Phenotypic grouping of 141 BmNPVs lacking viral gene sequences

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Running title: Phenotypic grouping of 141 knockout BmNPVs

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ABSTRACT

We constructed a series of gene knockout BmNPVs (KOVs) for each of 141 genes using the BmNPV T3 bacmid system and lambda red recombination system. In a subsequent analysis of the properties needed for infection using a marker gene, *egfp* (enhanced green fluorescent protein gene), inserted into the polyhedrin locus, the knockout viruses (KOVs) were subdivided into four phenotypic types, A to D. Type-A (86 KOVs) showed the ability to expand infections equivalent to the control while type-B (8 KOVs) spread infections more slowly. Type-C (37 KOVs) expressed *egfp* in transfected-BmN cells but the production of infectious viruses was not observed. Type-D (10 KOVs) showed no ability to express *egfp* even in the transfection experiments. KOVs lacking genes (*pkip* (*Bm15*), *gp41* (*Bm66*), *bro-d* (*Bm131*), *Bm20*, *48*, *65*, *91*, *93*, or *101*) previously identified as being essential, were placed in the viable type-A and B categories.

Keywords: BmNPV, bacmid, lambda red recombination system, gene knockout

Highlights: > We constructed a series of gene knockout BmNPVs (KOVs) for each of 141 genes using BmNPV T3 bacmid system. > KOVs were subdivided into four phenotypic types by *polyhedrin* promoter-driven EGFP expression and infectious behavior. > The catalogue of BmNPV genes will be a helpful reference in the functional analysis of viral genes.

1. Introduction

Nucleopolyhedrovirus (NPV) is one of the largest DNA viruses. Its genome contains over 100 genes, which are expressed in a stage-dependent manner; immediate-early, delayed-early, late and very-late (Friesen and Miller, 1986). Genes of NPV are involved in transcriptional regulation (Guarino and Summers, 1986; Yoo and Guarino, 1994), viral RNA polymerase components (Guarino *et al*., 1998), nucleocapsid formation (Thiem and Miller, 1989; Vanarsdall *et al*., 2006), host gene regulation (Nobiron *et al*., 2003) and so on. About half of them are predicted to be essential for viral propagation through gene expression, DNA replication and virion components (Rohrmann, 2011). On the other hand, there are some accessory genes or non-essential genes that remain in the viral genome during passage. These observations suggested that each viral gene would work to the advantage at least in certain situations through interaction with other viral/host genes.

The function of baculoviral genes in replication has been studied vigorously by using gene knock-out (KO) technology in baculoviruses such as *Autographa californica* multiple NPV (AcMNPV) and *Bombyx mori* NPV (BmNPV) (Rohrman *et al.*, 2011), however, the function of more than one-third of viral genes are still unknown. In addition, KO viruses have been generated based on different genetic backbones using various methods, that is; different species (AcMNPV or BmNPV), strains and methods for mutation (temperature-sensitive mutant, deletion or insertion into a wild-type baculoviral genome or bacmid). In the 1980s, KO viruses were obtained by conventional homologous recombination in insect cells, sometimes making it difficult to distinguish the negative results caused by a failure of the recombination from those caused by the knock-out of an essential gene. This problem was solved when the bacmid system was established in

AcMNPV (Luckow *et al*., 1993) since the viral (bacmid) DNAs could be amplified in *E. coli* even if they lacked essential genes for replication in the host insect cells. Now this technique is available for other baculoviruses such as BmNPV (Motohashi *et al*., 2005) and *Helicoverpa armigera* SNPV (HearNPV) (Wang *et al.*, 2003).

In general, early genes are transcribed by host RNA polymerase II and mainly involved in regulating replication as trans-regulators, however, late genes are transcribed by viral-derived RNA polymerase and involved in forming viral structure as capsid proteins. In addition, there have been reports that the expression of host genes was not only down-regulated (i.e. shut off), but up-regulated during baculovirus infections (Nobiron *et al*., 2003; Sagisaka *et al*., 2010.). These observations suggested that the baculovirus controlled the host cell mechanisms to produce progeny viruses using host factors and machinery through a complex gene regulatory mechanism among not only viral genes but host genes. To understand the mechanisms of viral replication, insight into the functions of baculoviral genes and host genes responding to viral infections is essential. Among lepidopteran insects, which include many hosts of baculoviruses, *Bombyx mori* is well-studied physiologically, biochemically and molecular biologically. It is worth noting that the genome has been sequenced through international collaboration between Japan and China (International Silkworm Genome Consortium, 2008), suggesting that the informational environment for analyzing the interaction between host and virus is being put into place in the BmNPV-silkworm infection system.

