTCGTGCTGCTAAA A	TAQMAN
>gb|AY035918.1| Porcine reproductive and respiratory syndrome virus strain SDRPII7A major envelope glycoprotein GP5 gene, complete cds	AY035918.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTCT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGCG GGCGGTATTTACTCTGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTGTGTTTTGTCATTCGTGCTGCTAAAA	TAQMAN
>gb|AY035919.1| Porcine reproductive and respiratory syndrome virus strain SDRPIV4A major envelope glycoprotein GP5 gene, complete cds	AY035919.1	199	394	196	Plus - Minus - Minus	GAGACCTTTGTGTTTTACCCGGTTGCCACTCATATCCTCT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGCG GGCGGTATGTACTCAGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTGTGTTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|U40692.1|PRU40692 Porcine reproductive and respiratory syndrome virus viral membrane-associated protein gene, partial cds	U40692.1	173	368	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTTGCAACTCATATTCTCT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTGTCCATAACAGGGTTTTATGATA AACGGTATGTACTCAGCAGTGTCTACGGCGCTTGTGCTC TCGCAGCGTTCGTGTGCTTTGTCATCCGTGCTGCTAAAA	TAQMAN
>gb|U40695.1|PRU40695 Porcine reproductive and respiratory syndrome	U40695.1	173	368	196	Plus - Minus - Minus	GAGACCTTTGTGCTTTACCCGGTCGCCACTCACATCCTC TCACTGGGTTTTCTCACAACAAGCCATTTTTTTGATGCG	TAQMAN

	virus viral membrane-associated protein gene, partial cds						CTCGGTCTCGGCGCAGTATCCACTGCAGGACTTGTTGAC GGGCGGTACGTACTCAGCAGCGTCTACGGCGCTTGTGCT TTTGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAA A	
	>gb|U40697.1|PRU40697 Porcine reproductive and respiratory syndrome virus viral membrane-associated protein gene, partial cds	U40697.1	173	368	196	Plus - Minus - Minus	GAGACCTTTGTGTTTTACCCGGTTGCCACTCATATCCTCT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGCG GGCGGTATGTACTCAGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTGTGTTTTGTCATCCGTGCTGCTAAAA GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTCT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGCG GGCGGTATTTACTCTGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTGTGTTTTGTCATCCGTGCTGCTAAAA GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTCT CACTGGGTTTTCTCACAACAAGCCATTTTTTTTGCACAC TCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGCG GGCGGTATGTACACTGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTGTGTTTTGTCATCCGTGCTGCTAAAA GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTCT CGCTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGC TCGGTCTCAGCGCTGTATCCACTGCAGGATTTGTTGGCG GGCGGTATGTACTCAGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTCT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGC TCGGTCTCAGCGCTGTATCCACTGCAGGATTTGTTGGCG GGCGGTATGTGCTCTGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTATGTTTTGTCATCCGTGCTGCTAAAA GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTCT CACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCGC TCGGTCTCGGCGCTGTATCCACCGCAGGATTTGTTGGCG GGCGGTATGTACTCTGCAGCGTCTACGGCGCTTGTGCTT TCGCAGCGTTCGTGTGTTTTGTCATCCGTGCTGCAAAAA GAGACCTTTGTGCTTTACCCGGTTGCCACTCATATCCTC GCACTGGGTTTTCTCACAACAAGCCATTTTTTTGACGCG CTCGGTCTCGGCGCTGTATCCACTGCAGGATTTGTTGGC GGGCGGTATGTACTCTGCAGCGTCTACGGCGCTTGTGCT TTCGCAGCGTTCGTGTGTTTTGTCATCCGTGCTGCTAAAA A	TAQMAN
	>gb|U40698.1|PRU40698 Porcine reproductive and respiratory syndrome virus viral membrane-associated protein gene, partial cds	U40698.1	173	368	196	Plus - Minus - Minus		TAQMAN
	>gb|U40699.1|PRU40699 Porcine reproductive and respiratory syndrome virus viral membrane-associated protein gene, partial cds	U40699.1	173	368	196	Plus - Minus - Minus		TAQMAN
	>gb|U40700.1|PRU40700 Porcine reproductive and respiratory syndrome virus viral membrane-associated protein gene, partial cds	U40700.1	173	368	196	Plus - Minus - Minus		TAQMAN
	>gb|U40701.1|PRU40701 Porcine reproductive and respiratory syndrome virus viral membrane-associated protein gene, partial cds	U40701.1	173	368	196	Plus - Minus - Minus		TAQMAN
	>gb|U40702.1|PRU40702 Porcine reproductive and respiratory syndrome virus membrane-associated protein gene, partial cds	U40702.1	173	368	196	Plus - Minus - Minus		TAQMAN
	>gb|AY035920.1| Porcine reproductive and respiratory syndrome virus strain SDRPV4A major envelope glycoprotein GP5 gene, complete cds	AY035920.1	199	394	196	Plus - Minus - Minus		TAQMAN
	>gb|AY035921.1| Porcine reproductive and respiratory syndrome virus strain 2.25 major envelope glycoprotein GP5 gene, complete cds	AY035921.1	199	394	196	Plus - Minus - Minus		TAQMAN
	>gb|AY035922.1| Porcine reproductive and respiratory syndrome virus strain 2.35 major envelope glycoprotein GP5 gene, complete cds	AY035922.1	199	394	196	Plus - Minus - Minus		TAQMAN
	>gb|AY035923.1| Porcine reproductive and respiratory syndrome virus strain 2.46 major envelope glycoprotein GP5 gene, complete cds	AY035923.1	199	394	196	Plus - Minus - Minus		TAQMAN
	>gb|AY035924.1| Porcine reproductive and respiratory syndrome virus strain 2.72 major envelope glycoprotein GP5 gene, complete cds	AY035924.1	199	394	196	Plus - Minus - Minus		TAQMAN
	>gb|AY035925.1| Porcine reproductive and respiratory syndrome virus strain 2.96 major envelope glycoprotein GP5 gene, complete cds	AY035925.1	199	394	196	Plus - Minus - Minus		TAQMAN
	>gb|AY035926.1| Porcine reproductive and respiratory syndrome virus strain 1/93 major envelope glycoprotein GP5 gene, complete cds	AY035926.1	199	394	196	Plus - Minus - Minus		TAQMAN
[redacted]								
sig_candidate_1810388	>gb|AY395080.1| Porcine reproductive and respiratory syndrome virus isolate SD-01-08 glycoprotein GP2, protein E, glycoprotein GP3, glycoprotein GP4, glycoprotein GP5, membrane protein M, and nucleocapsid protein N genes, complete cds	AY395080.1	2115	2304	190	Plus - Minus - Minus	CCGTACCCGGTTCACCAACTTCATTGTGGACGACCGGGG GGGAGTTCATAGATGGAAGTCTCCAATAGTGGTAGAAA AACTGGGCAAAGCCGAAATTGGCGGCAACCTTGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGAACTTCGGCCGAGCAATGGGAGGCCTAG	TAQMAN
111 total sequence hits	>gb|AY749389.1| Porcine reproductive and respiratory syndrome virus strain MN-03-10_EU GP5 protein gene, complete cds	AY749389.1	417	606	190	Plus - Minus - Minus	CCGTACTCGGTTTACCAACTTCATTGTGGACGACCGTGG TGTAGTTCATCGATGGAAGTCTCCAATAGTGGTAGAAA AATTGGGCAAAGCCGAAGTCGGCGGCAGCCTTGTCACC ATCAAACACGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG	TAQMAN
	>gb|AY422798.1| Porcine reproductive and respiratory syndrome virus isolate MN-02-02_EU GP5 protein (ORF5) gene, complete cds	AY422798.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG GGGAGTTCATAGATGGAAATCTCCAATAGTGGTAGAAA AACTAGGCAAAGCCGAAATTGGCGGCAACCTTGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGAACTTCGGCCGAGCAATGGGAGGCCTAG	TAQMAN
	>gb|AY749397.1| Porcine reproductive and respiratory syndrome virus strain MN-04-09_EU GP5 protein gene, complete cds	AY749397.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG AGGAGTTCATCGATGGAAGTCCCCAATAGTGGTGGAAA AATTGGGCAAAGCCGAAGTTGACGGCAGCCTTGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCCGAGCAATGGGAGGCCTAG	TAQMAN
	>gb|AY422799.1| Porcine reproductive and respiratory syndrome virus isolate MN-01-03_EU GP5 protein (ORF5) gene, complete cds	AY422799.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG GGGAGTTCATAGATGGAAATCTCCAATAGTGGTAGAAA AACTGGGCAAAGCCGAAATTGGCGGCAACCTTGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGAACTTCGGCTGAGCAATGGGAGGCCTAG	TAQMAN
	>gb|AY749411.1| Porcine reproductive and respiratory syndrome virus strain SDPRRS 04-48 GP5 protein gene, complete cds	AY749411.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG GGGAGTTCATAGAATGAAGTCTCCAATAGTGGTAGAAA AACTGGGCAAAGCCGAAATTGGCGGCAACCTTGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACAAGAACTTCGGCCGAGCAATGGGAGGCCTAG	TAQMAN
	>gb|AY875855.1| Porcine respiratory and reproductive syndrome virus envelope glycoprotein gene, complete	AY875855.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAATTTCATCGTGGACGACCGGGG GAGGATCCATCGATGGAAGTCCCCAATAGTGGTAGAAA AATTGGGCAAAGCTGAAGTCGGTGGTGGCCTTGTCACC	TAQMAN

cds						ATCAAGCATGTCGTCCTTGAAGGGGTTAAAGCTCAACCC TTGACGAGGACTTCGGCTGAGCAATGGGAAGCCTAG CCGTACCCGGTTTACCAACTTCATTGTAGACAACCGGGG GAGAGTTCATCGATGGAAGTCTCCAATAGTGGTAGAAA AATTGGGCAAAGCCGAAGTCGACGGCAACCTCGTCACC	
>gb|AY875856.1| Porcine respiratory and reproductive syndrome virus envelope glycoprotein gene, complete cds	AY875856.1	417	606	190	Plus - Minus - Minus	ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG ACCCGGTTTACCAACTTCATTGTGGACGACCGAGGGGG	TAQMAN
>gb|AY875857.1| Porcine respiratory and reproductive syndrome virus envelope glycoprotein gene, complete cds	AY875857.1	421	606	186	Plus - Minus - Minus	AGTTCATCGATGGAAGTCTCCAATAGTGGTAGAAAAAA TGGGCAAAGCCGAAATTGGCGGCAACCTTGTCAACATC AAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACCCTTA ACGAGGACTTCGGCCGAGCAATGGGAGGCCTAG CCGTACCCGGTTTACCAATTTCATTGTAGACGACCGGGG	TAQMAN
>gb|DQ345753.1| Porcine respiratory and reproductive syndrome virus isolate Spain 26/2003 envelope glycoprotein gene, complete cds	DQ345753.1	417	606	190	Plus - Minus - Minus	GAGGATCCATCGATGGAAGTCTCCGATAGTGGTAGAGA AATTGGGCAAAGCTGAAGTTGGTGGCGACCTCGTCACC ATTAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACCC TTGACGAGGACTTCGGCTGAGCAATGGGAAGCCTAG CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG	TAQMAN
>gb|DQ009640.1| Porcine respiratory and reproductive syndrome virus strain CRESA16 envelope glycoprotein (ORF5) gene, complete cds	DQ009640.1	417	606	190	Plus - Minus - Minus	GAGGATCCATCGATGGAAGTCTCCAATAGTGGTAGAGA AATTGGGCAAAGCTGAAGTCGGTGGCGACCTCATCACC ATCAAACATGTCGTCCTAGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAAGCCTAG ACCCGGTTTACCAACTTCATTGTGGATGACCGGGGGGG	TAQMAN
>gb|DQ475238.1| Porcine respiratory and reproductive syndrome virus isolate PRRSV0000614 envelope glycoprotein gene, complete cds	DQ475238.1	421	606	186	Plus - Minus - Minus	AGTTCATCGATGGAAGTCTCCAATAGTGGTAGAAAAAT TGGGCAAAGCCGAAGTCGGCGGCAGCCTCGTCACCATC AAACATGTCGTCCTCGAAGGGGTAAAAGCTCAACCCTT GACAAGGACTTCAGCTGAGCAATGGGAGGCCTAG CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG	TAQMAN
>gb|DQ009641.1| Porcine respiratory and reproductive syndrome virus strain CRESA17 envelope glycoprotein (ORF5) gene, complete cds	DQ009641.1	417	602	186	Plus - Minus - Minus	GAGAGTTCATCGATGGAAGTCTCCAATAGTGGTAGAAA AAATGGGCAAGGCCGAAGTTGACGGCAACCTTGTCACC ATTAAACATGTCGTCCTCGAAGGGGTCACAGCTCAACCC TTGACGAGGACTTCGGCTGAGCAATGGGAGGC CCGTACCCGGTTTACCAATTTCGTTGTGGACGACCGGGG	TAQMAN
>gb|DQ475447.1| Porcine respiratory and reproductive syndrome virus isolate PRRSV0000870 envelope glycoprotein gene, complete cds	DQ475447.1	417	602	186	Plus - Minus - Minus	GACGGTCCATCGATGGAAGTCTCCAATAGTGGTAGAAA AATTGGGCAAAGCCGAAATCGACGGCAACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGGCGAGGACTTCGGCTGAGCAATGGGAGGC ACCCGGTTTACCAACTTCATTGTGGACGACCGGGGGAG	TAQMAN
>gb|DQ009642.1| Porcine respiratory and reproductive syndrome virus strain CRESA19 envelope glycoprotein (ORF5) gene, complete cds	DQ009642.1	421	606	186	Plus - Minus - Minus	GATCCATCGATGGAAGTCTCCAATAGTGGTAGAGAAAT TGGGCAAAGCTGAAGTCGGTGGCGACCTCGTCACCATC AAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACCCTTG ACGAGGACTTCGGCTGAGCAATGGGAAGCCTAG CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG	TAQMAN
>gb|DQ475472.1| Porcine respiratory and reproductive syndrome virus isolate PRRSV0000902 envelope glycoprotein gene, complete cds	DQ475472.1	417	606	190	Plus - Minus - Minus	GGGAGTTCATAGATGGAAATCTCCAATAGTGGTAGAAA AACTGGGCAAAGCCGAAATTGGCGGCAACCTTGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGAACTTCGGCTGAGCAATGGGAGGCCTAG ACCCGGTTTACCAACTTCATTGTAGACGACCGGGGGAG	TAQMAN
>gb|DQ009643.1| Porcine respiratory and reproductive syndrome virus strain CRESA20 envelope glycoprotein (ORF5) gene, complete cds	DQ009643.1	421	606	186	Plus - Minus - Minus	GATCCATCGATGGAAGTCTCCAATAGTTGTAGAGAAATT GGGCAAAGCTGAAGTCGGTGGCGACCTTGTTACCATCA AACATGTTGTCCTCGAAGGGGTCAAAGCTCAACCTTTGA CGAGGACTTCGGCTGAGCAATGGGAAGCCTAG CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG	TAQMAN
>gb|DQ475490.1| Porcine respiratory and reproductive syndrome virus isolate PRRSV0000930 envelope glycoprotein gene, complete cds	DQ475490.1	417	606	190	Plus - Minus - Minus	AGGAGTTCATCGATGGAAGTCCCCAATAGTGGTGGAAA AATTGGGCAAAGCCGAAGTCGACGGCAGCCTTGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCCGAGCAATGGGAGGCCTAG ACCCGGTTTACCAACTTCATTGTGGATGACCGGGGAGG	TAQMAN
>gb|DQ476364.1| Porcine respiratory and reproductive syndrome virus isolate PRRSV0002045 envelope glycoprotein gene, complete cds	DQ476364.1	421	606	186	Plus - Minus - Minus	AGTTCATCGATGGAAGTCTCCGATAGTGGTGGAAAAAA TGGGCAAAGCCGAAGTCGACGGTAGCCTCGTTACCATC AAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACCCTTG ACAAGGACTTCGGCTGAGCAATGGGAGGCCTAG CCGTACCCGGTTTACCAACTTTATTGTGGACGACCGGGG	TAQMAN
>gb|DQ477659.1| Porcine respiratory and reproductive syndrome virus isolate PRRSV0003620 envelope glycoprotein gene, complete cds	DQ477659.1	417	606	190	Plus - Minus - Minus	AGGAGTTCATCGGTGGAAGTCTCCAATAGTGGTAGAAA AATTGGGCAAAGCCGACATCGACGGCAGCCTTGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACAAGGACTTCGGCTGAGCAATGGGAGGCCTAG CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG	TAQMAN
>gb|DQ477805.1| Porcine respiratory and reproductive syndrome virus isolate PRRSV0003791 envelope glycoprotein gene, complete cds	DQ477805.1	417	606	190	Plus - Minus - Minus	GAGAGTTCATCGATGGAAGTCTCCAATAGTGGTAGAAA AATTGGGCAAAGCCGAAGTCGATGGCAACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG CCGTACCCGGTTTCACCAACTTCATTGTGGACGACCGGGG	TAQMAN
>gb|DQ489311.1| Porcine reproductive and respiratory syndrome virus strain SD01-08, complete genome	DQ489311.1	13859	14048	190	Plus - Minus - Minus	GGGAGTTCATAGATGGAAGTCTCCAATAGTGGTAGAAA AACTGGGCAAAGCCGAAATTGGCGGCAACCTTGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGAACTTCGGCCGAGCAATGGGAGGCCTAG ACCCGGTTTACCAACTTCATTGTGGACGACCGGGGGAG	TAQMAN
>gb|DQ864705.1| Porcine reproductive and respiratory syndrome virus strain 01CB1, complete genome	DQ864705.1	13837	14022	186	Plus - Minus - Minus	AGTCCATCGATGGAAGTCTCCAATAGTGGTAGAAAAAT TGGGCAAAGCCGAAGTCGACGGCAACCTCGTCACCATT AAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACCCTTG ACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG CCGTACCCGGTTCACCAACTTCATTGTGGACGACCGGGG	TAQMAN
>gb|AY422800.1| Porcine reproductive and respiratory syndrome virus isolate MN-01-04_EU GP5 protein (ORF5) gene, complete cds	AY422800.1	417	606	190	Plus - Minus - Minus	GGGAGTTCATAGATGGAAGTCTCCAATAGTGGTAGAAA AACTGGGCAAAGCCGAAATTGGCGGCAACCTTGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGAACTTCGGCCGAGCAATGGGAGGCCTAG CCGTACCCGGTTTACCAATTTCGTTGTGGACGACCGGGG	TAQMAN
>gb|AY422801.1| Porcine reproductive and respiratory syndrome virus isolate MN-01-05_EU GP5 protein (ORF5) gene, complete cds	AY422801.1	417	602	186	Plus - Minus - Minus	GACGGTCCATCGATGGAAGTCTCCAATAGTGGTAGAAA AATTGGGCAAAGCCGAAATCGACGGCAACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGGCGAGGACTTCGGCTGAGCAATGGGAGGC CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG	TAQMAN
>gb|AY422802.1| Porcine reproductive and respiratory syndrome virus isolate MN-00-06_EU GP5 protein (ORF5) gene, complete cds	AY422802.1	417	606	190	Plus - Minus - Minus	GGGGGTTCATAGATGGAAATCTCCAATAGTGGTAGAAA AACTGGGCAAAGCCGAAATTGGCGGCAACCTTGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG ACCCGGTTTACCAACTTCATTGTGGATGACCGGGGGGG	TAQMAN
>gb|AY422803.1| Porcine reproductive and respiratory syndrome virus isolate MN-00-07_EU GP5 protein (ORF5) gene, complete cds	AY422803.1	421	606	186	Plus - Minus - Minus	AGTTCATCGATGGAAGTCTCCAATAGTGGTAGAAAAAT TGGGCAAAGCCGAAGTCGGCGGCAGCCTCGTCACCATC AAACATGTCGTCCTCGAAGGGGTAAAAGCTCAACCCTT GACAAGGACTTCAGCTGAGCAATGGGAGGCCTAG CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG	TAQMAN
>gb|AY422804.1| Porcine reproductive and respiratory syndrome virus isolate MN-00-08_EU GP5 protein (ORF5) gene, complete cds	AY422804.1	417	606	190	Plus - Minus - Minus	GGGAGTTCATAGATGGAAATCTCCAATAGTGGTAGAAA AACTGGGCAAAGCCGAAATTGGCGGCAACCTTGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG	TAQMAN
>gb|AY422805.1| Porcine reproductive and respiratory syndrome	AY422805.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGATGACCGGGG GGGAGTTCATAGATGGAAATCTCCAATAGTGGTAGAAA	TAQMAN

virus isolate MN-00-09_EU GP5 protein (ORF5) gene, complete cds						AACTGGGCAAAGCCGAAATCGGCGGCAACCTTGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG	
>gb|AY422806.1| Porcine reproductive and respiratory syndrome virus isolate MN-00-10_EU GP5 protein (ORF5) gene, complete cds	AY422806.1	417	602	186	Plus - Minus - Minus	GGGAGTTCATAGATGGAAATCTCCAATAGTGGTAGAAA AACTGGGCAAAGCCGAAATTAGCGGCAACCTTGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAGGC	TAQMAN
>gb|AY422807.1| Porcine reproductive and respiratory syndrome virus isolate MN-00-11_EU GP5 protein (ORF5) gene, complete cds	AY422807.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGATGACCGGGG GGGAGTTCATAGATGGAAATCTCCAATAGTGGTAGAAA AACTGGGCAAAGCCGAAATCGGCGGCAACCTTGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG CCGTACCCGGTTTACCAACTTCATTGTGGATGACCGGGG	TAQMAN
>gb|DQ009647.1| Porcine respiratory and reproductive syndrome virus strain CRESA-VP21 envelope glycoprotein (ORF5) gene, complete cds	DQ009647.1	417	606	190	Plus - Minus - Minus	GAGGATCCATCGATGGAAGTCTCCAATAGTGGTAGAGA AATTGGGCAAAGCTGAAGTCGGTGGCGACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAAGCCTAG CCGTACCCGGTTTACCAACTTCATTGTGGATGACCGGGG	TAQMAN
>gb|AY422808.1| Porcine reproductive and respiratory syndrome virus isolate MN-00-12_EU GP5 protein (ORF5) gene, complete cds	AY422808.1	417	606	190	Plus - Minus - Minus	GGGAGTTCATAGATGGAAATCTCCAATAGTGGTAGAAA AACTGGGCAAAGCCGAAATCGGCGGCAACCTTGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG CCGTACCCGGTTTACCAACTTCATTGTAGACAACCGGGG	TAQMAN
>gb|DQ064787.1| Porcine respiratory and reproductive syndrome virus strain V3 envelope glycoprotein (GP5) gene, complete cds	DQ064787.1	417	606	190	Plus - Minus - Minus	GAGAGTTCATCGATGGAAGTCTCCAATAGTGGTAGAAA AATTGGGCAAAGCCGAAGTCGACGGCAACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG	TAQMAN
>gb|AY588319.1| PRRSV LV4.2.1, complete genome	AY588319.1	13910	14099	190	Plus - Minus - Minus	GAGAGTTCATCGATGGAAGTCTCCAATAGTGGTAGAAA AATTGGGCAAAGCCGAAGTCGATGGCAACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG	TAQMAN
>gb|DQ064788.1| Porcine respiratory and reproductive syndrome virus strain CReSA-V1 envelope glycoprotein (GP5) gene, complete cds	DQ064788.1	417	606	190	Plus - Minus - Minus	GAGGATCCATCGATGGAAGTCTCCAATAGTGGTAGAGA AATTGGGCAAAGCTGAAGTCGGTGGCGACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAAGCCTAG CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG	TAQMAN
>gb|DQ345725.1| Porcine respiratory and reproductive syndrome virus strain VP-046 bis envelope glycoprotein gene, complete cds	DQ345725.1	417	606	190	Plus - Minus - Minus	GAGGATCCATCGATGGAAGTCTCCAATAGTGGTAGAGA AATTGGGCAAAGCTGAAGTCGGTGGCGACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAAGCCTAG CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG	TAQMAN
>gb|DQ345726.1| Porcine respiratory and reproductive syndrome virus strain All-183 envelope glycoprotein gene, complete cds	DQ345726.1	417	606	190	Plus - Minus - Minus	GAGGATCCATCGATGGAAGTCTCCAATAGTGGTAGAGA AATTGGGCAAAGCTGAAGTCGGTGGCGACCTCGTCGCC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAAGCCTAG CCGTACCCGGTTTACCAACTTCATTGTAGACAACCGGGG	TAQMAN
>gb|DQ345727.1| Porcine respiratory and reproductive syndrome virus strain DV envelope glycoprotein gene, complete cds	DQ345727.1	417	606	190	Plus - Minus - Minus	GAGAGTTCATCGATGGAAGTCTCCAATAGTGGTAGAAA AATTGGGCAAAGCCGAAGTCGACGGCAACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG	TAQMAN
>gb|L04493.1|PRWPOLGLYM Porcine reproductive and respiratory syndrome virus (PRRSV) RNA polymerase, 3' end; complete ORF3, ORF4, ORF5; glycoprotein gene, membrane protein gene, nucleocapsid protein gene, complete cds's	L04493.1	3728	3917	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG GAGAGTCCATCGATGGAGGTCTCCAATAGTGGTAGAAA AATTGGGCAAAGCCGAAGTCGACGGCAACCTCGTCACC ATTAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACCC TTGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG	TAQMAN
>gb|DQ345728.1| Porcine respiratory and reproductive syndrome virus isolate Spain 1/1991 envelope glycoprotein gene, complete cds	DQ345728.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG GAGGATCCATCGATGGAAGTCTCCAATAGTGGTAGAGA AATTGGGCAAAGCTGAAGTCGGTGGCGACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAAGCCTAG CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG	TAQMAN
>gb|M96262.2|LEYPOLYENV Lelystad virus, complete genome	M96262.2	13910	14099	190	Plus - Minus - Minus	GAGAGTTCATCGATGGAAGTCTCCAATAGTGGTAGAAA AATTGGGCAAAGCCGAAGTCGATGGCAACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG	TAQMAN
>gb|DQ345729.1| Porcine respiratory and reproductive syndrome virus isolate Spain 2/1991 envelope glycoprotein gene, complete cds	DQ345729.1	417	606	190	Plus - Minus - Minus	GAGGATCCATCGATGGAAGTCTCCAATAGTGGTAGAGA AATTGGGCAAAGCTGAAGTCGGTGGCGACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAAGCCTAG CCGTACCCGGTTTACTAACTTCATTGTGGACGACCGGGG	TAQMAN
>gb|DQ345730.1| Porcine respiratory and reproductive syndrome virus isolate Spain 3/1992 envelope glycoprotein gene, complete cds	DQ345730.1	417	606	190	Plus - Minus - Minus	GAGGATCCATCGATGGAAGTCTCCAATAGTGGTAGAGA AATTGGGCAAAGCTGAAGTCGGTGGCGACCTCGTCACC ATTAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACCC TTGACGAGGACTTCGGCTGAGCAATGGGAAGCCTAG	TAQMAN
>gb|DQ345732.1| Porcine respiratory and reproductive syndrome virus isolate Spain 5/1992 envelope glycoprotein gene, complete cds	DQ345732.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG GAGGATTCATCGATGGAAGTCTCCAATAGTGGTAGAGA AATTGGGCAAAGCTGAAGTCGGTGGCGACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACAAGGACTTCGGCTGAGCAATGGGAAGCCTAG	TAQMAN
>emb|X92942.1|PRRSENVNP Porcine reproductive and respiratory syndrome virus envelope protein and nucleoprotein genes	X92942.1	2180	2365	186	Plus - Minus - Minus	ACCCGGTTTACCAACTTCATTGTGGACGACCGGGGGAG AATCCATCGGTGGAAGTCTCCAATAGTGGTAGAGAAAT TGGGCAAAGCTGAAGTCGGTGGCGACCTTGTCACCATC AAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACCCTTG ACGAGGACTTCGGCTGAGCAATGGGAAGCCTAG	TAQMAN
>gb|AF253532.1|AF253532 Porcine reproductive and respiratory syndrome virus isolate V-502 major envelope glycoprotein GP5 gene, complete cds	AF253532.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG GAGAGTTCATCGATGGAAGTCTCCAATAGTGGTAGAAA AATTGGGCAAAGCCGAAGTCGATGGCAACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG	TAQMAN
>gb|AF253533.1|AF253533 Porcine reproductive and respiratory syndrome virus isolate V-516 major envelope glycoprotein GP5 gene, complete cds	AF253533.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG GAGAGTTCATCGATGGAAGTCTCCAATAGTGGTAGAAA AATTGGGCAAAGCCGAAGTCGATGGCAACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG	TAQMAN
>gb|AF486468.1| Porcine reproductive and respiratory syndrome virus isolate PRRSV-BS162 major envelope glycoprotein (ORF5) gene, complete cds	AF486468.1	417	602	186	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGATGACCGGGG GAGGATCCATCGATGGAAATCTCCAATAGTGATAGAGA AATTTGGCAAAGCCGAAGTTGGCAGAGACCTCGTTACC ATCAAACATGTCGTCCTCGAAGGGGTCAAAGCTCAACC CTTGACGAGGACTTCTGCTGAGCAATGGGAGGC	TAQMAN
>gb|AF253534.1|AF253534 Porcine reproductive and respiratory syndrome virus isolate V-548 major envelope glycoprotein GP5 gene, complete cds	AF253534.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG GAGAGTTCATCGATGGAAGTCTCCAATAGTGGTAGAAA AATTGGGCAAAGCCGAAGTCGATGGCAACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC	TAQMAN

						CTTGACGAGGACTTCGGATGAGCAATGGGAGGCCTAG	
>gb|AY035900.1| Porcine reproductive and respiratory syndrome virus strain 92V58 major envelope glycoprotein GP5 gene, complete cds	AY035900.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG GAGAGTTCATCGATGGAAGTCTCCAATAGTGGTAGAGA AATTGGGCAAAGCCGAAGTCGACGGCAACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG	TAQMAN
>gb|AF253535.1|AF253535 Porcine reproductive and respiratory syndrome virus isolate V-546 major envelope glycoprotein GP5 gene, complete cds	AF253535.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG GAGAGTTCATCGATGGAAGTCTCCAATAGTGGTAGAAA AATTGGGCAAAGCCGAAGTCGATGGCAACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGATGAGCAATGGGAGGCCTAG	TAQMAN
>gb|AY035901.1| Porcine reproductive and respiratory syndrome virus strain AV30 major envelope glycoprotein GP5 gene, complete cds	AY035901.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG GAGAGTTCATCGATGGAAGTCTCCAATAGTGGTAGAGA AATTGGGCAAAGCCGAAGTCGACGGCAACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG	TAQMAN
>gb|AF253536.1|AF253536 Porcine reproductive and respiratory syndrome virus isolate V-547 major envelope glycoprotein GP5 gene, complete cds	AF253536.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG GAGAGTTCATCGATGGAAGTCTCCAATAGTGGTAGAAA AATTGGGCAAAGCCGAAGTCGATGGCAACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG	TAQMAN
>gb|AY035904.1| Porcine reproductive and respiratory syndrome virus strain 12985 major envelope glycoprotein GP5 gene, complete cds	AY035904.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACAAACTTTATTGTGGACGACCGGGG GAGAATCCACAGGTGGAAGTCCCCAATAGTGGTGGAGA AATTGGGCAAAGCTGAAGTCGGCGGCGGCCTCATTACC ATCAAACACGTCGTTCTCGAAGGGGTTAAAGCTCAACC CTTGACCAGGACTTCGGCTGAGCAATGGGAAGCCTAG	TAQMAN
>gb|AF486452.1| Porcine reproductive and respiratory syndrome virus isolate PRRSV-BS26 major envelope glycoprotein (ORF5) gene, complete cds	AF486452.1	421	606	186	Plus - Minus - Minus	ACCCGGTTTACCAACTTCATTGTGGATGACCGTGGGAGG ATCCATAGATGGAAGTCTCCAATAGTGGTGGAGAAATT GGGCAAGGCTGAAGTTGGTGGTGACCTCGTTACCATCA AACATGTCGTCCTTGAAGGGGTTAAAGCTCAACCCTTGA CGAGGACCTCGGCTGAGCAATGGGAAGCCTAG	TAQMAN
>gb|AY035917.1| Porcine reproductive and respiratory syndrome virus strain 5767-6 major envelope glycoprotein GP5 gene, complete cds	AY035917.1	421	606	186	Plus - Minus - Minus	ACCCGGTTTACCAACTTCATCGTGGACGACCGGGGGAG GATTCATCGATGGAAGTCTCCAATAGTGGTGGAGAAAT TGGGCAAAGCCGAAGTCGGTAGTGACCTTGTCACCATC AAACATGTCGTCCTCGAAGGGGTCAAAGCTCAACCCTT GACGAGGACTTCGGCTGAGCAATGGGAAGCCTAG	TAQMAN
>gb|AF486463.1| Porcine reproductive and respiratory syndrome virus isolate PRRSV-BS77 major envelope glycoprotein (ORF5) gene, complete cds	AF486463.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACTAACTTCATTGTGGACGACCGGGG GAGGATCCACAGGTGGAAATCACAAATAGTGGTGGAAA AATTGGGCAAAGCTGAAATCGGCGGCGACCTTGTCACC ATTAAACATGTTGTCCTCGAAGGGGTCAAAGCTCAACCT TTAACGAGAACTTCGGCTGAGCAATGGGAAGCCTAG	TAQMAN
>gb|AY035918.1| Porcine reproductive and respiratory syndrome virus strain SDRPII7A major envelope glycoprotein GP5 gene, complete cds	AY035918.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG GAGAGTTCATCGATGGAAGTCTCCAATAGTGGTAGAAA AATTGGGCAAAGCCGAAGTCGACGGCAACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG	TAQMAN
>gb|AF486464.1| Porcine reproductive and respiratory syndrome virus isolate PRRSV-BS82 major envelope glycoprotein (ORF5) gene, complete cds	AF486464.1	421	606	186	Plus - Minus - Minus	ACCCGGTTTACCAACTTCATTGTGGACGACCGGGGGAG AATCCATCGATGGAAGTCTCCAATAGTGGTGGAAAAAT TAGGCAAGGCTGAAATCGGCAGTGACCTTGTTACCATC AAGCACGTCGTCCTCGAAGGGGTTAAAGCTCAACCCTT GACAAGGACTTCGGCTGAGCAATGGGAGGCCTAG	TAQMAN
>gb|AY035919.1| Porcine reproductive and respiratory syndrome virus strain SDRPIV4A major envelope glycoprotein GP5 gene, complete cds	AY035919.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG GAGAGTTCATCGATGGAAGTCTCCAATAGTGGTAGAAA AATTGGGCAAAGCCGAAGTCGACGGCAACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG	TAQMAN
>gb|AY035920.1| Porcine reproductive and respiratory syndrome virus strain SDRPV4A major envelope glycoprotein GP5 gene, complete cds	AY035920.1	418	606	189	Plus - Minus - Minus	CGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGGG AGAGTTCATCGATGGAAGTCTCCAATAGTGGTAGAAAA ATTGGGCAAAGCCGAAGTCGACGGCAACCTCGTCACCA TCAAACATGTCGTCCTCGAAGGGGTCAAAGCTCAACCCT TGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG	TAQMAN
>gb|AY615786.1| Porcine respiratory and reproductive syndrome virus strain SE44 envelope glycoprotein (ORF5) gene, complete cds	AY615786.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTAGACAACCGGGG GAGAGTTCATCGATGGAAGTCTCCAATAGTGGTAGAAA AATTGGGCAAAGCCGAAGTCGACGGCAACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG	TAQMAN
>gb|AY035921.1| Porcine reproductive and respiratory syndrome virus strain 2.25 major envelope glycoprotein GP5 gene, complete cds	AY035921.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG GAGAGTTCATCGATGGAAGTCTCCAATAGTGGTAGAAA AATTGGGCAAGGCCGAAGTCGACGGCAACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG	TAQMAN
>gb|AY615787.1| Porcine respiratory and reproductive syndrome virus strain SE53 envelope glycoprotein (ORF5) gene, complete cds	AY615787.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTAGACAACCGGGG GAGAGTTCATCGATGGAAGTCTCCAATAGTGGTAGAAA AATTGGGCAAAGCCGAAGTCGACGGCAACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG	TAQMAN
>gb|AY035922.1| Porcine reproductive and respiratory syndrome virus strain 2.35 major envelope glycoprotein GP5 gene, complete cds	AY035922.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG GAGAGTTCATCGATGGAAGTCTCCAATAGTGGTAGAAA AATTGGGCAAGGCCGAAGTCGACGGCAACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG	TAQMAN
>gb|AY615793.1| Porcine respiratory and reproductive syndrome virus strain SE553 envelope glycoprotein (ORF5) gene, complete cds	AY615793.1	420	606	187	Plus - Minus - Minus	TACCCGGTTTACCAACTTCATTGTGGACGACCGGGGGGG AGTCCATCGATGGAAGTCTCCAATAGTGGTAGAAAAAA TGGGCAAAGCCGAAGTTGGCGGCAACCTCGTCAACATC AAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACCCTTA ACGAGGACTTCGGCCGAGCAATGGGAGGCCTAG	TAQMAN
>gb|AY633973.1| Porcine reproductive and respiratory syndrome virus isolate B13 major envelope glycoprotein gene, complete cds	AY633973.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG GAGAGTTCATCGATGGAAGTCTCCAATAGTGGTAGAAA AATTGGGCAAAGCCGAAGTCGATGGCAACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG	TAQMAN
>gb|AY739965.1| Porcine reproductive and respiratory syndrome virus isolate IT9 GP5 gene, complete cds	AY739965.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAATTTCATTGTGGACGATCGGGG GATGATCCACAGATGGAAGTCTCCAATAGTGGTGGAAA AATTAGGCAAAGCTGAAGTTGGCGGCGACCTTGTCACT ATCAAACATGTTGTCCTCGAAGGGGTCAAAGCTCAACC CTTAACGAGAACTTCGGCTGAGCAATGGGAAGCCTAG	TAQMAN
>gb|AY739969.1| Porcine reproductive and respiratory syndrome virus isolate IT13 GP5 gene, complete cds	AY739969.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTCACCAACTTTATTGTGGACGACCGGGG GAAGATCCATAGATGGAAGTCTCCAATAGTGGTGGAGA AATTAGGCAAAGCTGAAGTCGGCGGCGACCTTGTCACT ATCAAGCATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTAACGAGAACCTCGGCTGAGCAATGGGAAGCCTAG	TAQMAN
>gb|AY739972.1| Porcine reproductive and respiratory syndrome virus isolate IT16 GP5 gene, complete cds	AY739972.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTCACCAACTTTATTGTGGACGACCGGGG GAAGATCCATAGATGGAAGTCTCCAATAGTGGTGGAGA AATTAGGCAAAGCTGAAGTCGGCGGCGACCTTGTCACT ATCAAGCATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTAACGAGAACTTCGGCTGAGCAATGGGAAGCCTAG	TAQMAN
>gb|AY739978.1| Porcine reproductive and respiratory syndrome	AY739978.1	421	602	182	Plus - Minus - Minus	ACCCGGTTTACCAACTTCATCGTGGACGATCGGGGGAG GGTCCATCGGTGGAAGTCTCCCATAGTGGTGGAAAAAA	TAQMAN