To establish a platform for the comprehensive analysis of the BmNPV gene network and/or interaction between viral and host genes, we constructed a series of gene knock-out BmNPVs (KOVs) for each of 135 genes (Gomi *et al*., 1999) and another 6 ORFs (Katsuma *et al*., 2011) using the BmNPV T3 bacmid system (Ono *et al*., 2007) and lambda red

recombination system (Datsenko and Wanner, 2000). Subsequently the growth properties of KOVs in BmN cells, and the gene knockout-specific effects on the production of infectious progeny and polyhedrin gene expression were analyzed using a marker (GFP) controlled by the *polh* promoter.

2. Materials and methods

2. 1. Cells, bacmids and transfection.

BmN cells were maintained in TC-100 medium (Applichem) containing 10% FBS at 26^{o}C. Transfection of BmN cells with bacmid DNAs was performed by lipofection using FuGENE HD Transfection Reagent (Promega) according to the manufacturer's instructions.

Escherichia coli (*E coli.*) strains BW25113 (containing pKD46 encoding the genes for the lambda red recombination system) and BW25141 (pKD3 encoding a chloramphenicol acetyltransferase gene (*cat*)) (Datsenko and Wanner, 2000) were provided by the *E. coli* Genetic Stock Center (Yale University, USA).

2. 2. Generation of knockout BmNPV bacmids expressing EGFP.

The BmNPV bacmid system (T3 strain, [Ono *et al.*, 2007]) was used to generate knockout viruses with the lambda red recombination system (Datsenko and Wanner, 2000).

We first generated a transfer vector for the *6olyhedron* (*polh*) locus of the bacmids. The enhanced green fluorescent protein (EGFP) coding sequence (*egfp*) was excised from pEGFP-1 (Clontech) by digestion with *Bam*H I and *Not* I and ligated into the *Bam*H I-*Not* I site of pFastBac1 (Invitrogen), yielding pFastBac-GFP. The BmNPV T3 bacmid expressing EGFP under the control of the *polh* promoter was produced by transposition in *E coli*. (BmT3DH10Bac; containing the BmNPV genome bacmid and a helper plasmid pMON7124 encoding a transposase) with pFastBac-GFP. The bacmid DNA carrying *egfp* was isolated from a kanamycin-, gentamycin-resistant and lacZ-negative colony, and designated Bmbac^{+egfp} (BmGFP) as described elsewhere (Ono *et al*., 2007).

For knocking out each gene, we used the lambda red recombination system (Datsenko

and Wanner, 2000). KOVs were generated by homologous recombination in *E. coli* containing pKD46 as a helper plasmid encoding a lambda red recombinase to replace each target gene with a chloramphenicol acetyltransferase (*cat*) gene from pKD3 for antibiotic selection. Briefly, DH10B cells (Invitrogen) containing pKD46 were transfected with BmGFP DNA by electroporation using a GenePulser (Biorad) (25 μF, 2.5 kV, 200 Ω) and designated BmT3DH10B^{+egfp}-pKD46. Then, the *cat* gene sequences wedged between the 5′ non-coding regions (50 nucleotides (nts)) and the 3′ non-coding regions (50 nts) of the BmNPV target genes were amplified by PCR with the primer sets (Table. 1) using pKD3 as a template. Primers for knocking out a gene were designed not to prevent the expression of adjacent genes, that is, promoter motifs (CAGT, TATA, and TAAG) and at least 30 nts upstream from the ATG translation start codon of the adjacent genes remained. The length of each deleted region was from 34 (Δ*Bm95a*) to 3605 nts (Δ*dnahel* (*Bm78*)). After *Dpn* I treatment to digest pKD3, PCR products (approximately 1 kbp, >500 ng) were purified using Wizard SV Gel and a PCR Clean-Up System (Promega) and transformed into BmT3DH10B^{+egfp}-pKD46 by electroporation as above. Then, cells were incubated with 0.01 % Arabinose SOC broth for 4-6 h at 37^{o}C. Each gene-knockout BmNPV bacmid was obtained from an chloramphenicol- and kanamycin-resistant colony, followed by verification of the absence of the viral gene ORF by PCR with gene-specific primer sets targeting the sequence (about 300 nts) inside of each gene, which were designed for a baculovirus DNA microarray (Yamagishi *et al*., 2003). On the other hand, the existence of the cat sequence in each KOV was confirmed by PCR with primers to amplify the sequence surrounding the 5'-terminus (cat-up: 5'-gaatcagctccagcctacac-3' and the gene-specific primers) or the 3'-terminus (cat-down: 5'-ctaaggaggatattcatatg-3' and the gene-specific primers) of the *cat* sequence. After incubation at 37 ^{o}C for 6 hours to remove the helper plasmid pKD46 from bacteria, Ampicillin-sensitive colonies were selected. Bacmid DNAs