virus isolate IT22 GP5 gene, complete cds						TGGGCAAAGCCGAAGTCGGTGGCGACCTTGTTGCCATC AAACATGTCGTCCTTGAAGGGGTTAAAGCTCAACCCTTG ACGAGGACTTCGGCTGAGCAATGGGAGGC	
>gb|DQ345734.1| Porcine respiratory and reproductive syndrome virus isolate Spain 7/1992 envelope glycoprotein gene, complete cds	DQ345734.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG GAGGATCCATCGATGGAAGTCTCCAATAGTGGTGGAGA AATTGGGCAAAGCTGAAATCGGTGGCGACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGAAGGCCTAG	TAQMAN
>gb|AY739979.1| Porcine reproductive and respiratory syndrome virus isolate IT23 GP5 gene, complete cds	AY739979.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTTATTGTGGACGACCGGGG GAAGATCCATAGATGGAAGTCTCCAATAGTGGTGGAGA AATTAGGCAAAGCCGAAGTTGGCGGCGACCTTGTCACT ATCAAGCATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTAACGAGAACTTCGGCTGAGCAATGGGAGGCCTAG	TAQMAN
>gb|DQ345735.1| Porcine respiratory and reproductive syndrome virus isolate Spain 8/1992 envelope glycoprotein gene, complete cds	DQ345735.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG GAGAGTTCATCGATGGAAGTCTCCAATAGTGGTAGAAA AATTGGGCAAAGCCGAAGTCGACGGTAACCTCTTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG	TAQMAN
>gb|AY739980.1| Porcine reproductive and respiratory syndrome virus isolate IT24 GP5 gene, complete cds	AY739980.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTTATTGTGGACGACCGGGG GAAGATCCATAGATGGAAGTCTCCAATAGTGGTGGAGA AATTAGGCAAAGCTGAAGTTGGCGGCGACCTTGTCACT ATCAAGCATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTAACGAGAACTTCGGCTGAGCAATGGGAAGCCTAG	TAQMAN
>gb|DQ345736.1| Porcine respiratory and reproductive syndrome virus isolate Spain 9/1993 envelope glycoprotein gene, complete cds	DQ345736.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTTGACGACCGGGG GAGGATCCATCGATGGAAGTCTCCAATAGTGATAGAGA AATTGGGCAAAGCCGAAGTCGGTGGCGACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAAGCCTAG	TAQMAN
>gb|DQ345740.1| Porcine respiratory and reproductive syndrome virus isolate Spain 13/2000 envelope glycoprotein gene, complete cds	DQ345740.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG GAGGATCCATCGATGGAAGTCTCCAATAGTGGTAGAGA AATTGGGCAAAGCTGAAGTCGGTGGCGACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAAGCCTAG	TAQMAN
>gb|DQ345741.1| Porcine respiratory and reproductive syndrome virus isolate Spain 14/2000 envelope glycoprotein gene, complete cds	DQ345741.1	421	606	186	Plus - Minus - Minus	ACCCGGTTTACCAACTTCATTGTGGACGACCGAGGGAG GATCCATCGATGGAAGTCTCCAATAGTGGTAGAAAAAT TGGGCAAAGCTGAGGTTGGCGGCAACCTCGTCACCATC AAACATGTCGTCCTCGAAGGGGTAAAAGCTCAACCCTT GACGAGAACTTCGGCTGAGCAATGGGAAGCCTAG	TAQMAN
>gb|DQ345744.1| Porcine respiratory and reproductive syndrome virus isolate Spain 17/2000 envelope glycoprotein gene, complete cds	DQ345744.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG GAGAATCCATCGATGGAAGTCTCCAATAGTGGTGGAAA AATTGGGCAAAGCCGAAATCGACGGCAACCTTGTCACC ATCAAACATGTTGTCCTCGAAGGGGTTAAAGCTCAACCC TTAACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG	TAQMAN
>gb|DQ345745.1| Porcine respiratory and reproductive syndrome virus isolate Spain 18/2000 envelope glycoprotein gene, complete cds	DQ345745.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG GAGGATCCATCGATGGAAGTCTCCAATAGTGGTAGAGA AATTGGGCAAAGCTGAAGTCGGTGGCGACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAAGCCTAG	TAQMAN
>gb|DQ345747.1| Porcine respiratory and reproductive syndrome virus isolate Spain 20/2002 envelope glycoprotein gene, complete cds	DQ345747.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG GAGGATCCATCGATGGAAGTCTCCAATAGTGGTAGAGA AATTGGGCAAAGCTGAAGTCGGTGGTGACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAAGCCTAG	TAQMAN
>gb|DQ345748.1| Porcine respiratory and reproductive syndrome virus isolate Spain 21/2002 envelope glycoprotein gene, complete cds	DQ345748.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG GAGAGTCCATCGATGGAAGTCTCCAATAGTGGTAGAAA AATTGGGCAAAGCCGAAGTAGACGGCAACCTCGTCACC ATCAAACATGTCATCCTTGAAGGGGTTAAAGCTCAACCC TTGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG	TAQMAN
>gb|DQ345752.1| Porcine respiratory and reproductive syndrome virus isolate Spain 25/2002 envelope glycoprotein gene, complete cds	DQ345752.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGATCGGGG GAGGATCCATCGATGGAAGTCTCCAATAGTGGTAGAGA AAATGGGCAAAGCTGAAGTCGGTGGCGACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAAGCCTAG	TAQMAN
>gb|AY035923.1| Porcine reproductive and respiratory syndrome virus strain 2.46 major envelope glycoprotein GP5 gene, complete cds	AY035923.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG GAGAGTTCATCGATGGAAGTCTCCAATAGTGGTAGAAA AATTGGGCAAGGCCGAAGTCGACGGCAACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG	TAQMAN
>gb|AY035924.1| Porcine reproductive and respiratory syndrome virus strain 2.72 major envelope glycoprotein GP5 gene, complete cds	AY035924.1	420	606	187	Plus - Minus - Minus	TACCCGGTTTACCAACTTCATTGTGGACGACCGGGGGAG AGTCCATCGATGGAAGTCTCCAATAGTGGTAGAAAAAT TGGGCAAAGCCGAAGTCGACGGCAACCTCGTCACCATT AAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACCCTTG ACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG	TAQMAN
>gb|AY035925.1| Porcine reproductive and respiratory syndrome virus strain 2.96 major envelope glycoprotein GP5 gene, complete cds	AY035925.1	421	606	186	Plus - Minus - Minus	ACCCGGTTTACCAACTTCATTGTGGACGACCGGGGGAG GATTCATCGATGGAAGTCTCCAATAGTGGTGGAGAAAT TGGGCAAAGCCGAAGTCGGTGGCGACCTCGTCACCATC AAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACCCTTG ACGAGGACTTCGGCTGAGCAATGGGAAGCCTAG	TAQMAN
>gb|AY035926.1| Porcine reproductive and respiratory syndrome virus strain 1/93 major envelope glycoprotein GP5 gene, complete cds	AY035926.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG GAGAGTTCATCGATGGAAGTCTCCAATAGTGGTAGAAA AATTGGGCAAAGCCGAAGTCGACGGCAACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTCAAAGCTCAACC CTTGACGAGAACTTCGGCTGAGCAATGGGAGGCCTAG	TAQMAN
>gb|AY035927.1| Porcine reproductive and respiratory syndrome virus strain 1751/93 major envelope glycoprotein GP5 gene, complete cds	AY035927.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG GAGAGTTCATCGATGGAAGTCTCCAATAGTGGTAGAAA AATTGGGCAAAGCCGAAGTCGACGGCAACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTCAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG	TAQMAN
>gb|AY035932.1| Porcine reproductive and respiratory syndrome virus strain 2567/96 major envelope glycoprotein GP5 gene, complete cds	AY035932.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTCACCAACTTCATTGTGGACGACCGGGG GAGGATCCACAGATGGAAGTCACCAGTAGTGGTGGAGA AATTGGGCAAAGCTGAAATCGGCGGCGACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCAGCCGAGCAATGGGAGGCCTAG	TAQMAN
>gb|AY035934.1| Porcine reproductive and respiratory syndrome virus strain 974/98 major envelope glycoprotein GP5 gene, complete cds	AY035934.1	421	606	186	Plus - Minus - Minus	ACCCGGTTTACCAACTTCATTGTGGATGACCGTGGGAGG ATCCATAGATGGAAGTCTCCAATAGTGGTGGAAAAATT AGGCAAAGCCGAAGTTGGTGGTGACCTTGTCACCATCA AACATGTCGTCCTCGAAGGGGTTAAAGCTCAACCCTTGA CGAGGACCTCGGCTGAGCAATGGGAGGCCTAG	TAQMAN
>gb|AY035936.1| Porcine reproductive and respiratory syndrome virus strain 65/2/91 major envelope glycoprotein GP5 gene, complete cds	AY035936.1	421	606	186	Plus - Minus - Minus	ACCCGGTTTACCAACTTCATTGTGGACGACCGGGGGAG AATCCATCGGTGGAAGTCTCCAATAGTGGTAGAGAAAT TGGGCAAAGCTGAAGTCGGTGGCGACCTTGTCACCATC AAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACCCTTG ACGAGGACTTCGGCTGAGCAATGGGAAGCCTAG	TAQMAN
>gb|AY035938.1| Porcine reproductive and respiratory syndrome virus strain H2-D768 major envelope glycoprotein GP5 gene, complete cds	AY035938.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG GAGAGTTCATCGATGGAAGTCTCCAATAGTGGTAGAAA AATTGGGCAAAGCCGAAGTCGACGGCAACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG	TAQMAN

	>gb|AY739981.1| Porcine reproductive and respiratory syndrome virus isolate IT25 GP5 gene, complete cds	AY739981.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTCACCAACTTTATTGTGGACGACCGGGG GAAGATCCATAGATGGAAGTCTCCAATAGTGGTGGAGA AATTAGGCAAAGCTGAAGTTGGCGGCGACCTTGTCACT ATCAAGCATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTAACGAGAACTTCGGCTGAGCAATGGGAAGCCTAG	TAQMAN
	>gb|AY035939.1| Porcine reproductive and respiratory syndrome virus strain L1-D767 major envelope glycoprotein GP5 gene, complete cds	AY035939.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG GAGAGTTCATCGATGGAAGTCTCCAATAGTGGTGGAAA AATTGGGCAAAGCCGAAGTCGACGGCAACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAAGCCTAG	TAQMAN
	>gb|AY739982.1| Porcine reproductive and respiratory syndrome virus isolate IT26 GP5 gene, complete cds	AY739982.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTCACCAACTTTATTGTGGACGACCGGGG GAGAGTCCATAGATGGAAGTCTCCAATAGTGGTGGAGA AATTAGGCAAAGCTGAAGTTGGCGGCGACCTTGTCACT ATCAAGCATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTAACGAGAACTTCGGCTGAGCAATGGGAAGCCTAG	TAQMAN
	>gb|AY739983.1| Porcine reproductive and respiratory syndrome virus isolate IT27 GP5 gene, complete cds	AY739983.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTCACCAACTTTATTGTGGACGACCGGGG GAAGATCCATAGATGGAAGTCTCCAATAGTGGTGGAGA AATTAGGCAAAGCTGAAGTTGGCGGCGACCTTGTCACT ATCAAGCATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTAACGAGAACTTCGGCTGAGCAATGGGAAGCCTAG	TAQMAN
	>gb|AY739984.1| Porcine reproductive and respiratory syndrome virus isolate IT28 GP5 gene, complete cds	AY739984.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTCACCAACTTCATTGTGGACGACCGGGG GAAGATCCATAGATGGAAGTCTCCAATAGTGGTGGAAA AATTAGGCAAAGCTGAAGTTGGCGGCGACCTTGTCACT ATCAAGCATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTAACGAGAACTTCGGCTGAGCAATGGGAAGCCTAG	TAQMAN
	>gb|AY739997.1| Porcine reproductive and respiratory syndrome virus isolate IT41 GP5 gene, complete cds	AY739997.1	421	602	182	Plus - Minus - Minus	ACCCGGTTTACCAACTTCATCGTGGACGATCGGGGGAG GGTCCATCGGTGGAAGTCTCCCATAGTGGTGGAAAAAA TGGGCAAAGCCGAAGTCGGTGGCGACCTTGTTGCCATC AAACATGTCGTCCTTGAAGGGGTTGAAGCTCAACCCTTG ACGAGGACTTCGGCTGAGCAATGGGAGGC	TAQMAN
	>gb|AY743931.1| Porcine reproductive and respiratory syndrome virus isolate Porcilis GP5 gene, complete cds	AY743931.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTAGACAACCGGGG GAGAGTTCATCGATGGAAGTCTCCAATAGTGGTAGAAA AATTGGGCAAAGCCGAAGTCGACGGCAACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG	TAQMAN
	>gb|AY743937.1| Porcine reproductive and respiratory syndrome virus isolate IT61 GP5 gene, complete cds	AY743937.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTCACCAACTTTATTGTGGACGACCGGGG GAAGATCCATAGATGGAAGTCTCCAATAGTGGTGGAGA AATTAGGCAAAGCTGAAGTTGGCGGCGACCTTGTCACT ATCAAGCATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTAACGAGAACTTCGGCTGAGCAATGGGAAGCCTAG	TAQMAN
	>gb|AY749377.1| Porcine reproductive and respiratory syndrome virus strain MN-03-01_EU GP5 protein gene, complete cds	AY749377.1	421	606	186	Plus - Minus - Minus	ACCCGGTTTACCAACTTCATTGTGGACGACCGGGGAGG AGTTCATCGATGGAAGTCCCCAATAGTGGTGGAAAAAT TGGGCAAAGCCGAAGTCGACGGCAGCCTTGTCACCATC AAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACCCTTA ACGAGGACTTCGGCCGAGCAATGGGAGGCCTAG	TAQMAN
	>gb|AY749381.1| Porcine reproductive and respiratory syndrome virus strain MN-03-05_EU GP5 protein gene, complete cds	AY749381.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG AGGAGTTCATCGATGGAAGTCCCCAATAGTGGTGGAAA AATTGGGCAAAGCCGAAGTTGACGGCAGCCTTGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCCGAGCAATGGGAGGCCTAG	TAQMAN
	>gb|AY749385.1| Porcine reproductive and respiratory syndrome virus strain MN-03-08_EU GP5 protein gene, complete cds	AY749385.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG AGGAGTTCATCGATGGAAGTCCCCAATAGTGGTGGAAA AATTGGGCAAAGCCGAAGTTGACGGCAGCCTTGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCCGAGCAATGGGAGGCCTAG	TAQMAN
	>gb|AY035940.1| Porcine reproductive and respiratory syndrome virus strain NY3-D769 major envelope glycoprotein GP5 gene, complete cds	AY035940.1	417	606	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTCATTGTGGACGACCGGGG GAAAGTTCATCGATGGAAGTCTCCAATAGTGGTAGAAA AAATGGGCAAAGCTGAAGTCGACGGGAACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACGAGGACTTCGGCTGAGCAATGGGAGGCCTAG	TAQMAN
	>gb|AY035941.1| Porcine reproductive and respiratory syndrome virus strain 1142/97 major envelope glycoprotein GP5, membrane protein M, and nucleocapsid protein genes, complete cds	AY035941.1	421	602	182	Plus - Minus - Minus	ACCCGGTTTACCAACTTCATCGTGGACGACCGGGGGAG GGTCCATCGATGGAAGTCTCCAATAGTAGTAGAAAAAT TGGGCAAAGCCGAGGTCGGCGGCGACCTTGTCACCATC AAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACCCTTA ACGAGAACTTCAGCTGAGCAATGGGAGGC	TAQMAN
	>gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome	AY366525.1	13859	14048	190	Plus - Minus - Minus	CCGTACCCGGTTTACCAACTTTATTGTGGACGACCGGGG AGGAGTTCATCGGTGGAAGTCTCCAATAGTGGTAGAAA AATTGGGCAAAGCCGACATCGACGGCAGCCTTGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGACAAGGACTTCGGCTGAGCAATGGGAGGCCTAG	TAQMAN
	>gb|AY395075.1| Porcine reproductive and respiratory syndrome virus isolate SD-03-17 major envelope glycoprotein GP5 mRNA, complete cds	AY395075.1	421	606	186	Plus - Minus - Minus	ACCCGGTTTACCAACTTCATTGTGGACGACCGGGGAGG AGTTCATCGATGGAAGTCCCCAATAGTGGTGGAAAAAT TGGGCAAAGCCGAAGTCGACGGCAGCCTTGTCACCATC AAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACCCTTA ACGAGGACTTCGGCCGAGCAATGGGAGGCCTAG	TAQMAN
	>gb|AY395076.1| Porcine reproductive and respiratory syndrome virus isolate SD-03-15 major envelope glycoprotein GP5 mRNA, complete cds	AY395076.1	421	606	186	Plus - Minus - Minus	ACCCGGTTTACCAACTTCATTGTGGACGACCGGGGAGG AGTTCATCGATGGAAGTCCCCAATAGTGGTGGAAAAAT TGGGCAAAGCCGAAGTCGACGGCAGCCTTGTCACCATC AAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACCCTTA ACGAGGACTTCGGCCGAGCAATGGGAGGCCTAG	TAQMAN
	>gb|AY395077.1| Porcine reproductive and respiratory syndrome virus isolate SD-03-16 major envelope glycoprotein GP5 mRNA, complete cds	AY395077.1	421	606	186	Plus - Minus - Minus	ACCCGGTTTACCAACTTCATTGTGGACGACCGGGGAGG AGTTCATCGATGGAAGTCCCCAATAGTGGTGGAAAAAT TGGGCAAAGCCGAAGTCGACGGCAGCCTTGTCACCATC AAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACCCTTA ACGAGGACTTCGGCCGAGCAATGGGAGGCCTAG	TAQMAN
	>gb|AY395079.1| Porcine reproductive and respiratory syndrome virus isolate SD-01-07 glycoprotein GP2, protein E, glycoprotein GP3, glycoprotein GP4, glycoprotein GP5, membrane protein M, and nucleocapsid protein N genes, complete cds	AY395079.1	2115	2300	186	Plus - Minus - Minus	CCGTACCCGGTTTACCAATTTCGTTGTGGACGACCGGGG GACGGTCCATCGATGGAAGTCTCCAATAGTGGTAGAAA AATTGGGCAAAGCCGAAATCGACGGCAACCTCGTCACC ATCAAACATGTCGTCCTCGAAGGGGTTAAAGCTCAACC CTTGGCGAGGACTTCGGCTGAGCAATGGGAGGC	TAQMAN
sig_candidate_1810391	>emb|X92942.1|PRRSENVNP Porcine reproductive and respiratory syndrome virus envelope protein and nucleoprotein genes	X92942.1	3214	3357	144	Plus - Minus - Minus	CGTGACTTCTACATCCGCCAGTCAGGGTGCAAGCTAATT TGACAGTCAGGTGAATGGCCGCGATTGACGTGTGGCCT CTAAGTCACCTATTCAATTAGGGCGATCACATGGGGGTC AAACTTAATCAGGCAGGAACCATGTGAC	TAQMAN
	>gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome	AY366525.1	14897	15040	144	Plus - Minus - Minus	CGTGACTTCTACATCCGCCAGTCAGGGTGCAAGTTAATT TGACAGTCAGGTGAATGGTCGCGATTGGCGTGTGACCTC TGAGTCACCTATTCAATTAGGGCGATCACATGGGGGTCA TACTTAATCAGGCAGGAACCATGTGAC	TAQMAN
6 total sequence hits	>gb|AY588319.1| PRRSV LV4.2.1, complete genome	AY588319.1	14948	15091	144	Plus - Minus - Minus	CGTGACTTCTACATCCGCCAGTCAGGGTGCAAGTTAATT TGACAGTCAGGTGAATGGCCGCGATTGGCGTGTGGCCT CTGAGTCACCTATTCAATTAGGGCGATCACATGGGGGTC ATACTTAATCAGGCAGGAACCATGTGAC	TAQMAN

>gb|DQ489311.1| Porcine reproductive and respiratory syndrome virus strain SD01-08, complete genome	DQ489311.1	14897	15040	144	Plus - Minus - Minus	CGTGACTTCTACATCCGCCAGTCAGGGTGCAAATTAATT TGACAGTCAGGTGAATGGCCGCGATTGGCGTGTGGCCT CTGAGTCACCTATTCAATTAGGGCGATCACATGGGGGTC ATACTTAATCAGGCAGGAACCATGTGAC	TAQMAN
>gb|L04493.1|PRWPOLGLYM Porcine reproductive and respiratory syndrome virus (PRRSV) RNA polymerase, 3' end; complete ORF3, ORF4, ORF5; glycoprotein gene, membrane protein gene, nucleocapsid protein gene, complete cds's	L04493.1	4766	4909	144	Plus - Minus - Minus	CGTGACTTCTACATCCGCCAGTCAGGGTGCAAGTTAATT TGACAGTCAGGTGAATGGCCGCGATTGGCGTGTGGCCT CTGAGTCACCTATTCAATTAGGGCGATCACATGGGGGTC ATACTTAATCAGGCAGGAACCATGTGAC	TAQMAN
>gb|M96262.2|LEYPOLYENV Lelystad virus, complete genome	M96262.2	14948	15091	144	Plus - Minus - Minus	CGTGACTTCTACATCCGCCAGTCAGGGTGCAAATTAATT TGACAGTCAGGTGAATGGCCGCGATTGGCGTGTGGCCT CTGAGTCACCTATTCAATTAGGGCGATCACATGGGGGTC ATACTTAATCAGGCAGGAACCATGTGAC	TAQMAN

[redacted]

APPENDIX II: Signature Targets

LLNL has developed several bioinformatics tools to analyze candidate signatures. Of these tools, the "Signature Targets" tool allows the user to analyze a triplet oligonucleotide against available databases (internal, and Genbank) to match the signatures against a specific genome. The benefit of this is to generate the complete "signature target" region on the reference genome. It also provides other helpful information such as amplicon size, oligo orientation, start and stop locations on genome, base-pair mismatches and annotations when available. The signature targets have been generated for all "final" signatures reported and are below.

BLUETONGUE VIRUS SIGNATURES

BTV-2 (BTV_1759932)

Genome Title/Description: Bluetongue virus gene for capsid protein VP1, genomic RNA.
Genome GI number: 58745
Signature Title: BTV_2 - rc(IO, R)
Forward: GCACCCTATATGTTTCCAGACCA
Internal: ACAGAAGATGATGATTGGCCCACGAGTTAG
Reverse: CGTGTGGCTGAAGAGTTAGCTG

Amplicon Size: 271
[F Start: 3276 End: 3298] [IO Start: 3323 End: 3352] [R Start: 3525 End: 3546]
Note: Purple characters represent allowed mismatches in the signature

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3276- G C A C C C T A T A T G T T T C C A G A C C A A A A T T T G T C T C C G C A G T T C T A T A T A C A
G A A G A T G A T G A T T G G C C C A C G A G T T A G C T C A C G A G T G C G G A A T T C T T A T G
T T G A T C G A A T T G A T G T G A T A T T A A G A A A G G A T G T C G T A A T G C G A G G T T T T
A T T A C T G C C A A T A C G A T T C T G A A C G T A A T T G A A A A A T T A G G G A C T A A T C A
C T C A G T G G G A G A T C T G G T T A C G G T C T T C A C G C T T A T G A A T A T C G A A A C A C
G T G T G G C T G A A G A G T T A G C T G -3546

BTV_1810199

Genome Title/Description: Bluetongue virus serotype 10 non-structural protein NS3 and non-structural protein NS3A genes, complete cds.
Genome GI number: 3643705
Signature Title: 1810199. (R & F Switched) - rc(F, IO)
Forward: GCGGAGAAGGCTGCATT
Internal: CATCGTACGCGGAAGCGTTTCGT
Reverse: GGTTAAGACAAATTAAGCGACATGTG

Amplicon Size: 78
[F Start: 236 End: 252] [IO Start: 255 End: 277] [R Start: 288 End: 313]
Note: Purple characters represent allowed mismatches in the signature

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236- G C G G A G A A G G C T G C A T T C G C A T C G T A C G C G G A A G C G T T T C G T G A T G A T
G T G A G G T T A A G A C A A A T T A A G C G A C A T G T G -313

BTV_1810200

Genome Title/Description: Bluetongue virus serotype 10 non-structural protein NS3 and non-structural protein NS3A genes, complete cds.
Genome GI number: 3643705
Signature Title: 1810200. (R & F Switched) - rc(F, IO)
Forward: AAAGCTGCATTCGCATCGT
Internal: CGCAGAAGCGTTTCGTGATGATGTG
Reverse: TTGAAAAGTGACCTAGGAGGCTTAA

Amplicon Size: 118
[F Start: 242 End: 260] [IO Start: 262 End: 286] [R Start: 335 End: 359]
Note: Purple characters represent allowed mismatches in the signature

242- A A G G C T G C A T T C G C A T C G T A C G C G G A A G C G T T T C G T G A T G A T G T G A G G
T T A A G A C A A A T T A A G C G A C A T G T G A A T G A G C A A A T T T T A C C A A A G T T G A A
A A G T G A C C T A G G A G G C T T A A -359

BTV_1810201

Genome Title/Description: Bluetongue virus serotype 10 non-structural protein NS3 and non-structural protein NS3A genes, complete cds.
Genome GI number: 3643705
Signature Title: no name provided - rc(R)
Forward: TAATGATGCGGTGAGGATGAGT
Internal: AGTCCCGCTAGATGGTTTCGAATTACCATTA
Reverse: CCTAAGATCAGTAGGTAGAGTGGCG

Amplicon Size: 96
[F Start: 634 End: 655] [IO Start: 673 End: 703] [R Start: 705 End: 729]
Note: Purple characters represent allowed mismatches in the signature

634- T A A T G A T G C G G T G A G G A T G A G T T T T A C G G A A T T T T C A T C A G T C C C G C T
A G A T G G T T T T G A A T T A C C A T T A A C C T A A G G T C G G T A G G T A G A G T G G C G -729

BTV_1810205

Genome Title/Description: Bluetongue virus 2 nonstructural protein NS3/NS3A (S10) gene, complete cds.
Genome GI number: 4959686
Signature Title: 1810205. (R & F Switched) - rc(F, IO)
Forward: GCGGAGAAGGCTGCATTC
Internal: CATCGTACGCGGAAGCGTTTCGT
Reverse: TGAATGAACAAATTCTACCAAAATTGA