were purified from 50-ml LB cultures using a Qiagen midi-plasmid kit (Qiagen) and each concentration was determined by NanoDrop2000 (Thermo scientific).

2. 3. KOV transfection and infection assay

BmN cells were washed with serum-free TC-100 medium and seeded into 96 well plates (5×10^4 cells/well). The cells were transfected with each bacmid (0.25 μg) as described above and subjected to fluorescence microscopic observation. The fluorescence intensity of EGFP was monitored by infinite M200PRO (Tecan). At 4 days post transfection (d.p.t.), the supernatant (10 μl) was collected and added to freshly seeded BmN cells. The culture was continued and the fluorescence intensity of these cells was measured daily as above.

3. Results

3. 1. Generation of BmNPV knockout bacmids.

BmNPV has about 140 genes, however, most of their functions were remain unknown. To investigate their role in the viral replication cycle, we generated 141 genes-knockout viruses. The absence of the target gene in each KOV was verified by PCR. PCR targeting a sequence of 0.3 kbp inside each deleted ORF was carried out for the knockout bacmids, resulting in negative for each ORF (Fig. 1(b): results for Δ*Bm20*, Δ*Bm48*, Δ*Bm91*, Δ*Bm93* and Δ*Bm101* are shown as examples). On the other hand, PCR with the primer sets targeting the 5'-terminal region (Fig. 1(c)) or the 3'-terminal region (Fig. 1(d)) of the *cat* sequence resulted in the amplification of DNA fragments of expected size for the 5'-terminal region (1500 nucleotides (nts) for Δ*Bm20*, 800 nts for Δ*Bm48*, 1200 nts for Δ*Bm91*, 2000 nts for Δ*Bm93*, 500 nts for Δ*Bm101*) or the 3'-terminal region (650 nts for Δ*Bm20*, 300 nts for Δ*Bm48*, 750 nts for Δ*Bm91*, 450 nts for Δ*Bm93*, 700 nts for Δ*Bm101*). We confirmed the knocked out region of other KOVs in the same way (data not shown). The KOV (Δ*Bm95a*) failed possibly because the sequence to be deleted for *Bm95a* was very small (34 nts). Therefore, we analyzed a KOV lacking both *Bm95a* and *Bm96* (Δ*Bm95a-96*).

3. 2. The growth properties of each KOV.

To define each KOV phenotype, we introduced the bacmids into BmN cells and analyzed the EGFP expression every 24 hours. In addition, at 96 hours post transfection (h.p.t.), the culture medium was collected and inoculated into the new cell layer in order to determine whether the infectious virion was produced or not. Because EGFP was driven by the *polh*

promoter in each KOV-transfected cell, we observed the infection and expression of *polh* based on the intensity of EGFP in the cells.