Amplicon Size: 103
[F Start: 217 End: 234] [IO Start: 236 End: 258] [R Start: 293 End: 319]
Note: Purple characters represent allowed mismatches in the signature

217- G C G G A G A A G G C T G C A T T C G C A T C G T A C G C G G A A G C G T T T C G T G A T G A T
G T G A G G C T G A G A C A A A T T A A A C G A C A T G T G A A T G A A C A G A T T C T G C C A A

A A T T G A -319

BTV_1810207

Genome Title/Description: Bluetongue virus 2 NS3 (S10) gene, complete cds.
Genome GI number: 21637336
Signature Title: 1810207. - rc(R)
Forward: CAAACACAAAAGGCGGAGAAG
Internal: TCGCATCGTACGCAGAAGCGTTTC
Reverse: ACGTAAGACTAAGACAGATTAAACGCC

Amplicon Size: 85
[F Start: 205 End: 225] [IO Start: 233 End: 256] [R Start: 263 End: 289]
Note: Purple characters represent allowed mismatches in the signature

205- C A A A C A C A A A A A G C G G A G A A G G C T G C A T T C G C A T C G T A C G C G G A A G C
G T T T C G T G A T G A T G T A A G A T T G A G A C A G A T T A A A C G C C -289

BOVINE HERPES VIRUS 1 SIGNATURES

BHV-1 (BVH_94666-68)

Genome Title/Description: Bovine herpesvirus type 1 31-kb DNA (left genome end).
Genome GI number: 995626
Signature Title: BHV_1 - rc(IO, R)
Forward: GTGCCAGCCGCGTAAAAG
Internal: CTCCATGTTAGCGCTCTGGAACCAGGA
Reverse: AAAAGAGCCCGGAGTCGTC

Amplicon Size: 140
[F Start: 17101 End: 17118] [IO Start: 17124 End: 17150] [R Start: 17222 End: 17240]
Note: Purple characters represent allowed mismatches in the signature

17101- G T G C C A G C C G C G T A A A A G C G G C G C T C C A T G T T A G C G C T C T G G A A C C A G G A
G A C G T C G C A G C G C A G G T T G G G C G G G T G G G C G G T T G G C G T C G C G T C C T C G A
G C G T A A G G A C G G A C G T G C G C G A A A A G A G C C C G G A G T C G T C -17240

BHV-3 (BVH_94666-68)

Genome Title/Description: gi|2653291|emb|AJ004801.1|BHV1CGEN Bovine herpesvirus type 1.1 complete genome
Genome GI number: 2653291
Signature Title: BHV_3 - rc(IO, R)
Forward: TGAGGCCTATGTATGGGCAGTT
Internal: CGCGAATCTTATTTAAGTGCACACCGTGTTATTT
Reverse: TTTACTTATGTTTGGCGCGC

Amplicon Size: 186
[F Start: 58190 End: 58211] [IO Start: 58234 End: 58267] [R Start: 58356 End: 58375]
Note: Purple characters represent allowed mismatches in the signature

58190- T G A G G C C T A T G T A T G G G C A G T T C G G G T G C C A A T A A T A A A T T T T G C G C G A A

TCTTATTTAAGTGCACACCGTGTTATTTGCGGCTGTTTGTTTTTCTTGGA
GGCGGGACGTGCGCGCGAGCTCGGCCGGATTAGGGTTCGGCGCCACCCGG
GCACGGCAGGGCGCCCTTTACTTATGTTTGGCGCGC-58375

BOVINE PAPULAR STOMATITIS VIRUS SIGNATURES

BPSV-1 (BPSV_95719-21)

Genome Title/Description: gi|40019124|gb|AY386265.1| Bovine papular stomatitis virus strain BV-AR02, complete genome
Genome GI number: 40019124
Signature Title: BPSV_1 - rc(IO, R)
Forward: GCAGATGCGCTCCTGGTT
Internal: CACGTTCTCCACGTCGGAGTCGG
Reverse: TTGCAGCAGCAGAGGTGC

Amplicon Size: 178
[F Start: 110778 End: 110795] [IO Start: 110910 End: 110932] [R Start: 110938 End: 110955]
Note: Purple characters represent allowed mismatches in the signature

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110778-GCAGATGCGCTCCTGGTTCTGGCAGAACACCGAGTCTTCGATGATCAACA
CTCTCCTGCTCCCGGCCGACCGCATGATGGCCATGGCCCGGATGAGCCTC
TTCTTCGAGCCCCGGATGGACATGGACCGGAGCACGTTCTCCACGTCGGA
GTCGGACACGTTGCAGCAGCAGAGGTGC-110955

BPSV-2 (BPSV_95722-24)

Genome Title/Description: gi|40019124|gb|AY386265.1| Bovine papular stomatitis virus strain BV-AR02, complete genome
Genome GI number: 40019124
Signature Title: BPSV_2 - rc(IO, R)
Forward: GATGGCCGTGCAGCTCTT
Internal: CGGAAGCCCATGAGCCCGTACA
Reverse: AGTTGGCCGTGATCTTGTACG

Amplicon Size: 95
[F Start: 25826 End: 25843] [IO Start: 25875 End: 25896] [R Start: 25900 End: 25920]
Note: Purple characters represent allowed mismatches in the signature

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25826-GATGGCCGTGCAGCTCTTGGCGGAGGCGTACGAGAAGAGCGCGCTGTTCC
GGAAGCCCATGAGCCCGTACACGGAGTTGGCCGTGATCTTGTACG-25920

BPSV-4 (BPSV_95731-33)

Genome Title/Description: gi|40019124|gb|AY386265.1| Bovine papular stomatitis virus strain BV-AR02, complete genome
Genome GI number: 40019124
Signature Title: BPSV_4 - rc(IO, R)
Forward: GCAGCAGTGCACCACGTAGT
Internal: CGCTTGTTGTCCGCCTCGAAGTC
Reverse: AGGATGTACGGGTTCAGCG

Amplicon Size: 167
[F Start: 86710 End: 86729] [IO Start: 86750 End: 86772] [R Start: 86858 End: 86876]
Note: Purple characters represent allowed mismatches in the signature

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86710-GCAGCAGTGCACCACGTAGTACCCGGCGGTGGCGCGCAGACGCTTGTTGT
CCGCCTCGAAGTCGGCCTCCAGGCCCTCGTTGAAGTACTTGTCGAAGATG
ATGGGCAGGAAGGAGAGCTTGGACTCGGTGACCACCTTCCCGAAGTTGAG
GATGTACGGGTTCAGCG-86876

BOVINE VIRAL DIARRHEA DISEASE VIRUS SIGNATURES

BVD-1a mod

Genome Title/Description: Bovine viral diarrhea virus 1 isolate VID_nb_02 N-terminal autoprotease gene, 5'UTR and partial cds.
Genome GI number: 38260461
Signature Title: BVD-1a mod R is a reverse complement -> Probe Strand: minus
Forward: GTAGTCGTCAGTGGTTCG
Internal: CTCGAGATGCCACGTG
Reverse: ATCTCTGCTGTACATGGC

Amplicon Size: 195
[F Start: 69 End: 86] [IO Start: 105 End: 120] [R Start: 246 End: 263]
Note: Purple characters represent allowed mismatches in the signature

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69- GTAGTCGTCAGTGGTTCGACACCTCGGAAAGAAGGTCTCGAGATGCCACG
TGGACGAGGGCATGCCCACAGCACATCTTAACCTGGACAGGGGTCGCCCA
GGTGAAAGCAGTTTAACCAACTGTTATGGACACAGCCTGATAGGGTGCTG
CAGAGGCCCACTGAATTGCTATTAAAAATCTCTGCTGTACATGGC-263

MALIGNANT CATARRHAL FEVER VIRUS SIGNATURES

emcf_94975

Genome Title/Description: gi|10140926|ref|NC_002531.1| Alcelaphine herpesvirus 1, complete genome
Genome GI number: 10140926
Signature Title: emcf_94975. R is a reverse complement -> Probe Strand: plus
Forward: ATGCCAGTCACTGGCTCTCA
Internal: CCAGGGTGCCACCGTGATCAAC
Reverse: CCATTTCAGGATTCTACAACACCC

Amplicon Size: 76
[F Start: 21141 End: 21160] [IO Start: 21170 End: 21191] [R Start: 21193 End: 21216]
Note: Purple characters represent allowed mismatches in the signature

21141- ATGCCAGTCACTGGCTCTCAAGAGGGGTACCAGGGTGCCACCGTGA
TCAACCCCATTTCAGGATTCTACAACACCC-21216

emcf_95059

Genome Title/Description: gi|10140926|ref|NC_002531.1| Alcelaphine herpesvirus 1, complete genome
Genome GI number: 10140926
Signature Title: emcf_95059. R is a reverse complement -> Probe Strand: plus
Forward: GTTCTGGAAACTGACCAAACAGTGT
Internal: TGTATTTCCCTTATGCCTGCCAGAGTGC
Reverse: CAATAAAAGTTACACTCAAGTGCCACT

Amplicon Size: 99
[F Start: 75080 End: 75104] [IO Start: 75123 End: 75150] [R Start: 75152 End: 75178]
Note: Purple characters represent allowed mismatches in the signature

75080- GTTCTGGAAACTGACCAAACAGTGTTCTTATGTGCACTTATTTTGTA
TTTCCCTTATGCCTGCCAGAGTGCTCAATAAAAGTTACACTCAAGTGCCA

C T -75178

emcf_95155

Genome Title/Description: gi|10140926|ref|NC_002531.1| Alcelaphine herpesvirus 1, complete genome
Genome GI number: 10140926
Signature Title: emcf_95155. R is a reverse complement -> Probe Strand: plus
Forward: CCCTGGAAGCTGTCATACAAAA
Internal: TGAGACAACTGCAGCCCTGGACTCTACTG
Reverse: ACCTTGCAAGATATGCCAATGTTT

Amplicon Size: 127
[F Start: 106827 End: 106848] [IO Start: 106900 End: 106928] [R Start: 106930 End: 106953]
Note: Purple characters represent allowed mismatches in the signature

106827- C C C T G G A A G C T G T C A T A C A A A A A G C T T T C C T G C C A G G A G A C C C A G C T G A G G C T C T A A A C A G T A G C C A G T T T T G T G A G A C A A C T G C A G C C C T G G A C T C T A C T G C A C C T T G C A A G A T A T G C C A A T G T T T -106953

emcf_95416

Genome Title/Description: gi|10140926|ref|NC_002531.1| Alcelaphine herpesvirus 1, complete genome
Genome GI number: 10140926
Signature Title: emcf_95416. R is a reverse complement -> Probe Strand: plus
Forward: TGGCCTACTTAAATGCTACTGTATCAA
Internal: CCTGTCATTAGTTTGCTTTCACTTTCCAAGAAGGT
Reverse: GCTATGGGCACTTTGTGTGTTAGTATT

Amplicon Size: 164
[F Start: 3493 End: 3519] [IO Start: 3522 End: 3556] [R Start: 3630 End: 3656]
Note: Purple characters represent allowed mismatches in the signature

3493- T G G C C T A C T T A A A T G C T A C T G T A T C A A A A C C T G T C A T T A G T T T G C T T T C A C T T T C C A A G A A G G T A C T T A A A T T T G A G C A C T G T G G G G G A G A G G G T C A G T G T T T G G G G C T G A T A A C A G A G T T T G T A A T A C A T C C T G C A G C T A T G G G C A C T T T G T G T G T T A G T A T T -3656

emcf_95476

Genome Title/Description: gi|10140926|ref|NC_002531.1| Alcelaphine herpesvirus 1, complete genome
Genome GI number: 10140926
Signature Title: no name provided R is a reverse complement -> Probe Strand: plus
Forward: CAAAACTGGACAGATGTCTTTAGTTTG
Internal: CACAGATTTTACAGACCTCAGTGGTTGACTTTGCTA
Reverse: TGCATTTTAACTCACACTTTTTAACCA

Amplicon Size: 139
[F Start: 40851 End: 40877] [IO Start: 40906 End: 40941] [R Start: 40963 End: 40989]
Note: Purple characters represent allowed mismatches in the signature

40851- C A A A A C T G G A C A G A T G T C T T T A G T T T G A T C A C A T C C C A C A C T G T G G G
A G A A T T G G C A C A G A T T T T A C A G A C C T C A G T G G T T G A C T T T G C T A G G A A G G
G T A G G T G T C G C T C A G T G C A T T T T A A C T C A C A C T T T T T T A A C C A -40989

RINDERPEST VIRUS SIGNATURES

1811628

Genome Title/Description: ref|NC_006296.2|gnl|NCBI_GENOMES|17932|gi|56410431|Rinderpest virus (strain Kabete O), complete genome
Genome GI number: NC_006296.2
Signature Title: no name provided - rc(R)
Forward: CGGTGAAAAGGTTGAGGGAGT
Internal: AGATGCTGACTCTATCCTGGTTCAATCAGGC
Reverse: TCTGGGGAGGAGATGAGGAA

Amplicon Size: 94
[F Start: 2154 End: 2174] [IO Start: 2178 End: 2208] [R Start: 2228 End: 2247]
Note: Purple characters represent allowed mismatches in the signature

2154- C G G T G A A A A G G T T G A G G G A G T C G A A G A T G C T G A C T C T A T C C T G G T T C
A A T C A G G C G C T G A T G A T G G T G T C G A A G T C T G G G G A G G A G A T G A G G A A -2247

1814853

Genome Title/Description: ref|NC_006296.2|gnl|NCBI_GENOMES|17932|gi|56410431|Rinderpest virus (strain Kabete O), complete genome
Genome GI number: NC_006296.2
Signature Title: no name provided - rc(R)
Forward: GGATCGCTGAAATGATCTGTGA
Internal: TACATAGTGGAGGCAGGGTTGGCCAG
Reverse: CAAATGGGTGAACTGGCTCC

Amplicon Size: 187
[F Start: 853 End: 874] [IO Start: 885 End: 910] [R Start: 1020 End: 1039]
Note: Purple characters represent allowed mismatches in the signature

853- G G A T C G C T G A A A T G A T C T G T G A C A T T G A T A C C T A C A T A G T G G A G G C A G
G G T T G G C C A G T T T T A T A C T C A C T A T C A A A T T T G G T A T A G A A A C G A T G T A C
C C A G C A C T G G G C C T G C A T G A A T T C G C C G G A G A G C T C T C C A C A A T C G A G T C
T C T T A T G A A T C T G T A C C A G C A A A T G G G T G A A C T G G C T C C -1039

1814855

Genome Title/Description: ref|NC_006296.2|gnl|NCBI_GENOMES|17932|gi|56410431|Rinderpest virus (strain Kabete O), complete genome
Genome GI number: NC_006296.2
Signature Title: no name provided - rc(IO, R)
Forward: TGCATCTTATGTGACTTTGGTTCA
Internal: TACATCGGATCGACAACAGATGAGAGGACTG
Reverse: GTCAGCTGTGCGGATAGCC

Amplicon Size: 198
[F Start: 12750 End: 12773] [IO Start: 12855 End: 12885] [R Start: 12929 End: 12947]
Note: Purple characters represent allowed mismatches in the signature

12750- T G C A T C T T A T G T G A C T T T G G T T C A G C C A A T T A T G G T T G G T T T T T T G T A C C A T C G A A C T G T C A G T T G G A T G A C A T A G A T A G A G A G A C G T C A G C A C T C A G G G T C C C C T A C A T C G G A T C G A C A A C A G A T G A G A G G A C T G A T A T G A A G C T C G C A T T T G T T A A G T C A C C C A G T C G A A C C C T G C G G T C A G C T G T G C G G A T A G C C - 12947

1814856

Genome Title/Description: ref|NC_006296.2|gnl|NCBI_GENOMES|17932|gi|56410431|Rinderpest virus (strain Kabete O), complete genome
Genome GI number: NC_006296.2
Signature Title: no name provided - rc(IO, R)
Forward: AACTCCTGACCTCATTCCTTGC
Internal: TATTCAAGGTCTTTGTGAATGCACTGAGCCA
Reverse: TGGCATAGTGGGATTATAGAGCC

Amplicon Size: 115
[F Start: 13756 End: 13777] [IO Start: 13795 End: 13825] [R Start: 13848 End: 13870]
Note: Purple characters represent allowed mismatches in the signature

13756- A A C T C C T G A C C T C A T T C C T T G C A A G G A T G A G T A A G A G C G T A T T C A A G G T C T T T G T G A A T G C A C T G A G C C A C C C C A A G A T T T A C A G G A A G T T C T G G C A T A G T G G G A T T A T A G A G C C -13870

1814893

Genome Title/Description: ref|NC_006296.2|gnl|NCBI_GENOMES|17932|gi|56410431|Rinderpest virus (strain Kabete O), complete genome
Genome GI number: NC_006296.2
Signature Title: no name provided - rc(R)
Forward: AATAAACCGAGGATCGCTGAAATGAT
Internal: TGTGACATTGATACCTACATAGTGGAGGCAGG
Reverse: AGAACTCAATCCAGAACAAATTCAG

Amplicon Size: 239
[F Start: 843 End: 868] [IO Start: 870 End: 901] [R Start: 1057 End: 1081]
Note: Purple characters represent allowed mismatches in the signature

843- A A T A A A C C A A G G A T C G C T G A A A T G A T C T G T G A C A T T G A T A C C T A C A T A G T G G A G G C A G G G T T G G C C A G T T T T A T A C T C A C T A T C A A A T T T G G T A T A G A A A C G A T G T A C C C A G C A C T G G G C C T G C A T G A A T T C G C C G G A G A G C T C T C C A C A A T C G A G T C T C T T A T G A A T C T G T A C C A G C A A A T G G G T G A A C T G G C T C C T T A

VESICULAR STOMATITIS VIRUS SIGNATURES

VSV_1798941

Genome Title/Description: ref|NC_001560.1|gnl|NCBI_GENOMES|10405|gi|9627229|Vesicular stomatitis Indiana virus, complete genome
Genome GI number: NC_001560.1
Signature Title: no name provided - rc(IO, R)
Forward: AGAACCAGCGCAGATGACAA
Internal: TATACAGAGTGGGCAGAACACAAATGCCTGA
Reverse: AAAAGCTCATGGATGGGCTG

Amplicon Size: 105
[F Start: 439 End: 458] [IO Start: 485 End: 515] [R Start: 524 End: 543]
Note: Purple characters represent allowed mismatches in the signature

439- A G A A C C A G C G C A G A T G A C A A A T G G T T G C C T T T G T A T C T A C T T G G C T T A T A C A G A G T G G G C A G A A C A C A A A T G C C T G A A T A C A G A A A A A A G C T C A T G G A T G G G C T G -543

VSV_1798942

Genome Title/Description: ref|NC_001560.1|gnl|NCBI_GENOMES|10405|gi|9627229|Vesicular stomatitis Indiana virus, complete genome
Genome GI number: NC_001560.1
Signature Title: no name provided - rc(R)
Forward: AAATGATGCTTCCAGGCCAA
Internal: AAATTGACAAGGCCGATTCATACATGCCTTA
Reverse: AGAGCAAGGAATGCCCGAC

Amplicon Size: 174
[F Start: 842 End: 861] [IO Start: 863 End: 893] [R Start: 997 End: 1015]
Note: Purple characters represent allowed mismatches in the signature

842- A A A T G A T G C T T C C A G G C C A A G A A A T T G A C A A G G C C G A T T C A T A C A T G C C T T A T T T G A T C G A C T T T G G A T T G T C T T C T A A G T C T C C A T A T T C T T C C G T C A A A A A C C C T G C C T T C C A C T T C T G G G G G C A A T T G A C A G C T C T T C T G C T C A G A T C C A C C A G A G C A A G G A A T G C C C G A C -1015

VSV_1798943

Genome Title/Description: ref|NC_001560.1|gnl|NCBI_GENOMES|10405|gi|9627229|Vesicular stomatitis Indiana

virus, complete genome
Genome GI number: NC_001560.1
Signature Title: no name provided - rc(R)
Forward: CGCCACAAGGCAGAGATGT
Internal: CAGAAAACCGACTCCTGATATGATGCAGTATGC
Reverse: GCAAGTATGCTAAGTCAGAATTTGACA

Amplicon Size: 159
[F Start: 1169 End: 1187] [IO Start: 1221 End: 1253] [R Start: 1301 End: 1327]
Note: Purple characters represent allowed mismatches in the signature

1169- C G C C A C A A G G C A G A G A T G T G G T C G A A T G G C T C G G A T G G T T T G A A G A T
C A A A A C A G A A A A C C G A C T C C T G A T A T G A T G C A G T A T G C G A A A A G A G C A G
T C A T G T C A C T G C A A G G C C T A A G A G A G A A G A C A A T T G G C A A G T A T G C T A A G
T C A G A A T T T G A C A -1327

VSV_1798944

Genome Title/Description: ref|NC_001560.1|gnl|NCBI_GENOMES|10405|gi|9627229|Vesicular stomatitis Indiana virus, complete genome
Genome GI number: NC_001560.1
Signature Title: no name provided - rc(R)
Forward: CCTCATGATCATCCCTTTAAAAGTC
Internal: TTCAAGATTTTGGAGATAAATGGCATGAACTTCC
Reverse: TGAATAGGTCAGAGGTGTTGAAACA

Amplicon Size: 191
[F Start: 5981 End: 6005] [IO Start: 6042 End: 6075] [R Start: 6147 End: 6171]
Note: Purple characters represent allowed mismatches in the signature

5981- C C T C A T G A T C A T C C C T T T A A A A G T C A T G T T A A A G A A A A T A C A T G G C C
C A C A G C T G C T C A A G T T C A A G A T T T T G G A G A T A A A T G G C A T G A A C T T C C G C
T G A T T A A A T G T T T T G A A A T A C C C G A C T T A C T A G A C C C A T C G A T A A T A T A C
T C T G A C A A A A G T C A T T C A A T G A A T A G G T C A G A G G T G T T G A A A C A -6171

VSV_1798945

Genome Title/Description: ref|NC_001560.1|gnl|NCBI_GENOMES|10405|gi|9627229|Vesicular stomatitis Indiana virus, complete genome
Genome GI number: NC_001560.1
Signature Title: no name provided - rc(R)
Forward: ACCAGACTTGATGCGTGTTCA
Internal: TCAATTCAACCTCCCAACGAGTTTGTTGG
Reverse: GTCTTGGCACAAGGTGATAATCA

Amplicon Size: 192
[F Start: 6688 End: 6708] [IO Start: 6723 End: 6751] [R Start: 6857 End: 6879]
Note: Purple characters represent allowed mismatches in the signature

6688- A C C A G A C T T G A T G C G T G T T C A C A A C A A C A C A C T G A T C A A T T C A A C C T
C C C A A C G A G T T T G T T G G C A A G G A C A A G A G G G T G G A C T G G A A G G T C T A C G
G C A A A A A G G A T G G A C T A T C C T C A A T C T A C T G G T T A T T C A A A G A G A G G C T A
A A A T C A G A A A C A C T G C T G T C A A A G T C T T G G C A C A A G G T G A T A A T C A -6879

VSV_1798946

Genome Title/Description: gi|9627229|ref|NC_001560.1| Vesicular stomatitis Indiana virus, complete genome
Genome GI number: 9627229
Signature Title: VSV_1798946. rc(IO, R)
Forward: CCGATTTTCCGTGGAGTGAT
Internal: AGAGGGTTAGAGACCAAGAGATGGTCACGAGT
Reverse: TGTCACCAATGACCAAATACCC

Amplicon Size: 81
[F Start: 7070 End: 7089] [IO Start: 7091 End: 7123] [R Start: 7129 End: 7150]
Note: Purple characters represent allowed mismatches in the signature

7070- C C G A T T T T C C G T G G A G T G A T T A G A G G G T T A G A G A C C A A G A G A T G G T C A C G A G T G A C T T G T G T C A C C A A T G A C C A A A T A C C C -7150

VSV_1798947

Genome Title/Description: ref|NC_001560.1|gnl|NCBI_GENOMES|10405|gi|9627229|Vesicular stomatitis Indiana virus, complete genome
Genome GI number: NC_001560.1
Signature Title: no name provided - rc(IO, R)
Forward: CCCAATCAATGCCATGATACA
Internal: GATACCGGGCTTGCACAGTTCTACTTTCAAA
Reverse: TTTGGACCCTTCCATTGGAG

Amplicon Size: 173
[F Start: 7216 End: 7236] [IO Start: 7324 End: 7354] [R Start: 7369 End: 7388]
Note: Purple characters represent allowed mismatches in the signature

7216- C C C A A T C A A T G C C A T G A T A C A G T A C A A T T A T T T T G G G A C A T T T G C T A G A C T C T T G T T G A T G A T G C A T G A T C C T G C T C T T C G T C A A T C A T T G T A T G A A G T T C A A G A T A A G A T A C C G G G C T T G C A C A G T T C T A C T T T C A A A T A C G C C A T G T T G T A T T T G G A C C C T T C C A T T G G A G -7388

VSV_1798948

Genome Title/Description: ref|NC_001560.1|gnl|NCBI_GENOMES|10405|gi|9627229|Vesicular stomatitis Indiana virus, complete genome
Genome GI number: NC_001560.1
Signature Title: no name provided - rc(IO, R)
Forward: TCATGCCGAGGACAGTTCTCTA
Internal: AGAAGTCTGGCTCATTTGAAGAGGCCG
Reverse: CGCAGTGTACGGAGGTTTGA

Amplicon Size: 173
[F Start: 8869 End: 8890] [IO Start: 8990 End: 9016] [R Start: 9022 End: 9041]
Note: Purple characters represent allowed mismatches in the signature

8869- T C A T G C C G A G G A C A G T T C T C T A T T T C C T C T A T C T A T A C A A G G T C G T A T
T A G A G G T C G A G G T T T C T T A A A A G G G T T G C T A G A C G G A T T A A T G A G A G C A A
G T T G C T G C C A A G T A A T A C A C C G G A G A A G T C T G G C T C A T T T G A A G A G G C C G
G C C A A C G C A G T G T A C G G A G G T T T G A -9041

VSV_1798949

Genome Title/Description: ref|NC_001560.1|gnl|NCBI_GENOMES|10405|gi|9627229|Vesicular stomatitis Indiana virus, complete genome
Genome GI number: NC_001560.1
Signature Title: no name provided - rc(R)
Forward: GGCGCTCATTATAAAATTCGGA
Internal: ACATGGAATGGGAATCCATTACAGGGACTTC
Reverse: CTGCTGCATTACTACGAGAAAATGT

Amplicon Size: 116
[F Start: 9671 End: 9692] [IO Start: 9700 End: 9730] [R Start: 9762 End: 9786]
Note: Purple characters represent allowed mismatches in the signature

9671- G G C G C T C A T T A T A A A A T T C G G A G T A T A T T A C A T G G A A T G G G A A T C C A
T T A C A G G G A C T T C T T G A G T T G T G G A G A C G G C T C C G G A G G G A T G A C T G C T G
C A T T A C T A C G A G A A A A T G T -9786

VSV_1811405

Genome Title/Description: ref|NC_001560.1|gnl|NCBI_GENOMES|10405|gi|9627229|Vesicular stomatitis Indiana virus, complete genome
Genome GI number: NC_001560.1
Signature Title: no name provided - rc(R)
Forward: AAGAGATGGTCACGAGTGAC
Internal: TGTGTCACCAATGACCAAATACCCA
Reverse: GCTCGGTTTCCACAAATGCTC

Amplicon Size: 85
[F Start: 7106 End: 7125] [IO Start: 7127 End: 7151] [R Start: 7170 End: 7190]
Note: Purple characters represent allowed mismatches in the signature

7106- A A G A G A T G G T C A C G A G T G A C T T G T G T C A C C A A T G A C C A A A T A C C C A C
T T G T G C T A A T A T A A T G A G C T C A G T T T C C A C A A A T G C T C -7190

VSV_1811406

Genome Title/Description: gi|9627229|ref|NC_001560.1| Vesicular stomatitis Indiana virus, complete genome
Genome GI number: 9627229
Signature Title: no name provided R is a reverse complement -> Probe Strand: plus
Forward: GCTTGCACAGTTCTACTT
Internal: TGTTGTATTTGGACCCTTCCATTGGAGG
Reverse: GTGTCAGGCATGTCTTTGTC

Amplicon Size: 79
[F Start: 7332 End: 7349] [IO Start: 7362 End: 7389] [R Start: 7391 End: 7410]
Note: Purple characters represent allowed mismatches in the signature

7332- G C T T G C A C A G T T C T A C T T T C A A A T A C G C C A T G T T G T A T T T G G A C C C T T C C A T T G G A G G A G T G T C G G G C A T G T C T T T G T C -7410

VSV_1811407

Genome Title/Description: gi|9627229|ref|NC_001560.1| Vesicular stomatitis Indiana virus, complete genome
Genome GI number: 9627229
Signature Title: no name provided R is a reverse complement -> Probe Strand: plus
Forward: CGGAGGATTGACGACTAATGC
Internal: CCGCCACAAGGCAGAGATGTGGT
Reverse: GAATGGCTCGGATGGTTTGA

Amplicon Size: 66
[F Start: 1146 End: 1166] [IO Start: 1168 End: 1190] [R Start: 1192 End: 1211]
Note: Purple characters represent allowed mismatches in the signature

1146- C G G A G G A T T G A C G A C T A A T G C A C C G C C A C A A G G C A G A G A T G T G G T C G A A T G G C T C G G A T G G T T T G A -1211

VSV_1811408

Genome Title/Description: Vesicular stomatitis virus nucleocapsid protein (N) mRNA, complete cds.
Genome GI number: 37528739
Signature Title: no name provided - rc(R)
Forward: CTCACAACATGGGTCCTGAA
Internal: AGGGAAGTYGCAGACGARCTATGCC
Reverse: ATGATGTATCCAGGTCAAGAA

Amplicon Size: 69
[F Start: 733 End: 752] [IO Start: 754 End: 779] [R Start: 780 End: 801]
Note: Purple characters represent allowed mismatches in the signature

733- C T C A C A A C A T G G G T C C T G A A T A G G G A A G T T G C A G A C G A G C T A T G C C A G A T G A T G T A T C C G G G T C A A G A A -801

VSV_1811409

Genome Title/Description: Vesicular stomatitis virus nucleocapsid protein (N) mRNA, complete cds.
Genome GI number: 37528739

Signature Title: no name provided - rc(R)
Forward: CTCACAACATGGGTCCTGAA
Internal: AGGGAAGTCGCAGATGAGCTGTGCC
Reverse: ATGATGTATCCGGGGCAAGAA

Amplicon Size: 69
[F Start: 733 End: 752] [IO Start: 754 End: 779] [R Start: 780 End: 801]
Note: Purple characters represent allowed mismatches in the signature

733- C T C A C A A C A T G G G T C C T G A A T A G G G A A G T T G C A G A C G A G C T A T G C C A G A T G A T G T A T C C G G G G T C A A G A A -801

FOOT-AND-MOUTH DISEASE VIRUS SIGNATURES

FMDV.TC

Genome Title/Description: Foot-and-mouth disease virus Asia 1 isolate Lebanon/88 RNA polymerase (3D) gene, partial cds.
Genome GI number: 22770791
Signature Title: FMDV.TC - rc(IO, R)
Forward: ACTGGGTTTTACAAACCTGTGA
Internal: TCCTTTGCACGCCGTGGGAC
Reverse: TCCGTGGCAGGACTCGC

Amplicon Size: 107
[F Start: 182 End: 203] [IO Start: 233 End: 252] [R Start: 272 End: 288]
Note: Purple characters represent allowed mismatches in the signature

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182- A C T G G G T T T T A C A A A C C T G T G A T G G C C T C G A A G A C C C T C G A G G C C A T C C T C T C C T T T G C A C G C C G T G G G A C C A T A C A G G A G A A G T T G A T C T C C G T G G C A G G A C T C G C -288

FMDV-Pir

***Could not locate FMDV.Pir using this particular informatics tool due to high level of degeneracies.

SWINE VESICULAR DISEASE VIRUS SIGNATURES

SVD-1 (SVD_1727049)

Genome Title/Description: gi|37993797|gb|AY429470.1| Swine vesicular disease virus strain HKprime70, complete genome
Genome GI number: 37993797
Signature Title: SVD_1 - rc(R)
Forward: CAGGATAATTTCTTCCAAGGGC
Internal: CGTCACAAGTTGTACCATCAGACACAATGCA
Reverse: GGAAGCTCGAAATGTTCACGT

Amplicon Size: 349
[F Start: 2430 End: 2451] [IO Start: 2563 End: 2593] [R Start: 2758 End: 2778]
Note: Purple characters represent allowed mismatches in the signature

2430- C A G G A T A A T T T C T T C C A A G G G C C C C C A G G A G A G G T G A T G G A A A G A G C C A T
T G C C C G C G T C G C T G A T A C T A T T G G G A G C G G A C C A G T T A A C T C G G A A T C C A
T T C C A G C C C T A A C C G C C G C G G A G A C A G G G C A C A C G T C A C A A G T T G T A C C A
T C A G A C A C A A T G C A A A C T A G G C A C G T G A A G A A T T A T C A T T C A A G A T C A G A
G T C G A C A G T G G A G A A C T T C C T G T G C A G A T C T G C A T G C G T C T T C T A C A C C A
C A T A T A A G A A C C A T G A C T C T G A T G G C G A T A A C T T C G C C T A C T G G G T G A T C
A A C A C A C G G C A A G T T G C T C A A C T G C G T C G G A A G C T C G A A A T G T T C A C G T -2778

SVD-2 (SVD_1727050)

Genome Title/Description: gi|37993797|gb|AY429470.1| Swine vesicular disease virus strain HKprime70, complete genome
Genome GI number: 37993797
Signature Title: SVD_2 - rc(IO, R)
Forward: GACTTGTTGTGGCTGGAGGA
Internal: GTGAGAAATCACGATGACCTCATTACGGTCA
Reverse: CTACCTCACCATGGCGCTG

Amplicon Size: 281
[F Start: 3711 End: 3730] [IO Start: 3915 End: 3945] [R Start: 3973 End: 3991]
Note: Purple characters represent allowed mismatches in the signature

3711- G A C T T G T T G T G G C T G G A G G A C G A T G C C A T G G A G C A A G G A G T T A G G G A C T A
T G T G G A A C A A C T C G G C A A T G C C T T C G G C T C A G G A T T C A C C A A T C A G A T T T
G C G A A C A G G T T A C C C T T C T A A A A G A G T C G T T A A T T G G A C A G G A T T C T A T C
C T T G A G A A G T C T C T C A A G G C C C T C G T C A A G G T A G T A T C A G C A C T C G T G A T
C G T G G T G A G A A A T C A C G A T G A C C T C A T T A C G G T C A C C G C C A C A C T G G C G T
T A A T A G G A T G T A C T A C C T C A C C A T G G C G C T G -3991

SVD-3 (SVD_1727051)

Genome Title/Description: gi|37993797|gb|AY429470.1| Swine vesicular disease virus strain HKprime70, complete genome
Genome GI number: 37993797
Signature Title: SVD_3 - rc(IO, R)
Forward: GACAAAGTGGCCAAGGGAAA
Internal: TAGCGTTTGACTATTCAGGCTATGACGCCAG
Reverse: AGCCCAGTGTGGTTTACGTG

Amplicon Size: 248
[F Start: 6408 End: 6427] [IO Start: 6601 End: 6631] [R Start: 6636 End: 6655]
Note: Purple characters represent allowed mismatches in the signature

6408- G A C A A A G T G G C C A A G G G A A A G T C C A G G C T C A T C G A G G C T T C T A G C C T C A A
C G A C T C A G T A G C A A T G A G G C A G A C A T T T G G A A A C C T A T A C A A G A C T T T C C
A C C T C A A C C C G G G C A T C G T T A C G G G T A G C G C C G T T G G G T G T G A C C C A G A T
G T T T T T T G G A G C A A G A T C C C C G T C A T G C T C G A T G G A C A T C T C A T A G C G T T
T G A C T A T T C A G G C T A T G A C G C C A G T C T C A G C C C A G T G T G G T T T A C G T G -6655

VESICULAR EXANTHEMAOF SWINE VIRUS SIGNATURES

VESV-1 (VESV_95653)

Genome Title/Description: gi|10141008|gb|U76874.2|VEU76874 Vesicular exanthema of swine virus stain A48, complete genome
Genome GI number: 10141008
Signature Title: VESV_1 - rc(R)
Forward: GCCTTCTCCCTTCCCAAAA
Internal: CCAAATTGCACATCTAAGGTTATCAACGATGATG
Reverse: ACTGACGGAACCATTCCTTCA

Amplicon Size: 153
[F Start: 404 End: 422] [IO Start: 497 End: 530] [R Start: 536 End: 556]
Note: Purple characters represent allowed mismatches in the signature

404- GCCTTCTCCCTTCCCAAAACGGACGGACCCACCGGAAACGAACCCGAATT
CATCGCTGAGGCTTGCCCTAGCTGCGCTCTTTACGACACGTGTCCAAATT
GCACATCTAAGGTTATCAACGATGATGGCTCAACTGACGGAACCATTCCT
TCA-556

VESV-3 (VESV_95680)

Genome Title/Description: gi|10141008|gb|U76874.2|VEU76874 Vesicular exanthema of swine virus stain A48, complete genome
Genome GI number: 10141008
Signature Title: VESV_3 - rc(R)
Forward: GGGAATGAGGTGTGCATCATT
Internal: CGACTCATCTGACAAGGTTGATTATGCCAATTT
Reverse: GTCAAGCCAACATCAAGACGTG

Amplicon Size: 199
[F Start: 1898 End: 1918] [IO Start: 1927 End: 1959] [R Start: 2075 End: 2096]
Note: Purple characters represent allowed mismatches in the signature

1898- GGGAATGAGGTGTGCATCATTGATGAATTCGACTCATCTGACAAGGTTGA
TTATGCCAATTTTGTAGTCAACATGGTTAACACCAACCCCATGGTCTTAA
ATTGTGATCTAATTGAAAACAAAGGCAAGACATTCACCTCAAAATACGTC
ATCATGACGTCCAACACGGAAACACCAGTCAAGCCAACATCAAGACGTG-2096

VESV-4 (VESV_95686)

Genome Title/Description: gi|10141008|gb|U76874.2|VEU76874 Vesicular exanthema of swine virus stain A48, complete genome
Genome GI number: 10141008
Signature Title: VESV_4 - rc(R)
Forward: GGTCGCTCTCACTGATGATGAGTA
Internal: CTTCCTCCAACTCAGGCACCGAGC
Reverse: GCAATGGGTGCTGATAACACC

Amplicon Size: 124
[F Start: 3274 End: 3297] [IO Start: 3352 End: 3375] [R Start: 3377 End: 3397]
Note: Purple characters represent allowed mismatches in the signature

3274- GGTCGCTCTCACTGATGATGAGTACAATGATTGGAAACAGTCCAAAGCTG
AAAAAAACCTCGACCTCACGGTCAAGGACTTCCTCCAACTCAGGCACCGA
GCTGCAATGGGTGCTGATAACACC-3397

VESV-5 (VESV_95692)

Genome Title/Description: gi|10141008|gb|U76874.2|VEU76874 Vesicular exanthema of swine virus stain A48, complete genome
Genome GI number: 10141008
Signature Title: VESV_5 - rc(R)
Forward: ACCACCTCTGGAAACATCTATGG
Internal: TGGTGACAAATGCCCGTCCCG
Reverse: ATTCGTGACACGTGCACAAA

Amplicon Size: 200
[F Start: 3869 End: 3891] [IO Start: 3991 End: 4011] [R Start: 4049 End: 4068]
Note: Purple characters represent allowed mismatches in the signature

3869- ACCACCTCTGGAAACATCTATGGAGCCTGCGGCTCATCGTGTTCACTGAC
GAGACAGGGTGACTGCGGTCTCCCCTACGTCGACGATCACGGTGTTGTCG
TTGGACTCCATGCTGGGTCTGGTGGTGACAAATGCCCGTCCCGAAAACTC
ATTGTTCCCTACGTCAAGGTGGATATGAGAATTCGTGACACGTGCACAAA-4068

PRRS_1807661

Genome Title/Description: gi|7650192|gb|AF184212.1|AF184212 Porcine reproductive and respiratory syndrome virus strain SP, complete genome
Genome GI number: 7650192
Signature Title: PRRS_1807661. R is a reverse complement -> Probe Strand: minus
Forward: CCAGGACATCAGCTGCCTTA
Internal: TCTTGCCTTTTCTATGCTTCTGAGATGAGTGAAA
Reverse: CCAGCTACGTCCAACATGTCA

Amplicon Size: 275
[F Start: 13490 End: 13509] [IO Start: 13650 End: 13683] [R Start: 13744 End: 13764]
Note: Purple characters represent allowed mismatches in the signature

13490- C C A G G A C A T C A G C T G C C T T A G G C A T G G C G A C C C G T C C T C T G C G G C G A
T T C G C A A A A G C T C T C A G T G C C G C A C G G C G A T A G G G A C A C C C G T G T A T A T C
A C C A T T A C A G C C A A T G T G A C A G A T G A G A A T T A T T T A C A C T C C T C T G A T C T
C C T C A T G C T T T C T T C T T G C C T T T T C T A T G C T T C T G A G A T G A G T G A A A A G G G
A T T T A A G G T G G T A T T T G G C A A T G T G T C A G G C A T C G T G G C T G T G T G T G T T A
A T T T T A C C A G C T A C G T C C A A C A T G T C A -13764

PRRS_1807662

Genome Title/Description: gi|7650192|gb|AF184212.1|AF184212 Porcine reproductive and respiratory syndrome virus strain SP, complete genome
Genome GI number: 7650192
Signature Title: PRRS_1807662. R is a reverse complement -> Probe Strand: minus
Forward: GGTCGCGCTCACTATGGG
Internal: AAATGATAACCACGCATTTGTCGTCCGG
Reverse: GTGTTGGGTGGCAGAAAAGC

Amplicon Size: 273
[F Start: 14693 End: 14710] [IO Start: 14864 End: 14891] [R Start: 14946 End: 14965]
Note: Purple characters represent allowed mismatches in the signature

14693- G G T C G C G C T C A C T A T G G G A G C A G T A G T T G C A C T C C T T T G G G G G G T G T
A C T C A G C C A T A G A A A C T T G G A G G T T C A T C A C C T C T A G A T G C C G T T T G T G C
T T G T T A G G C C G C A G G T A C A T T C T G G C C C C T G C C C A C C A C G T T G A A A G T G C
C G C A G G C T T T C A T C C G A T T A C G G C A A A T G A T A A C C A C G C A T T T G T C G T C C
G G C G T C C C G G C T C C A C T A C G G T T A A C G G C A C A T T G G T G C C C G G G T T G A A G
A G C C T C G T G T T G G G T G G C A G A A A A G C -14965

PRRS_1807703

Genome Title/Description: gi|12240324|gb|AF331831.1|AF331831 Porcine reproductive and respiratory syndrome virus BJ-4, complete genome
Genome GI number: 12240324
Signature Title: PRRS_1807703. R is a reverse complement -> Probe Strand: minus

Forward: CTAACCCGTTTGCCGTCC
Internal: GCTTGCAATTGCCAGCTATGTTTGGGTA
Reverse: TGTGTTTCCTTGCTGGTTGC

Amplicon Size: 273
[F Start: 5043 End: 5060] [IO Start: 5248 End: 5275] [R Start: 5296 End: 5315]
Note: Purple characters represent allowed mismatches in the signature