The KOV (bacmid)s were roughly subdivided into four phenotypes (A to D); type-A and -B KOVs produced infectious viruses but type-C and -D did not (Fig. 2). At 48 h.p.t., EGFP expression was detected in the BmN cells transfected with 131 KOV DNAs (Fig. 2) in addition to the cells transfected with BmGFP DNA, a control (*polh* replaced with *egfp*) bacmid. At 96 h.p.t., the expansion of the cells expressing EGFP was observed in the cells transfected with 86 KOV DNAs (see Fig. 2, photograph of Δ*Bm101* bacmid-transfected BmN cells; type-A KOV). The ability to expand an infection was confirmed by experiments using the culture medium of the cells transfected with the 86 KOVs as inoculums. Many type-A KOVs were able to spread infections (=cell-to-cell infection) as fast as BmGFP (a control), however, 8 KOVs (Δ*Bm5*, Δ*lef-6* (*Bm19*), Δ*ubiquitin* (*Bm26*), Δ*lef-12* (*Bm32*), Δ*Bm36*, Δ*odv-e66* (*Bm37*), Δ*Bm56*, Δ*iap2* (*Bm58*)) showed less activity for EGFP expression (<30%, compared to BmGFP at 96 h.p.t.). On the other hand, the EGFP levels in the transfected cells were almost the same as in the BmGFP-transfected cells but the infection did not spread to all cells in the case of the 8 KOVs (<40%, compared to BmGFP at 96 h.p.t.; see Fig. 2, photograph of Δ*pkip* (*Bm15*) bacmid-transfected BmN cells; type-B KOV). In addition, in a second infection in newly seeded cells, the fluorescence intensity of EGFP was low. Then, for 37 KOVs, EGFP expression was verified but no cell-to-cell infection was observed at least not at 96 h.p.t. (see Fig. 2, photograph of Δ*dbp* (*Bm16*) bacmid-transfected BmN cells; type-C KOV). In the subsequent infection experiment, we couldn't detect any EGFP in the cells (data not shown). Finally, there were 10 KOVs for which EGFP expression wasn't observed not only in transfected BmN cells, but also in infected cells (see Fig. 2, photograph of Δ*lef-9* (*Bm50*) bacmid-transfected BmN cells; type-D KOV).

4. Discussion

We generated KOVs for 141 genes of BmNPV using a bacmid system and the lambda red recombination system and characterized their phenotype (Table 2). Forty-five of these KOVs have not been reported for not only BmNPV but also AcMNPV previously (Table.2).

Type-A KOVs (n=86) were able to spread infections as fast as a control BmGFP, though much less GFP than BmGFP (<30% at 96 h.p.t.) was expressed for eight KOVs (Δ*Bm5*, Δ*lef-6* (*Bm19*), Δ*ubiquitin* (*Bm26*), Δ*lef-12* (*Bm32*), Δ*Bm36*, Δ*odv-e66* (*Bm37*), Δ*Bm56*, Δ*iap2* (*Bm58*)). It was previously reported that the deletion of *Bm56* in BmNPV affected the occlusion body's morphogenesis (Xu *et al*., 2008). In addition, the deletion of *lef-6* or *lef-12* in AcMNPV resulted in a decrease in the expression of late and very-late genes including *polh* (Lin and Blissard, 2002; Guarino *et al*., 2002). These observations were consistent with our results. On the other hand, though AcMNPV *lef*-7 was also identified as late expression factor and reported to stimulate DNA replication (Morris *et al*., 1994) and polyhedra formation in SF21 cells (Chen and Thiem, 1997), we observed no significant differences in viral production and Polh expression between *lef*-7 KOV and the control bacmid BmGFP

Type-B KOVs (n=8) were characterized by a markedly slow spreading of the infection probably because of the low production of infectious viruses and/or low infectivity of the progeny viruses. Viruses lacking *pkip* (*Bm15*) (temperature-sensitive mutant, AcMNPV L1 [McLachlin *et al*., 1998]), *Bm65* (failed in homologous recombination, BmNPV T3 [Rohrmann, 2011]), *gp41* (*Bm66*) (temperature-sensitive mutant, AcMNPV L1 [Olszewski

and Miller, 1997]) and *bro-d* (*Bm131*) (homologous recombination failed, BmNPV T3 [Kang *et al*., 1999]) were also viable (type-B).