5043- C T A A C C C G T T T G C C G T C C C T G G C T A C G G A C C T G G C T C T C T C T G C A C G T
C C A G A T T G T G C A T T T C C C A A C A C G G C C T T A C C C T G C C C T T G A C A G C A C T T
G T G G C G G G A T T C G G T A T T C A A G A A A T T G C C T T G G T C G T T T T G A T T T T T G T T
T C C A T C G G A G G C A T G G C T C A T A G G T T G A G C T G T A A G G C T G A C A T G C T G T G
T G T T T T G C T T G C A A T T G C C A G C T A T G T T T G G G T A C C T C T T A C C T G G C T G C T
T T G T G T G T T T C C T T G C T G G T T G C -5315

PRRS_1807706

Genome Title/Description: gi|12240324|gb|AF331831.1|AF331831 Porcine reproductive and respiratory syndrome virus BJ-4, complete genome
Genome GI number: 12240324
Signature Title: PRRS_1807706. R is a reverse complement -> Probe Strand: minus
Forward: ATTGGTTTGCTCCGCGATAC
Internal: GAAATGGTGTCGCGTCGAATGTACCG
Reverse: TTGGATGTGGTGGCTCATTT

Amplicon Size: 286
[F Start: 12228 End: 12247] [IO Start: 12395 End: 12420] [R Start: 12494 End: 12513]
Note: Purple characters represent allowed mismatches in the signature

12228- A T T G G T T T G C T C C G C G A T A C T C C G T A C G C G C C C T G C C A T T C A C T C T G
A G C A A T T A C A G A A G A T C T T A T G A G G C C T T T C T T T C C C A G T G C C A A G T G G A
C A T T C C C A C C T G G G G A A C T A A A C A T C C T T T G G G G A T G C T T T G G C A C C A T A
A G G T G T C A A C C C T G A T T G A T G A A A T G G T G T C G C G T C G A A T G T A C C G C A T C
A T G G A A A A A T C A G G G C A G G C T G C C T G G A A A C A G G T G G T G A G C G A G G C T A
C G C T G T C T C G C A T T A G T A G T T T G G A T G T G G T G G C T C A T T T -12513

PRRS_1807709

Genome Title/Description: gi|12240324|gb|AF331831.1|AF331831 Porcine reproductive and respiratory syndrome virus BJ-4, complete genome
Genome GI number: 12240324
Signature Title: no name provided R is a reverse complement -> Probe Strand: minus
Forward: GAGCGGCAATTGTGTCTGTC
Internal: GAGTTTAGTTTGCCTACGCATCATACTGTGCG
Reverse: GTCACAGCATCACCCTCAGC

Amplicon Size: 158
[F Start: 15097 End: 15116] [IO Start: 15193 End: 15224] [R Start: 15235 End: 15254]
Note: Purple characters represent allowed mismatches in the signature

15097- G A G C G G C A A T T G T G T C T G T C G T C A A T C C A G A C C G C C T T T A A T C A A G G
C G C T G G G A C T T G C A C C C T G T C A G A T T C A G G G A G G A T A A G T T A C A C T G T G G
A G T T T A G T T T G C C T A C G C A T C A T A C T G T G C G C C T G A T C C G C G T C A C A G C A
T C A C C C T C A G C -15254

PRRS_1810342

Genome Title/Description: gi|38146324|gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome
Genome GI number: 38146324
Signature Title: no name provided R is a reverse complement -> Probe Strand: plus
Forward: CCAAGTCTTTTGCACACGGT
Internal: CTTCTCTCTCCAGAGCTTCAGGACACTGACC
Reverse: AATGTACTCCATCCGGGTGC

Amplicon Size: 163
[F Start: 284 End: 303] [IO Start: 321 End: 351] [R Start: 427 End: 446]
Note: Purple characters represent allowed mismatches in the signature

284- C C A A G T C T T T T G C A C A C G G T G T G T C A G T G C G C G G G C T C T T C T C T C T C C
A G A G C T T C A G G A C A C T G A C C T C G G T G C G G T T G G A T T G T T T T A C A G G C C T A
G G G A T A A G C T A C A C T G G A A A G T C C C T A T C G G C A T C C C C C A G G C G G A A T G T
A C T C C A T C C G G G T G C -446

PRRS_1810344

Genome Title/Description: gi|38146324|gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome
Genome GI number: 38146324
Signature Title: no name provided R is a reverse complement -> Probe Strand: plus
Forward: ACGTCTCATTCTTGCGGTCA
Internal: ACGTGTTTGATGGCAAGTGCTGGCTC
Reverse: GCCTTCGGTTACCAAACCAA

Amplicon Size: 140
[F Start: 993 End: 1012] [IO Start: 1030 End: 1055] [R Start: 1113 End: 1132]
Note: Purple characters represent allowed mismatches in the signature

993- A C G T C T C A T T C T T G C G G T C A T C T C G T C C G A A A C T C C A A C G T G T T T G A T
G G C A A G T G C T G G C T C A C C T G C T T T T T G G G C C A G T C G G T C G A A G T G C G C T G
C C A T G A A G A A C A T C T A G C C A A C G C C T T C G G T T A C C A A A C C A A -1132

PRRS_1810346

Genome Title/Description: gi|38146324|gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain

EuroPRRSV, complete genome
Genome GI number: 38146324
Signature Title: no name provided R is a reverse complement -> Probe Strand: plus
Forward: TGACTTCACGTCCCCTCTGA
Internal: CAGTACAACAGACCAGAGGATGATTGGGCTT
Reverse: CTGCTACCGTGGTTCGGAAT

Amplicon Size: 115
[F Start: 1553 End: 1572] [IO Start: 1575 End: 1605] [R Start: 1648 End: 1667]
Note: Purple characters represent allowed mismatches in the signature

1553- T G A C T T C A C G T C C C C T C T G A C T C A G T A C A A C A G A C C A G A G G A T G A T T G G G C T T C A G A T T A T G A T C T T G C T C A G G C G A T T C A A T G T C T A C A A C T A C C T G C T A C C G T G G T T C G G A A T -1667

PRRS_1810347

Genome Title/Description: gi|38146324|gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome
Genome GI number: 38146324
Signature Title: no name provided R is a reverse complement -> Probe Strand: minus
Forward: TCCTCGCTCCCTTTCTCGT
Internal: CGTCTGCTTACAGACTACCCTCCGATTGTGT
Reverse: GCTCTGGTATTGCTGACTTTCTTG

Amplicon Size: 164
[F Start: 1745 End: 1763] [IO Start: 1852 End: 1882] [R Start: 1885 End: 1908]
Note: Purple characters represent allowed mismatches in the signature

1745- T C C T C G C T C C C T T T C T C G T G A A T G T G T G G T C G G C G T T T G T T C T G A A G G C T G C G T C G C G C C G C C T T A C C C A G C G G A T G G G C T T C C T A A G C G T G C A C T C G A G G C C T T G G C G T C T G C T T A C A G A C T A C C C T C C G A T T G T G T T T G C T C T G G T A T T G C T G A C T T T C T T G -1908

PRRS_1810348

Genome Title/Description: gi|38146324|gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome
Genome GI number: 38146324
Signature Title: no name provided R is a reverse complement -> Probe Strand: plus
Forward: ATCCTTTCGAATTTGCCGAA
Internal: CGTTTCTCCGCACAAGCCTTAATTGACC
Reverse: TGTGGGATAGGGTGGACATG

Amplicon Size: 200
[F Start: 2935 End: 2954] [IO Start: 2967 End: 2994] [R Start: 3115 End: 3134]
Note: Purple characters represent allowed mismatches in the signature

2935- A T C C T T T C G A A T T T G C C G A A C T C A A G C G C C C G C G T T T C T C C G C A C A A G
C C T T A A T T G A C C G A G G C G G T C C G C T T G C C G A T G T C C A T G C G A A A A T A A A G
A A C C G G G G T G T A T G A A C G G T G C C T C C A A G C T T G T G A G C C C G G T A G T C G T G C
A A C C C C A G C C A C C A A G G A G T G G C T C G A C A A G A T G T G G G A T A G G G T G G A C
A T G -3134

PRRS_1810351

Genome Title/Description: gi|38146324|gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome
Genome GI number: 38146324
Signature Title: PRRS_1810351. R is a reverse complement -> Probe Strand: plus
Forward: TTCTTGTGACCACGATTCGC
Internal: TGTCATGCTGAGCTTTTGGCTCTTGAGC
Reverse: GGCAAGTTACTCGGTGGGTC

Amplicon Size: 141
[F Start: 3773 End: 3792] [IO Start: 3798 End: 3825] [R Start: 3894 End: 3913]
Note: Purple characters represent allowed mismatches in the signature

3773- T T C T T G T G A C C A C G A T T C G C C G G A A T G T C A T G C T G A G C T T T T G G C T C T
T G A G C A G C G C C A A C T T T G G G A A C C T G T G C G C G G C C T T G T G G T C G G C C C C T
C G G G C C T C T T A T G T G T C A T T C T T G G C A A G T T A C T C G G T G G G T C -3913

PRRS_1810355

Genome Title/Description: gi|38146324|gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome
Genome GI number: 38146324
Signature Title: no name provided R is a reverse complement -> Probe Strand: plus
Forward: GAAGGCACTTATATGGCCGC
Internal: ACTTTAATCTTCACCCCGTCTGCAGTTGGAT
Reverse: AACCCTGCCTTAACACCGTG

Amplicon Size: 129
[F Start: 5160 End: 5179] [IO Start: 5208 End: 5238] [R Start: 5269 End: 5288]
Note: Purple characters represent allowed mismatches in the signature

5160- G A A G G C A C T T A T A T G G C C G C C G T C C G G A G A G C T G C T T T A A C T G G G C G
A A C T T T A A T C T T C A C C C C G T C T G C A G T T G G A T C C C T T C T C G A A G G T G C C T T
C A G G A C T C A A A A A C C C T G C C T T A A C A C C G T G -5288

PRRS_1810360

Genome Title/Description: gi|38146324|gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome
Genome GI number: 38146324
Signature Title: no name provided R is a reverse complement -> Probe Strand: plus

Forward: GCGGCTCCAAATTCAGTGTT
Internal: ATCCCACTCCAGACACCAACCCCTCTTT
Reverse: GAGAATGGTCCGCGTCATC

Amplicon Size: 111
[F Start: 6769 End: 6788] [IO Start: 6831 End: 6858] [R Start: 6861 End: 6879]
Note: Purple characters represent allowed mismatches in the signature

6769- G C G G C T C C A A A T T C A G T G T T T G C A C T G T T G T G T C C A A C A C A C C C G T G G
A T G C C T T A A C C G G C A T C C C A C T C C A G A C A C C A A C C C C T C T T T T T G A G A A T
G G T C C G C G T C A T C -6879

PRRS_1810362

Genome Title/Description: gi|38146324|gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome
Genome GI number: 38146324
Signature Title: no name provided R is a reverse complement -> Probe Strand: minus
Forward: CACGCTGTTGTGGCAAACTT
Internal: GTCATCTTGATGAGACCTCACCCACCGT
Reverse: GACGTTCTTCTGAAACCCGG

Amplicon Size: 86
[F Start: 7517 End: 7536] [IO Start: 7547 End: 7574] [R Start: 7583 End: 7602]
Note: Purple characters represent allowed mismatches in the signature

7517- C A C G C T G T T G T G G C A A A C T T A T G T T C G G G T G T C A T C T T G A T G A G A C C T
C A C C C A C C G T C C C T T G T T G A C G T T C T T C T G A A A C C C G G -7602

PRRS_1810365

Genome Title/Description: gi|38146324|gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome
Genome GI number: 38146324
Signature Title: no name provided R is a reverse complement -> Probe Strand: minus
Forward: TTGGCCATATTGGTAAGGCG
Internal: CCAACAAAGGACGTTCAGAGCATACCTGAAA
Reverse: CAAGGAGAATTGGCAAACTGTG

Amplicon Size: 167
[F Start: 8154 End: 8173] [IO Start: 8243 End: 8273] [R Start: 8299 End: 8320]
Note: Purple characters represent allowed mismatches in the signature

8154- T T G G C C A T A T T G G T A A G G C G C C G C C A T T G T T C C T T C C A T C A A C C T A T C
C C G C C A A A A A C T C T A T G G C A G G G A T C A A T G G C C A G A G G T T T C C A A C A A A G
G A C G T T C A G A G C A T A C C T G A A A T T G A T G A A A T G T G T G C C C G C G C C G T C A A

G G A G A A T T G G C A A A C T G T G -8320

PRRS_1810367

Genome Title/Description: gi|38146324|gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome
Genome GI number: 38146324
Signature Title: no name provided R is a reverse complement -> Probe Strand: minus
Forward: CCCGCCATTGTAAGATGGTT
Internal: ACTTGCCTAGCTATGTGCTTAATTGCTGCCA
Reverse: CAACACAGGATGGTGCCTTC

Amplicon Size: 123
[F Start: 8558 End: 8577] [IO Start: 8619 End: 8649] [R Start: 8661 End: 8680]
Note: Purple characters represent allowed mismatches in the signature

8558- C C C G C C A T T G T A A G A T G G T T C G T C G C C A A C C T C C T G T A T G A A C T T G C A
G G A T G T G A A G A G T A C T T G C C T A G C T A T G T G C T T A A T T G C T G C C A T G A C C T
T G T G G C A A C A C A G G A T G G T G C C T T C -8680

PRRS_1810368

Genome Title/Description: gi|38146324|gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome
Genome GI number: 38146324
Signature Title: PRRS_1810368. R is a reverse complement -> Probe Strand: plus
Forward: CGTCACCAGTGTGTCCAACA
Internal: TGTCGGCCTTGAAAATGGGTCATGAAAT
Reverse: TTGTCTTGTACGCTGAAAGACCC

Amplicon Size: 190
[F Start: 8713 End: 8732] [IO Start: 8772 End: 8799] [R Start: 8880 End: 8902]
Note: Purple characters represent allowed mismatches in the signature

8713- C G T C A C C A G T G T G T C C A A C A C C G T A T A T T C A C T G G T G A T T T A T G C C C A
G C A C A T G G T G T T G T C G G C C T T G A A A A T G G G T C A T G A A A T C G G T C T C A A G T
T C C T C G A G G A A C A G C T C A A A T T T G A G G A C C T C C T C G A A A T T C A G C C T A T G
C T G G T A T A C T C T G A T G A C C T T G T C T T G T A C G C T G A A A G A C C C -8902

PRRS_1810369

Genome Title/Description: gi|38146324|gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome
Genome GI number: 38146324
Signature Title: no name provided R is a reverse complement -> Probe Strand: plus
Forward: GGTTTCAGAACGGACCCAAA

Internal: TCATAACTGATAAACCCAGCTTCCTCGGCTG
Reverse: GTATTATGCGTCTGCTGCCG

Amplicon Size: 169
[F Start: 8951 End: 8970] [IO Start: 8979 End: 9009] [R Start: 9100 End: 9119]
Note: Purple characters represent allowed mismatches in the signature

8951- G G T T T C A G A A C G G A C C C A A A G A A A A C T G T C A T A A C T G A T A A A C C C A G
C T T C C T C G G C T G C A G A A T T G A G G C A G G G C G A C A G C T G G T T C C C A A T C G C G
A C C G C A T C C T G G C T G C T C T C G C A T A C C A C A T G A A G G C G C A G A A C G C C T C A
G A G T A T T A T G C G T C T G C T G C C G -9119

PRRS_1810374

Genome Title/Description: gi|38146324|gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome
Genome GI number: 38146324
Signature Title: PRRS_1810374. - rc(R)
Forward: TTGCATCTACCATCGCCAAA
Internal: CAAGACACGGGTTGTTCATTTATGACCCTCA
Reverse: ACCTTGTGTTCAGCCGTGG

Amplicon Size: 161
[F Start: 10307 End: 10326] [IO Start: 10368 End: 10398] [R Start: 10449 End: 10467]
Note: Purple characters represent allowed mismatches in the signature

10307- T T G C A T C T A C C A T C G C C A A A A T C T C T A A A C A A A T C C C G A G C A C T T G T
G G C C A T C A C T C G G G C A A G A C A C G G G T T G T T C A T T T A T G A C C C T C A T A A T C
A G C T T C A G G A G T T T T T C A A C C T A A C C C C T G A G C G T A C T G A T T G C A A C C T T
G T G T T C A G C C G T G G -10467

PRRS_1810375

Genome Title/Description: gi|38146324|gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome
Genome GI number: 38146324
Signature Title: no name provided R is a reverse complement -> Probe Strand: plus
Forward: TGCGGATAATGCAGTCACAA
Internal: TCAGACCCGAGGTGCAAGTCTCTCTTAGC
Reverse: TATGCCACTACCGCAAGTGG

Amplicon Size: 143
[F Start: 10489 End: 10508] [IO Start: 10553 End: 10581] [R Start: 10612 End: 10631]
Note: Purple characters represent allowed mismatches in the signature

10489- T G C G G A T A A T G C A G T C A C A A C T G T G G C G A A G G C C C T A G A A A C A G G T

C C A T C T C G A T T T C G A G T G T C A G A C C C G A G G T G C A A G T C T C T C T T A G C C G C
T T G T T C G G C C A G T C T G G A G G G G A G C T G T A T G C C A C T A C C G C A A G T G G -10631

PRRS_1810382

Genome Title/Description: gi|38146324|gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome
Genome GI number: 38146324
Signature Title: no name provided R is a reverse complement -> Probe Strand: minus
Forward: GTGGTTGGTGAGGCCACTCT
Internal: CGCTTTCTCAGCTCACGACTCGTGATG
Reverse: AATCTTGCCGTTGGCAATGT

Amplicon Size: 143
[F Start: 12123 End: 12142] [IO Start: 12213 End: 12239] [R Start: 12246 End: 12265]
Note: Purple characters represent allowed mismatches in the signature

12123- G T G G T T G G T G A G G C C A C T C T C A C G A A G C T T T C A G G G C T C G A C A T A G T
T A C C C A T T T C C A A C A C C T G G C C G C A G T G G A G G C G G A T T C T T G T C G C T T T C T
C A G C T C A C G A C T C G T G A T G C T A A A G A A T C T T G C C G T T G G C A A T G T -12265

PRRS_1810383

Genome Title/Description: gi|38146324|gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome
Genome GI number: 38146324
Signature Title: PRRS_1810383. R is a reverse complement -> Probe Strand: minus
Forward: CAGTGTGCACGCTTCCATTT
Internal: TGGCTTCGAATTCCAGCTCTACGCTATGTTT
Reverse: ACGGCAACACATCATTCGAG

Amplicon Size: 129
[F Start: 12362 End: 12381] [IO Start: 12423 End: 12453] [R Start: 12471 End: 12490]
Note: Purple characters represent allowed mismatches in the signature

12362- C A G T G T G C A C G C T T C C A T T T T T T C C T C T G T G G C T T C A T C T G T T A C T T T
G T T C A C A G T G C T T T G G C T T C G A A T T C C A G C T C T A C G C T A T G T T T T T G G T T T
C C A T T G G C C C A C G G C A A C A C A T C A T T C G A G -12490

PRRS_1810384

Genome Title/Description: gi|38146324|gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome
Genome GI number: 38146324
Signature Title: no name provided R is a reverse complement -> Probe Strand: plus
Forward: GTTAATGTCCATCCCGTCCG
Internal: CAAACTTGAGGGTTATTATGCTTGGCTGGCT
Reverse: TCATTTGTGCCGAGCATGAT

Amplicon Size: 175
[F Start: 12622 End: 12641] [IO Start: 12655 End: 12685] [R Start: 12777 End: 12796]
Note: Purple characters represent allowed mismatches in the signature

12622- G T T A A T G T C C A T C C C G T C C G G G T A C G A C A A C C T C A A A C T T G A G G G T T
A T T A T G C T T G G C T G G C T T T T T T G T C T T T T T C C T A C G C G G C C C A A T T C C A T C
C A G A G T T G T T C G G G A T C G G A A A T G T G T C G C G C G T C T T C G T G G A C A A G T G G
C A C C A G T T C A T T T G T G C C G A G C A T G A T -12796

PRRS_1810386

Genome Title/Description: gi|38146324|gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome
Genome GI number: 38146324
Signature Title: PRRS_1810386. R is a reverse complement -> Probe Strand: minus
Forward: GCTTTCTGCGTGCCTTTTCT
Internal: TCAGAAATGAGCGAGAAAGGCTTCAAAGTCA
Reverse: GGGAATGTCTCTGGCGTTGT

Amplicon Size: 81
[F Start: 13200 End: 13219] [IO Start: 13225 End: 13255] [R Start: 13261 End: 13280]
Note: Purple characters represent allowed mismatches in the signature

13200- G C T T T C T G C G T G C C T T T T C T A C G C T T C A G A A A T G A G C G A G A A A G G C T
T C A A A G T C A T C T T T G G G A A T G T C T C T G G C G T T G T -13280

PRRS_1810387

Genome Title/Description: gi|38146324|gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome
Genome GI number: 38146324
Signature Title: no name provided R is a reverse complement -> Probe Strand: minus
Forward: GAGACCTTTGTGCTTTACCCG
Internal: GCTTGTGCTTTCGCAGCGTTCGTATG
Reverse: TTGTCATCCGTGCTGCTAAAA

Amplicon Size: 196
[F Start: 13641 End: 13661] [IO Start: 13788 End: 13813] [R Start: 13816 End: 13836]
Note: Purple characters represent allowed mismatches in the signature

13641- G A G A C C T T T G T G C T T T A C C C G G T C G T C A C T C A T A T C C T C T C A C T G G G
T T T T C T C A C G A C A A G T C A T T T T T T T G A C G C G C T C G G T C T C G G C G C T G T G T C
C A C C G C A G G A T T T A T T G A C G G G C G G T A T G T G C T C A G C A G C A T C T A C G G C G
C T T G T G C T T T C G C A G C G T T C G T A T G T T T T G T C A T C C G T G C T G C T A A A A -13836