pkip codes for a protein kinase interacting protein (PKIP) which stimulates a virus-encoded protein kinase (Pk1) activity in vitro (McLachlin *et al*., 1998) which may explain our observations (ΔPk-1 (*Bm3*) was grouped into type-C and *pkip* (*Bm15*) was type-B)

The *gp41 (Bm66)* product is not a component of budded viruses (BVs) but is required for the egress of nucleocapsids from the nucleus in the process of BV synthesis (Whitford and Faulkner, 1992). Olszewski and Miller (1997) reported that a ts mutant of *gp41* was able to form occlusion bodies in Sf21 cells, but couldn't produce infectious progeny. These reports suggested *gp41* to play important roles in the process of virus production that is consistent with our observations though *gp41*-KO BmNPV was not like complete defective in the productivity of infectious progeny.

The functions of *Bm65* and *bro-d* in virion production are still unclear. The *orf79* of AcMNPV, a homolog of the *Bm65* of BmNPV, was found to code for a protein associating with ODV and predicted to be a member of the UvrC superfamily of endonucleases involved in DNA repair (Aravind *et al*., 1999). The baculovirus repeated ORF (bro) family is unique and involved in nuclear export, shuttling mRNA between the nucleus and cytoplasm (Kang *et al*., 2006). BmNPV contains five bro genes with *bro-d* thought to play an important role in viral infection because of a failure to isolate a *bro-d* deletion mutant. The lack of such functions might explain the defective (type-B) infection of *Bm65* KOV or *bro-d* KOV (Δ*Bm65* or Δ*bro-d*) observed in this study but more experiments are required.

Type-C KOVs (n=37) expressed GFP in the BmN cells transfected with the bacmid DNAs but no spreading of the infection was observed. Most of the genes knocked out in type-C KOVs code for virion structural proteins (shown in Table 2, (Braunagel *et al*., 2003; Wang *et al*., 2010). In type-C KOV-transfected cells, very-late gene expression was observed but cell-to-cell infection (the production of infectious progeny) was not. These observations suggested the genes to play important roles in the morphogenesis of BmNPV. Interestingly, *lef-1* (Bm6), *lef-2* (*Bm135*) and *dnapol* (*Bm53*) that were required for transient DNA replication (Kool *et al*., 1994.; Lu and Miller, 1995) were not necessary for *polh* expression (type-C KOVs), indicating that DNA replication and very-late gene expression were regulated independently. In addition, we observed the expression of GFP in the cells transfected with *dbp* (*Bm16*) KOV (Δ*dbp*). The *dbp* gene is required for the maturation of the virogenic stroma (Mikhailov *et al*., 2008; Nagamine *et al*., 2006; Vanarsdall *et al*., 2007), suggesting that the very-late gene expression was independent of the formation of the virogenic stroma.

In the cells transfected with type-D bacmid DNAs (n=10), neither the expression of GFP nor the production of infectious viruses was detected. The 10 viral genes lacking in type-D KOVs were essential not only for viral replication, but for *polh* expression. This observation is consistent with reports that they were essential for DNA replication or late gene expression as components of viral RNA polymerase (Kool *et al*., 1994; Lu *et al*., 1995; Guarino *et al*., 1998).

Some 57 essential genes have been recognized in BmNPV (Gomi *et al*., 1997; Kang *et al*., 1999; Rohrmann, 2011), mainly based on the results of homologous recombination experiments and information on the function of homologues in AcMNPV. We here adopted

the bacmid system which allows the construction of KOV DNA lacking even essential genes to reevaluate the necessity of BmNPV genes in viral reproduction.

Our results indicated that viruses lacking one of 9 BmNPV genes (*Bm20*, *48*, *65*, *91*, *93*, *101*, *pkip* (*Bm15*), *gp41* (*Bm66*) and *bro-d* (*Bm131*)), which were originally identified as essential, generated infectious progeny (type-A, B). The discrepancy could be due to difficulties in generating these insertion/deletion mutants in the homologous recombination system.