PRRS_1810388

Genome Title/Description: gi|38146324|gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome
Genome GI number: 38146324
Signature Title: no name provided R is a reverse complement -> Probe Strand: minus
Forward: CCGTACCCGGTTTACCAACT
Internal: ATCAAACATGTCGTCCTCGAAGGGGTTAAAG
Reverse: CTGAGCAATGGGAGGCCTAG

Amplicon Size: 190
[F Start: 13859 End: 13878] [IO Start: 13974 End: 14004] [R Start: 14029 End: 14048]
Note: Purple characters represent allowed mismatches in the signature

13859- C C G T A C C C G G T T T A C C A A C T T T A T T G T G G A C G A C C G G G G A G G A G T T C
A T C G G T G G A A G T C T C C A A T A G T G G T A G A A A A A T T G G G C A A A G C C G A C A T C
G A C G G C A G C C T T G T C A C C A T C A A A C A T G T C G T C C T C G A A G G G G T T A A A G C
T C A A C C C T T G A C A A G G A C T T C G G C T G A G C A A T G G G A G G C C T A G -14048

PRRS_1810391

Genome Title/Description: gi|38146324|gb|AY366525.1| Porcine reproductive and respiratory syndrome virus strain EuroPRRSV, complete genome
Genome GI number: 38146324
Signature Title: no name provided R is a reverse complement -> Probe Strand: minus
Forward: CGTGACTTCTACATCCGCCA
Internal: AGTCACCTATTCAATTAGGGCGATCACATGG
Reverse: TCAGGCAGGAACCATGTGAC

Amplicon Size: 144
[F Start: 14897 End: 14916] [IO Start: 14977 End: 15007] [R Start: 15021 End: 15040]
Note: Purple characters represent allowed mismatches in the signature

14897- C G T G A C T T C T A C A T C C G C C A G T C A G G G T G C A A G T T A A T T T G A C A G T C
A G G T G A A T G G T C G C G A T T G G C G T G T G A C C T C T G A G T C A C C T A T T C A A T T A
G G G C G A T C A C A T G G G G G T C A T A C T T A A T C A G G C A G G A A C C A T G T G A C -
15040

APPENDIX III: Protocols

Extraction of Nucleic Acids

Total DNA extraction from Virus-infected cell culture by phenol/chloroform/isoamyl alcohol precipitation

1) Add 5 x 10^{-2} nanograms of Puc18 per ml of virus cell culture (to 15 ml add 0.75 ng Puc19)

2) Add Triton X-100 to a final concentration of 0.5% V/V (to 15 ml add 75ul Triton)

2) Add 0.5M EDTA to a final concentration of 20 mM (15 ml add 600 ul 0.5M EDTA)

3) Mix vigorously and vortex, let sit 5 min at room temp

4) Spin tube at < 1000 rpm for 10 minutes

5) Discard pellet and to the supernatant add

6) Add 10% (W/V) Sodium Dodecyl Sulfate (SDS) solution to a final concentration of 1% (to 15 ml add 1.5ml of 10% SDS)

7) Add proteinase K to a final concentration of 0.4 U per ml (to 15 ml add 2.4 mg of 2.5 U/mg Roche or 60 ul of a .1mg/ul solution of proteinase K in water)

8) Incubate the tubes at 55^{o} C for one hour mixing every 10 minutes

9) Cool tubes to room temperature

10) Add 5 M NaCl to a final concentration of 150 mM (to 16.5 ml add 510 ul 5 M NaCl)

11) Add an equal volume of room temperature phenol/chloroform/isoamyl alcohol (approx. 15 ml)

12) Mix by inversion and swirling till phases are completely mixed

13) Let sit 5 min then spin at 3,000 rpm for 10 min

14) Remove the upper aqueous layer and distribute 500 ul into 1.5 ml microcentrifuge tubes (for 15 mls need 30 tubes)

15) Discard the lower layer in phenol/ chloroform waste

16) Add two volumes (1 ml) of 100% ethanol to each microcentrifuge tube and leave at -20°C one hour

17) Spin in microfuge at top speed refrigerated for 10 minutes

18) Discard supernatant and wash pellet once with 70% ethanol 150 mM NaCl

19) Remove all ethanol and dissolve the pellet in either TE what is TE? Define at first use or water. For 15 ml extraction dissolve each pellet in each tube in 50 ul of liquid. Each tube should contain 50 fg/ul of puc 18.

RNA Extraction by Trizol (LLNL, DAD nucleic acids) move protocol to appendix
TRIZOL LS Invitrogen Cat. No. 10296-010

1.) Add 3X the volume Trizol to the volume of sample. (Upon completion of this step, sample can be stored at -80, or continue with extraction.) Lyse cells in the sample suspension by passing the suspension several times through a pipette, or by shaking vigorously. Incubate for 15 minutes at room temperature. (Typically, LLNL uses 2X the volume Trizol to water (e.g., 15 ml sample and 30 mls TRIZOL.)

2.) Add 200 ul chloroform per 1 ml solution in the fume hood, cap and shake vigorously for 15 seconds. Incubate at room temperature for 5-15 minutes.

3.) Centrifuge at 3000g for 15 minutes, at 4°C

4.) Remove aqueous layer.

5.) Add 1 ml isopropyl alcohol per 500 ml aqueous layer. Gently mix by inverting several times. Incubate samples on the bench top for 10 minutes.

6.) Centrifuge at 12,000g for 10 minutes at 4 C.

7.) Carefully, pour off liquid.

8.) Wash pellet with 70% EtOH. (We added 1 ml to each 1.5 ml tube.) Vortex sample and re-centrifuge at 7,500g for 5 minutes at 4°C.

9.) Pour off the EtOH, cap, re-spin at 7,500g for 5 minutes and pipette off remaining liquid. Air dry briefly at 55°C, caution not to over-dry.

10.) Resuspend RNA in RNAse-free water and store at -80°C.

RNA Extraction by Ambion MagMax Extraction Kit (PIADC, FAD nucleic acids)

(catalog # 1839). **http://www.ambion.com/catalog/CatNum.php?1839**

Real-time PCR Limit of Detection Screening Protocol

Reagents and Equipment
*See Reagents/materials section of Appendices.

Procedure

- Ran all primers/probe sets designated to the particular signature at the following concentrations in triplicate against targets:
  100pg, 10pg, 1pg and 100fg
- Each primer/probe was run against the specified targets in triplicate.
- Reagent Mix Preparation:

Superscript mix:

Component	1x (ul)	Final Conc.
2x Reaction Mix	12.5	1x
Nuclease Free H_2O	2	N/A
50mM $MgSO_4$	1.4	4mM [$MgSO_4$ from 2X mix+50mM added]
F/R Primers (10uM)	2.6	0.5uM [Each Primer]
Probe (10uM)	0.5	0.2uM
RT/PlatinumTaq	1	N/A
Template (**/ul)	5	variable dilution range

PCR Amplification Procedure:

- All primers/probe sets for each signature were run at the following concentrations of targets:
  100pg, 10pg, 1pg and 100fg (total nucleic acid; not absolute values).
- Each primer/probe was run against the specified targets in triplicate, values are averaged.
- RT kit Cycling Parameters:

Cycle 1:(1X)		
Step 1:	50.0ºC	for 30:00
Step 2:	95.0ºC	for 02:00
Cycle 2:(39X)		
Step 1:	94.0ºC	for 00:15
Step 2:	55.0ºC	for 00:30 [Fluorescence detection step]
Step 3:	72.0ºC	for 00:15
Cycle 3:(1X)		
Step 1:	4.0ºC	HOLD

Gel electrophoresis

Product size was determined by running 15ul of PCR product with 5ul 10x loading dye (Teknova; Hollister, CA) on 4% argarose gels (Cambrex Rockland, IN) in Tris-borate-EDTA

buffer, pH=8.0 (Teknova). Band size was determined using Cambrex's Simpleload 20 base pair ladder. Bioimaging system (UVP BioImaging Systems Upland, CA) was used for visualization of the DNA.

Multiplexed RT-PCR assay protocol

Reagents and Equipment
*For complete listing, please see Reagents/materials section of Appendices.

Purpose
This protocol provides basic procedure for multiplex PCR detection of nucleic acids with subsequent fluorescent detection on a Luminex/Bioplex instrument.

Procedure

PCR amplification of DNA samples:
Set-up PCR master mix according to the below specified concentrations:

Example 1X reaction Singleplex	**Volume (ul)/reaction**	**Final Concentration**
SSIII 2 X rxn mix cat#12574-026	12.5	1X 0.4uM ea dNTP 3.2mM $MgSO_4$
$MgSO_4$ (50mM)	0.95	3.5mM *incl. SSIII 2x mix
SSIII/Plat Taq	1.0	
Primer1B*(100uM)	0.1each	0.400uM
Primer2 (100uM)	0.1each	0.400uM
Sample DNA (200-.002pg/ul)	5.0uL	"X"
Positive control (Alien RNA)	1.0uL	100 copies/rxn

+ pcr grade water to make up 25uL total per reaction
*100copies/uL diluted fresh in RNase free water

Preparation of Alien RNA:

Prepare positive control sample (Alien RNA) as per manufacturer's recommendation. Concentrated Alien RNA stock should be stored in a Tris-based buffer, TSM: [10 mM Tris (pH7.0), 100 mM NaCl and 1 mM $MgCl_2$] TSM buffer is provided with the armored Alien RNA from the vendor. A sub-stock should be prepared in TSM buffer and aliquotted for daily use. Alien RNA dilutions should be prepared fresh, prior to use and can be diluted to working concentration in PCR grade water. Prepare needed volume of Alien RNA in water to 100 copies per reaction concentration. Heat lyse this solution by heating for 3 min at 75°C. This can be done using a Heat Block or thermocycler. Once heat lysed the appropriate volume can be added to the PCR master mix.

PCR/RT-PCR Reaction:

1. Aliquot PCR mix (-sample) into amplification strip wells or a 96 well microtube plate. Then add 5ul of sample. (Always run 2 "blanks" (no DNA) sample with each run type.) Cover plate with appropriate sealant before placing in Tetrad.
2. Cycle reactions in a Tetrad on Program "MuxRT-PCR1", using the following cycling conditions;

Cycle 1:(1X)		
Step 1:	55.0ºC	for 30:00
Step 2:	95.0ºC	for 02:00
Cycle 2:(35X)		
Step 1:	95.0ºC	for 00:15
Step 2:	60.0ºC	for 00:30
Step 3:	72.0ºC	for 00:15
Cycle 3:(1X)		
Step 1:	72.0ºC	for 02:00
Cycle 4:(1X)		
Step 1:	4.0ºC	HOLD

Hybridization of PCR product to Conjugated Beads

3. Vortex concentrated bead mix (60 seconds).
4. Dilute ~3-6 ul (depending on concentration of bead stock) of conjugated bead into 997ul Tris-NaCl buffer for singleplex analysis. For multiplex analysis, dilute 3ul of each conjugated bead into total volume of 1000ul using Tris-NaCl buffer (1:300dilution). For example, if you are running a multiplex bead set (6-plex) you would add 3ul of each bead (18ul total) to a total volume of 1ml.
5. Vortex bead mix well.
6. Aliquot 22ul of diluted bead mix per well in a clean PCR reaction plate.
7. Aliquot 5ul of "cycled PCR product" into each well and seal.
8. Cycle reactions in Tetrad on Program "AME*MODH" or use following cycle parameters;

Cycle 1:(1X)		
Step 1:	95.0ºC	for 02:00
Cycle 2:(1X)		
Step 1:	55.0ºC	for 05:00
Cycle 3:(1X)		
Step 1:	4.0ºC	HOLD

Hybridization Wash and Fluorescent Staining

9. Pre-wet 96-well filter plate with 100ul Tris-NaCl buffer- aspirate briefly on vacuum manifold*.
10. Add 100ul Tris-NaCl buffer to each hybridization reaction in the 96-well solid support plate, then transfer the entire contents (123ul in each well) to the above mentioned pre-wetted filter plate.
11. Vacuum aspirate briefly.
12. Aliquot 100ul Tris NaCl buffer per well and aspirate again.
13. Repeat wash step (above) once more.

14. Add 60ul Streptavidin PE (3ng/ul) to each reaction. For example, to prepare enough SAPE for a 96 well plate add 65ul of SAPE stock (300ug/ml) to 6435ul Tris NaCl buffer.
15. Incubate in the dark at room temperature for 5 minutes.
16. Vacuum aspirate samples, wash with 100ul Tris NaCl buffer, aspirate briefly and remove from vacuum.
17. Add 100ul Tris NaCl buffer and pipette mix samples vigorously to resuspend beads. *Note* be careful to prevent filter from tearing*
18. Transfer to 96-well round bottom microtiter plate. (Several varieties of microtiter plate can be used on the XYP Bioplex Platform, so be sure to check probe height for specific plate desired-see Luminex probe height adjustment in manual)

Carbodiimide Coupling of Amino-Substituted Probes to Luminex Carboxylated Microspheres
S. Smith Version V, 4/18/06

Equipment and Reagents:

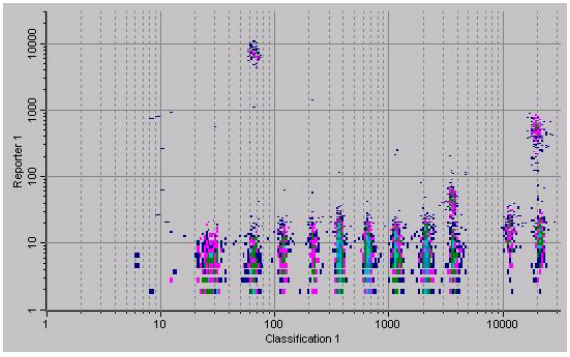
1. Vortexer
2. Sonicator
3. Micropipettors
4. Microcentrifuge
5. Timer
6. Analytical Balance (reads to milligram scale)
7. Microcentrifuge tubes (1.5 ml)..you will want colorless ones so you can see the pellets
8. EDC (solid powder form, make fresh resuspensions)

1. 0.02% (v/v) Tween-20
2. 0.1% (w/v) SDS
3. TE (Tris EDTA) buffer, pH=8
4. Luminex™ Microspheres (Photosensitive)
5. Amino-substituted probes

Protocol: (allow reagents to reach room temp before use)

1. Vortex microsphere beads stock vigorously so as to fully resuspend and homogenize beads. Prepare amino-substituted probes at appropriate concentration in MES (typically 50uM in MES).
2. Pipet naked microspheres into colorless microcentrifuge tubes. (Refer to scaling chart for volumes)
3. Centrifuge tubes at max centrifugal force (16.1 RCF) for 5 minutes.
4. Without disturbing the pellet, aspirate the supernatant.
5. Resuspend pellet in 50 µl of MES. Vortex 30 seconds. Sonicate 1 minute.
6. Add volume of oligo appropriate to starting bead volume. (See scaling chart). Vortex.

7. (Do this immediately before use!) Resuspend an aliquot of EDC powder to 10 mg/ml in pcr grade water. Then add appropriate volume of solution to each vial of spheres. Vortex tubes immediately after EDC addition. (See scaling chart).
8. Incubate tubes for 30 minutes, in the dark, at room temperature. (Put rack of tubes in drawer).
9. Add another freshly prepared aliquot of 10 mg/ml EDC (see scaling chart). Incubate another 30 minutes.
10. Add 0.5ml-1ml 0.02% Tween (see scaling chart). Vortex. Centrifuge 5 minutes at max RPM.
11. Aspirate supernatant. Add 0.5ml-1ml SDS (see scaling chart). Vortex. Centrifuge 5 minutes at max RPM.
12. Aspirate supernatant, add 100 µl TE. Vortex. Centrifuge at max RPM for 5 minutes.
13. Aspirate supernatant. Resuspend in appropriate volume of TE buffer. (See scaling chart) Votex. Store bead at 4C in the dark, or go onto step 14 for QC/validation.
14. Prepare a 1:100 dilution of bead stock in buffer (1uL bead stock to 99uL buffer) and run on Luminex. In the Luminex data analyzer plot window, sort beads by fluorescence intensity (reporter 1) and dye spectrum (classification 1), as seen below. Verify that beads are forming tight populations (indicates distribution of probes on beads) and that there are not multiple populations (verifies that bead stock does not contain multiple bead types).



15. Enumerate and sonicate bead mix before use.

Notes:

1. Probes are resuspended to 1mM in MES. (The oligos are supplied in a lyophilized form at nanomolar scale. To obtain 1 mM, look at amount of nm on label, then add that many microliters of MES, but working through the calculation is suggested).
2. Oligo conjugation concentration optimization is assay specific. This means that this information must be solicited from assay development group, or if it is a new signature, optimization of coupling will have to be performed. Our current optimized coupling concentration for oligos is 50uM, but again, this information should be solicited from assay development group.
3. EDC is unstable, and hygroscopic. It is kept in powder form at -20º C, preferably in a dessicator. Pre-aliquot no more than 10 mg of powder into tared amber vials for -20º storage. Write amount of EDC on tube. When ready for use, add enough sterile water to make a 10 mg/ml solution. (Move the mass's decimal point over 2 places to the right, but again, working through the calculation is suggested). Dispose of EDC IMMEDIATELY after addition to conjugation reactions. (You will use two separate vials of EDC per conjugation procedure, and much will be wasted).
4. Keep MES in the refrigerator (4C), but let it come to room temperature before use.
5. SDS and Tween are kept at room temperature.
6. Microspheres are kept at 4º C.
7. Oligos and other reagents are kept at -20º C. Avoid excessive (>4) freeze-thaw cycles.
8. All buffers and coupling reagents should be mass batch prepared and aliquotted for use.

Scaling Chart:

Volume Naked Beads (uL)	# Microspheres	Probe Input @ "X" concentration (uL)	10mg/ml EDC vol (uL)	0.1M MES Resuspension (uL) (step 5)	0.02% Tween 20 Wash Vol (uL)	0.1% SDS Wash Vol (uL)	Final Volume (uL) TE
1000	1.25E+07	10	5	50	1000	1000	250
500	6.25E+06	5	3	50	1000	1000	125
400	5.00E+06	4	2.5	50	1000	1000	100
250	3.13E+06	2.5	2.5	50	500	500	62.5
100	1.25E+06	1	2.5	50	500	500	25

Recipes for Multiplexed Assay Buffers

Tris-NaCl Buffer, pH =8.0
0.1M Tris (final concentration)
0.05% Triton X-100 (final concentration)
0.2M NaCl (final concentration)
*from Teknova, Cat# t1015

TSM Buffer, pH=7.0
10 mM Tris
100 mM NaCl
1 mM $MgCl_2$
*from Asuragen with purchase of Alien RNA

0.1M (2-{N-morpholino}ethanesulfonic acid)(MES) buffer, pH 4.5

MES 21.3g MES –dissolve in 900ml dH20 and adjust pH to 4.5 with NaOH, qs to 1L. Filter sterilize.

Tween 20 Buffer (0.02% v/v), prepared in reagent grade water for dilution. Used for covalent coupling of microspheres

SDS Buffer: Sodium Dodecyl Sulfate (0.1% w/v), prepared in reagent grade water for dilution Used for covalent coupling of microspheres.

Note: (1) Most buffers are available commercially by Teknova as custom orders. (2) All solutions are filter-sterilize and water diluent is reagent grade.

APPENDIX IV: Design of a Luminex Binding Assay From a Real-time PCR Signature

Illustration of the basic Luminex assay design where the forward primer is biotinylated in 3 locations, the reverse primer is unmodified and the binding sequence is the complement sequence of the forward strand. The probe sequence is conjugated to the microsphere at 50uM concentration of oligonucleotide (depicted below as a singular attachment, where it is actually in excess of 10^7 nearly saturating bead surface). Probe binds complimentary amplicon sequence that is labeled with biotins.

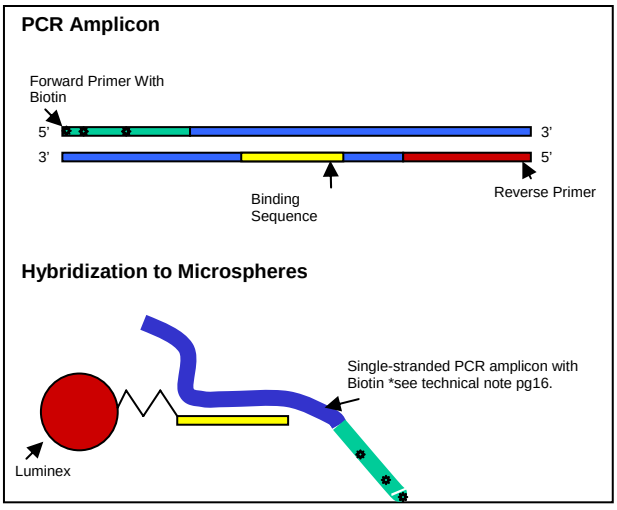
Conversion of a Real-time PCR assay to a Luminex binding-assay is accomplished by the modification of one of the primers and the probe. The modifications are as follows:

Forward Primer: 5' biotin label and 1-2 internal biotins
Example: Real-time PCR primer 5'-AATCGGATCAGATCCA-3" becomes 5'-/5Bio/AA/iBiodT/CGGA/iBiodT/CAGATCCA-3' for Luminex assay.

Reverse Primer: unmodified sequence

Probe: 5'amine and a spacer 18 modification
Example: If the real-time PCR probe sequence is 5' FAM-ATCCGCGCATAG-TAM3', the Luminex binding sequence becomes 5'/5AmMC12//iSp18//ATCCGCGCATAG-3'.

Technical Notes: (1) As a result of complex probe "hybridization networks", probe sequences can be adjusted to be either compliment to the forward sequence (FCP) or compliment to the reverse sequence (RCP). This can be optimized with each assay as needed, but for the purposes of this testing, all probes were optimized in the FCP orientation for multiplexed assay development. **In some cases the Real-time PCR assay probe will be the opposite orientation of the same target region of the genome.** (2) Optimal 10 base separation of internal biotins placed on the forward primer, biotin placement must be on a thymidine.(3) 5AmMC6 has in probe has also been used and is found to work just as well.



Appendix V: Assay Controls

Real-time PCR

Real-time PCR incorporates an internal positive control to prevent reporting of false negative sample results. The internal control used for real-time PCR is an agent assay specific for the bacteria *bacillus thuringiensis*. A CT value is reported at a specific concentration and controls for run-to-run variability and provides information on assay reliability.

Multiplexed PCR

Summary of Multiplexed Assay Internal Controls

In-built assay controls are integrated into each assay to provide confidence in test results. In the multiplexed assay panel there are several internal controls as described in detail below:

IC: Instrument Control
This control is a bead coupled to BSA conjugated to tetramethylrhodamine (TAMRA), a heat stable fluorophore. Due to the limited lifespan of the laser, continual monitoring of this control is important to verify instrument performance. Fluorescence from this bead provides a measure of reporter laser integrity and reproducibility of fluorescence detection. A decline in the signal, or large variations in signal imply a decline in the reporter laser integrity and would put results into question. Instrument must be serviced by trained personnel to correct this problem. This control is generally the most robust.

FC: Fluorescence Control/SAPE Addition Control
This control is a bead coupled to biotinylated BSA (b-BSA) and is used as a fluorescent or SAPE addition control. The biotin molecule has a very high binding affinity for streptavidin (biotin-avidin binding) and the Phycoerythrin (PE) component of SAPE is what is detected by the reporter laser (same as the fluorophore bound directly to the bead for the IC). If no signal is detected on the FC, then it is likely that SAPE was not added to the bead-probe-product hybrid.

NC: Negative Binding Control
This control is a bead bound to a DNA sequence specific to a sequence from the genome of an organism found near deep ocean vents (Maritima). This is NOT a ubiquitous organism, thus making this a good candidate for use as a negative binding control. There is an infinitesimally low probability that sequence complimentary to the Maritima genome will be present in environmental sample matrices. Under normal conditions, this control signal is expected to be low (2-10 MFI's).

PCR/RT-PCR PC: RNA Amplification Control / Inhibition Control
Alien RNA. This control is a bead coupled to an oligo specific for a synthetic sequence that has been screened against all Genbank entries for potential cross-reactivity. This sequence is referred to as "Alien" and was developed by Ambion Diagnostics as a target specific assay that is designed to be unique. This control is designed as a complete assay which requires a modified forward primer and reverse primer as well as the template for amplification. The "Alien RNA" template is produced as a packaged vector, similar to armored RNA technology and is purchased as an "off-the-shelf" item from Ambion. This design construct is optimal because the RNA material is protected from degradation and produces more stable results. The use of this control requires that the Alien RNA material be added to each RT-PCR reaction, and similar to the DNA positive controls, the RNA is co-amplified with the target assay. Alien RNA is added directly to the PCR reaction (for benchtop applications) or directly to extraction media (for actual clinical samples) targeting 100 copies per reaction detection range. For benchtop applications, Alien RNA is stored as a concentrate in TSM buffer and immediately before use is diluted in RT-PCR grade water.

APPENDIX VI: Background Confounders List

Soils: Nucleic acids extracted from soil collections distributed over geographically diverse locations. Screened 55 soils from various locations

Aerosols: Nucleic acid extractions from aerosol samples collected by environmental sampling systems using a air collector and filtration system. Screened 2304 samples (3 aerosol plates, each well = 8 pooled samples)

Prokaryotes (48):

Bacillus thuringiensis	Cytophaga marinoflava	Lampropedia hyalina
Borrellia burgdorferi	Geobacillus caldoxylosilyticus	Mogibacterium vescum
Escherichia coli	Halomonas halmophila	Mycoplasma phocacerebrale
Erwina herbicola	Lactobacillus garvieae	Natrialba magadii
Heamophilus influenza	Lactobacillus gasseri	Nitrospira multiformis
Listeria monocytogenes	Micrococcus luteus	Oceanospirillium ssp. Maris
Pseudomonas aeruginosae	Moraxella lacunatica	Porphyrobacter sanguineus
Salmonella typhimurium	Paenibacillus napthalaenovorans	Prevotella nigrescens
Staphylococcus aureus	Paracoccus dentrificans	Rhizobium leguminosarum
Streptococcus pneumoniae	Proteus mirabillis	Shewanella colwelliana
Clostridium butyricum	Pseudomonas oleovorans	Spiroplasma insolitum
Listeria seeligeri	Rhodococcus rhodochrous	Tatlockia maceachernii
Achromobacter spp xylosoxidans	Simonsiella muelleri	Thermomonospora chromogena
Actinobacillus suis	Sphingomonas sp. (Alcaligenes sp)	Treponema denticola
Aneurinbacillus migulanus	Vibrio paraheamolyticus	Coxiella burnetti
Alicylcobacillus acidocaldarius	Acidithiobacillus ferrooxidans	Campylobacter coli

Eukaryotes (16):

BOVINE	MONKEY
CAT	MOSQUITO
CHICKEN	MOUSE
DOG	PIG / PORCINE
DROSOPHILA MELANOGASTER	RABBIT
EQUINE	RAT
FLEA	SHEEP
HUMAN	TICK

Prokaryotes were spiked into PCR reactions at 200 pg/uL (5uL per reaction) = 1ng
Eukaryotes and soils were spiked into PCR reactions at 1ng/uL (5uL per reaction) = 5ng

APPENDIX VII: Oligo Ordering for Multiplexed Assays

Nomenclature for oligonucleotide ordering

- ".F" label at the end of the oligo name indicates that it is a forward primer
- ".R" label at the end of the oligo name indicates that it is a reverse primer
- "5Bio/" refers to biotinylation of the 5 prime end of the oligo
- "iBiodT" refers to the insertion of an internal biotin dT
- ".FCP" at the end of the oligo names indicates that the probe has been determined to be located on the strand that is the forward compliment orientation. Note: this may differ from what is used in real-time screening where probe orientation need not be specific to strand and the reverse compliment orientation is sufficient.
- "5AmM C6//iSp18/" refers to the addition of a amine attached to the 5 prime end of the oligo with a carbon 6 and internal 18 spacer.

Example Primer order sheet for multiplexed PCR assays.

Oligo Name	Sequence	Sequence w/ modification notation
vesv_95653.BF	GCCTTCTCCCTTCCCAAAA	5'-/5Bio/GCCT/iBiodT/CTCCCT/iBiodT/CCCAAAA-3'
vesv_95653.R	TGAAGGAATGGTTCCGTCAGT	5'-TGAAGGAATGGTTCCGTCAGT-3'
vesv_95680.BF	GGGAATGAGGTGTGCATCATT	5'-/5Bio/GGGAA/iBiodT/GAGGTGTGCA/iBiodT/CATT-3'
vesv_95680.R	CACGTCTTGATGTTGGCTTGAC	5'-CACGTCTTGATGTTGGCTTGAC-3'
vesv_95686.BF	GGTCGCTCTCACTGATGATGAGTA	5'-/5Bio/GGTCGC/iBiodT/CTCACTGATGA/iBiodT/GAGTA-3'
vesv_95686.R	GGTGTTATCAGCACCCATTGC	5'-GGTGTTATCAGCACCCATTGC-3'
vesv_95692.BF	ACCACCTCTGGAAACATCTATGG	5'-/5Bio/ACCACC/iBiodT/CTGGAAACATC/iBiodT/ATGG-3'
vesv_95692.R	TTTGTGCACGTGTCACGAAT	5'-TTTGTGCACGTGTCACGAAT-3'

Example Probe order sheet for multiplexed PCR assays. Includes nomenclature, specific sequence information and modification specifications

Internal name	Oligo Name	Probe strand	Sequence w/ modification notation
VESV_1	vesv_95653.FCP	FCP	5'- /5AmMC6//iSp18/CATCATCGTTGATAACCTTAGATGTGCAATTTGG-3'
VESV_3	vesv_95680.FCP	FCP	5'- /5AmMC6//iSp18/AAATTGGCATAATCAACCTTGTCAGATGAGTCG-3'
VESV_4	vesv_95686.FCP	FCP	5'- /5AmMC6//iSp18/GCTCGGTGCCTGAGTTGGAGGAAG-3'
VESV_5	vesv_95692.FCP	FCP	5'- /5AmMC6//iSp18/CGGGACGGGCATTTGTCACCA-3'

The International Union of Biochemistry Code for Bases and Mixed Bases.

Base	IUB Code	Complement	Comment
Adenosine	A	T	
Cytidine	C	G	
Guanosine	G	C	
Thymidine	T	A	DNA only
Uracil	U	A	RNA only
Adenosine or Guanosine (puRine)	R	Y	Purines
Cytidine or Thymidine (pYrimidine)	Y	R	Pyrimidines
Guanosine or Thymidine (Keto)	K	M	
Adenosine or Cytidine (aMino)	M	K	
Guanosine or Cytidine (Strong - 3 H bonds)	S	S	

Adenosine or Thymidine (Weak - 2 H bonds)	W	W	
Cytidine, Guanosine or Thymidine	B	V	All but A
Adenosine, Guanosine or Thymidine	D	H	All but C
Adenosine, Cytidine or Thymidine	H	D	All but G
Adenosine, Cytidine or Guanosine	V	B	All but T
Adenosine, Cytidine, Guanosine or Thymidine (aNy base)	N	N	All

APPENDIX VIII: Vendor/Reagent List

Reagents and Equipment Summary for Bioplex Assay

A. Bead Mixture at a 1X concentration (28-plex). Store in the dark at 2-8°C. DO NOT FREEZE. Stored at 4°C, this reagent is stable for at least 3 months.
B. Streptavidin R-Phycoerythrin (SAPE) at 10X concentration. Store in the dark at 2-8°C. DO NOT FREEZE. Stored at 4°C, this reagent is stable for at least 3 months. When working with SAPE keep covered with foil to prevent photobleaching.
C. PCR Reaction Reagents
    a. 1X Primer Mix prepared in Tris EDTA buffer (25 plex = 25 primer sets); store in small aliquots at –20°C and do not refreeze.
    b. Internal control, *Erwinia herbicola* (Eh) DNA, (1 pg/µL in PCR grade water), Store at -20°C in small aliquots. Once thawed keep at 4°C, stable for several months.
    c. PCR Grade Water. Store at room temperature.
    d. LightCycler-FastStart DNA Master HybProbes kit (Roche 12239272001 (2239272)). Store at –20°C, refer to manufacturer's instructions for expiration information.
D. Tris NaCl assay buffer (Teknova cat #t1015), pH=8.0. Store at room temperature (25 ± 5°C).
E. 10% bleach solution, freshly prepared in a squirt bottle (1:10 dilution of 5.25-6.0% m/v sodium hypochlorite), (Bioplex instrument maintenance).
F. Distilled water in a squirt bottle (Bioplex instrument maintenance).
G. 70% Isopropanol in a squirt bottle (Bioplex instrument maintenance).
H. Luminex *x*MAP Sheath Fluid (Luminex Corp.; Cat. # 40-50000).
I. Calibration Spheres (Bio-Rad; Cat. # 171-203060). Store at 4°C. DO NOT FREEZE.
J. Validation Kit (Bio-Rad; Cat. # 171-203001 for version 4.0) Store at 4°C. DO NOT FREEZE.
K. Multichannel Electronic Pipettes (see table below).

Table of recommended pipettes according to task.

Task	Location	Volume Range (ul)	Channel	Suggested Model
PCR	PCR clean	20-200	single-repeating	Rainin E3-200
PCR	PCR clean	1-10	multi (8 or 12)	Rainin E8-10
Hybridization	PCR dirty	10-1000	single-repeating	Rainin E3-1000
Hybridization	PCR dirty	1-10	multi (8 or 12)	Rainin E8-10
Hybridization	PCR dirty	20-200	multi (8 or 12)	Rainin E8-200
Hybridization	PCR dirty	100-1200	multi (8 or 12)	Rainin E8-1200

*The pipettes listed above can help to reduce analysis time and the number of pipetting iterations, as well as improved ergonomics.

L. 10ul filter pipette tips, (Rainin, Cat.# RT-L10F). Note: filter tips, or "aerosol resistant" tips, minimize the risk of PCR contamination.
M. 200ul filter pipette tips, (Rainin, Cat.# RT-L200F)
N. 1000ul filter pipette tips, (Rainin, Cat.# RT-L1000F)
O. Plastic reservoirs (50ml and 100ml) (VWR, Cat.# 3054-1006)
P. Multi-Tube Vortex Mixer (VWR, Cat.# 58816-115)
Q. Stop Watch or Timer (VWR, Cat.# 62344-585)
R. Falcon 5ml Tube (VWR, Cat.# 60819-295)
S. Falcon 15ml Tube (VWR, Cat.# 21008-929)
T. Foil Seal (AbGene, Cat.# AB-0626)
U. 96-well PCR plates, 0.2 mL, Semi Skirted (E&K Scientific, Cat.# 289096)
V. PCR Plate Seals (AbGene, Cat.#1040-39-4)
W. 1.2 uM vacuum filter plate, 96-well format (Millipore Multiscreen Filter Plate, Cat. #MABVN 1250)
X. Vacuum Manifold Kit (Millipore Cat.# MAVMM0960R)
Y. PCR Thermal cycler, MJ Tetrad,
Z. Falcon round-bottom microtiter plates (VWR Cat.# 62406-343)
AA. Vacuum Source (house vacuum or vacuum pump, outfitted with a trap to protect the pump and an in-line filter and associated tubing if pump is external to hood). Cole Parmer - Vacuum Pump, Cat# A-07061-40; Nalgene 2 Liter Bottle, Cat# BH06257-20; Nalgene Venting Cap, Cat# BH06258-10; Tygon Tubing 1/4" ID 1/2" OD, Cat# BH-95636-00.

Other Recommended Supplies:

i. Laboratory marking pen
ii. Disposable gloves
iii. Racks for 4.5mL tubes and for microcentrifuge tubes

Summary Table -Materials/Vendors

Consumables (brand/type required)	Catalog Number	Vendor
Rainin Tips, 1000µl-F, 768/8 racks	RT-L1000F	Rainin
RaininTips, 200µl-F, 960/10 racks	RT-L200F	Rainin
Rainin Tips, 10µl-F, 960/10 racks	RT-L10F	Rainin
Multiscreen 1.2 um filter plates, 50/pk	MABVN1250	Millipore

PCR Plate Standard Optical Plate, Half Skirt, clear (10/sleeve)	289096	E&K Scientific
BD Falcon Assays Plate U bottom, 50/case	62406343	VWR
Cycleseal PCR plate sealer 20/pk (Robbins Scientific)	1044-39-4	AB Gene
Consumables (brand/type suggested)	**Catalog Number**	**Vendor**
Eppendorf 1.5 ml microcentrifuge tubes, natural, pk500	20901551	VWR
Falcon 15 ml tubes, 50/rack, case 500	21008929	VWR
Falcon 5ml Tubes, pk 500	60819-295	VWR
100 ml reservoirs, 100/case	41428-950	VWR
50 ml resevoirs, 100/case	3054-1006	VWR
Adhesive PCR Foil seals 100/pk	AB-0626	AB Gene
Clear seals 100/pk	48461	Edge Bio
Teknova Glass Distilled Water (PCR Grade Water), 1L	W3350	Teknova
Reagents	**Catalog Number**	**Vendor**
LightCycler ® FastStart DNA Master HybProbe (480 rxns)	12239272001 (2239272)	Roche
Sheath fluid (20L)	40-50000	Luminex
Teknova Tris NaCl, pH 8.0	t1015	Teknova
SAPE, Strepdavadin PE, 2mL	SA-1004-4	CAL-TAG Labs
Bioplex Calibration Kit	171-203060	Bio-Rad
Bioplex Validation Kit	171-203000	Bio-Rad
70% isopropanol in squeeze bottle	N/A	N/A
10% bleach in squeeze bottle	N/A	N/A
Water in squeeze bottle	N/A	N/A
Equipment Needed (brand/type required)	**Catalog Number**	**Vendor**
MJ Tetrad		Biorad
Bioplex 100 system with version 4.0 software		Biorad
Multiscreen vacuum filtration manifold	MAVM0960R	Millipore
Multichannel Pipet EDP3+ ELEC LTS 8-CH PIPET 0.5-10UL	E8-10	Rainin
Multichannel Pipet EDP3+ ELECTRONIC LTS PIPET 8-CH 20-200UL	E8-200	Rainin
Multichannel Pipet EDP3+ ELEC LTS 8-CH PIPET 100-1200UL	E8-1200	Rainin
Multidispense Pipet EDP3+ ELECTRONIC LTS PIPET 100-1000UL	E3-1000	Rainin
Equipment Needed (brand/type suggested)	**Catalog Number**	**Vendor**
Laminar Flow Hoods		
Vacuum Pump	A-07061-40	Cole Parmer

Nalgene 2 Liter Bottle	BH06257-20	Cole Parmer
Nalgene Venting Cap	BH06258-10	Cole Parmer
Tygon Tubing 1/4" ID 1/2" OD	BH-95636-00	Cole Parmer
Timer	62344-585	VWR
Vortexer	58816-115	VWR

a.

Vendor Contact Information:

1. ABgene (Marsh Brand), 565 Blossom Road, Rochester New York 14610, USA. Phone (585) 654 4800 (or toll free 1 800 445 2812), Fax (585) 654 4810. infousa@abgene.com
2. Bio-Rad Laboratories, 1000 Alfred Nobel Dr., Hercules, CA 94547, **http://bio-rad.com**, Phone (510) 724-7000, (800) 262-1640, fax: (800) 428-2883.
3. Cole-Parmer Instrument Company, 625 East Bunker Court, Vernon Hills, Illinois 60061-1844, www.coleparmer.com, phone (800) 323-4340, fax (847) 247-2929.
4. Corning Costar, 1 Riverfront Plaza, Corning, NY 14831, **www.corning.com**, Phone (607) 974-9000.
5. E&K Scientific, 3575 Thomas Road, Santa Clara, CA 95054. Phone (800) 934-8114, Fax (408) 567-9671. **http://www.eandkscientific.com/**
6. Edge BioSystems, 201 Perry Parkway, Suite 5, Gaithersburg, MD 20877. Phone (800) 326-2685. **http://www.edgebio.com/**
7. Invitrogen Life Technologies (Caltag), 1600 Faraday Avenue, PO Box 6482, Calsbad, CA 92008. Phone (760) 603-7200, fax (760) 602-6500, orders (800) 955-6288. **http://www.lifetechnologies.com/content.cfm?pageid=1**
8. Fisher Scientific, 3970 John's Creek Ct Suite 500, Suwanee, GA 30024, **www.fishersci.com**, Phone (800) 766-7000, fax (800) 926-1166
9. Luminex Corporation, 12212 Technology Blvd. Austin, TX 78727, **http://www.luminexcorp.com**, Phone (888) 219-8020.
10. Millipore Corporation, 290 Concord Rd., Billerica, MA 01821, **http://www.millipore.com**, Phone (800) 645-5476.
11. Rainin Pipetting Solutions, 7500 Edgewater Drive, P.O. Box 2160, Oakland, CA 94621-0060. Phone (510) 564-1600, fax (510)564-1617. Ordering: 800-472-4646. **www.rainin.com**
12. Roche Applied Science, 9115 Hague Road, P.O. Box 50414, Indianapolis, IN 46250-0414 USA, www.roche-applied-science.com, phone: (800) 262-1640, Fax: (800) 428-2883
13. Teknova Inc., 2290 Bert Drive, Hollister, CA 95023, **www.teknova.com**, phone (831)637-1100, faxes (831)637 2355.
14. VWR International, 1310 Goshen Parkway, West Chester, PA 19380, orders phone1-800-932-5000. **http://www.vwrsp.com/**

Reagents Summary for Real-time Assay

1. Invitrogen Platinum Taq polymerase, Catalog #10966-083 (Carlsbad, CA)
2. Invitrogen 10 PCR Buffer, Catalog #10966-083 (Carlsbad, CA)
3. Invitrogen 50mM $MgCl_2$, Catalog # 10966-083 (Carlsbad, CA)
4. Sigma Chemical BSA, Catalog #B8687 (St Louis, MO)
5. Amersham dNTPs, Catalog #27-2035-02 (Piscataway, NJ)
6. Biosearch Technologies oligonucleotdes (Novato, CA)
7. Nuclease-Free water
8. Cambrex 4% agarose gel, Catalog #57225
9. Cambrex Simplyload 20 bp ladder, catalogue #50331
10. Teknova 10X TBE, Catalog# T0210
11. Teknova 10x Loading Dye, Catalog # F3062

APPENDIX IX: Cost Analysis for Multiplexed Assays

Cost Estimate for Bioplex Multiplexed Assays						
			Unit Price ($)	**# Units for 1 plate (96 samples**	**Total ($) per 96 reactions**	**Total ($) per reaction**
Consumables	**Vendor**					
Vacuum filtration plates	Millipore	50/pack	500.0	0.02	10.00	0.10
PCR amplification plate	VWR	20/pack	161.0	0.05	8.05	0.08
PCR hybridization plate	VWR	20/pack	161.0	0.05	8.05	0.08
Plate seals 96 well	ABGene	100/pack	106.0	0.01	1.06	0.01
96 well U bottom plates	VWR	50 per case	105.7	0.02	2.11	0.02
Rainin Aerosol Resistant LTS Pipette tips	Rainin	960 tips/10 racks	85.0	1.10	93.50	0.97
Total per plate					**122.77**	
Total per reaction well (per sample)						**1.28**
Reagents						
Luminex beads conjugated with probes	Luminex	1 reaction	1.2	96.00	118.08	1.23
Primers	IDT DNA	1 reaction	0.7	96.00	62.40	0.65
SAPE		1 reaction	0.1	96.00	9.60	0.10
Tris NaCl (Hyb buffer)	Teknova	1L	30.0	0.20	6.00	0.06
SuperScript™ III Platinum® One-Step qRT-PCR Kit	Invitrogen	100 rxns	400.2	1.00	400.20	4.17
Sheath fluid	Luminex	20L	25.0	0.05	1.25	0.01
Total per plate					**597.53**	
Total per reaction well (per sample)						**6.22**
TOTAL COST PER REACTION WELL (PER SAMPLE)						**7.50**