Bm20, *48*, *91*, *93* and *101* were originally identified as essential because no insertion/deletion mutants of these genes could be isolated from BmNPV strain T3 (Rohrmann, 2011). However, in this study using a bacmid system, the viruses lacking these genes produced infectious viruses as well as a control BmGFP in BmN cells (type-A). *Bm48* is the homologue of AcMNPV *orf60*, which is a homologue of *ChaB*, a putative membrane ion antiporter regulator from *Escherichia coli*. The *ChaB* homologues identified in *Spodoptera litura* (*Spli*) NPV were suggested to code for DNA-binding proteins (Li *et al*., 2006). Furthermore, a recent study of the homolog in HearNPV, *Ha51*, reported that was not essential for virus production (Zheng *et al*., 2011), and our result in BmNPV was consistent with thier report. The homolog *of Bm91* (in *Antheraea pernyi* NPV [Shi *et al*., 2007]) was an ODV-associated protein and that of *Bm101* (in AcMNPV [Wang *et al*., 2010]), a BV-associated protein. The results of this study suggested that the functions of these genes were not required for BmNPV reproduction in BmN cells though these genes are conserved among Group I NPV (Lepidoptera NPV) (Jehle *et al*., 2006). However, our results do not exclude the possibility that these genes have important roles in the viral infection cycle in vivo.

In this study, we generated KOVs for 141 BmNPV genes and sub-divided the KOVs into four phenotypic groups. The catalogue of BmNPV genes and their relationship to a criterion for their replication presented here will be a helpful reference in the functional analysis of viral genes though other experiments are required for evaluating the knockouts that showed an effect in detail. In addition, the series of sequence deletion mutants in BmNPV constructed here with the lambda red recombination system will provide a useful tool in systems biology for identifying the interaction among viral/host genes.

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FIGURE LEGENDS

Table 1. Oligonucleotide primers used in the PCR to generate knockout bacmids.

To construct KOVs using the lambda red recombination system (Datsenko and Wanner, 2000), the *cat* genes with sequences for gene-targeting recombination were synthesized by PCR with each primer set. Oligonucleotide primer sequences, the target gene name and the orf number of BmNPV are shown. 5' primer; *cat* gene 5'-terminal sequence in pKD3 (gtgtaggctggagctgcttc) combined with the upstream sequences of target genes. 3' primer: *cat* gene 5'-terminal sequence in pKD3 (catatgaatatcctccttag) combined with the downstream sequences of target genes.

Fig. 1. Structural confirmation of knockout BmNPV bacmids.

A schematic representation of the verification of gene-knocking out by PCR is shown in panel (a). PCR was carried out targeting the internal sequence of the knocked out gene (INT), upstream (cat-U) and downstream (cat-D) for each KOV. The PCR products were analyzed by agarose gel electrophoresis and the results for five KOVs (Δ*Bm20,* Δ*Bm48,* Δ*Bm91,* Δ*Bm93,* Δ*Bm101*) are presented (b, c, d). Lanes 1 to 5 contain PCR products for Δ*Bm20,* Δ*Bm48,* Δ*Bm91,* Δ*Bm93,* and Δ*Bm101,* respectively. The results for PCR targeting INT are shown in panel (b). Lanes 6 to 10 contain the PCR products using BmGFP as the template for a positive control of lane 1 to 5. The results for PCR targeting *cat*-U and *cat*-D are presented in panel (c) and (d), respectively.

Fig. 2. Four types of KOVs

The results for BmGFP, Δ*Bm101*, Δ*pkip* (*Bm15*), Δ*dbp* (*Bm16*) and Δ*lef-9* (*Bm50*) are presented as examples for type-A, -B, -C and -D, respectively. Δ ORF indicates the number of open reading frames knocked out in each KOV. Fluorescence micrographs of bacmid-transfected BmN cells are shown along with a schematic representation.

Table 2. Category (type-A to D) of BmNPV KOVs.

The knocked out genes (ORF No. or gene name. ORF No. of AcMNPV is parenthesized) and categories are presented. The numbers of ORFs refer to previous reports (Gomi *et al*., 1999; Katsuma *et al*., 2011; Ayres *et al*., 1994). "*" on the ORF No. indicates that there is no repot concerning KOV of the ORF so far.

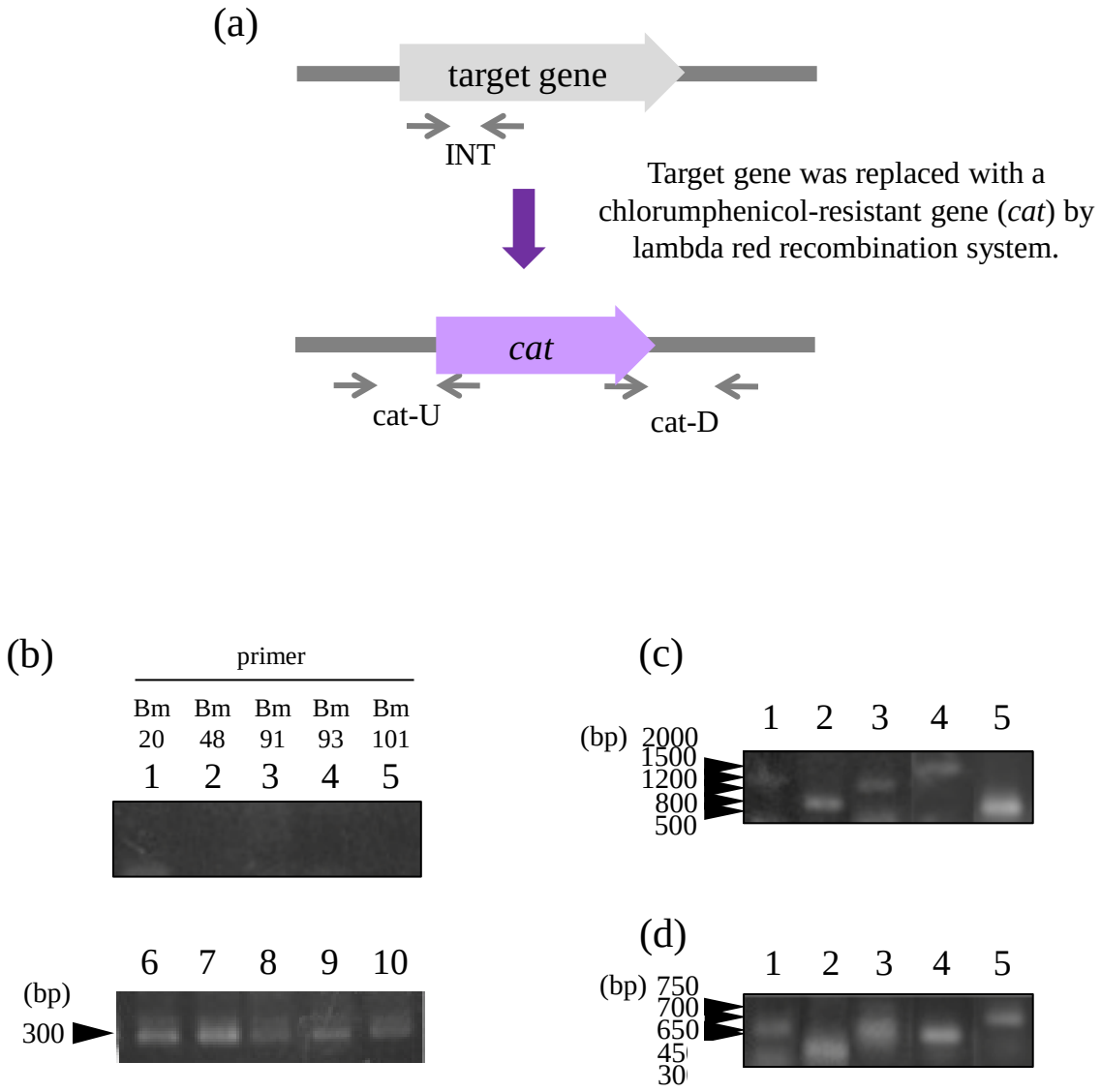
(a)
target gene
INT
Target gene was replaced with a chlorumphenicol-resistant gene (*cat*) by lambda red recombination system.
cat
cat-U
cat-D
(b)
primer
Bm 20
Bm 48
Bm 91
Bm 93
Bm 101
1 2 3 4 5
6 7 8 9 10
(bp)
300
(c)
1 2 3 4 5
(bp) 2000
1500
1200
800
500
(d)
1 2 3 4 5
(bp) 750
700
650
45
30

Fig. 1

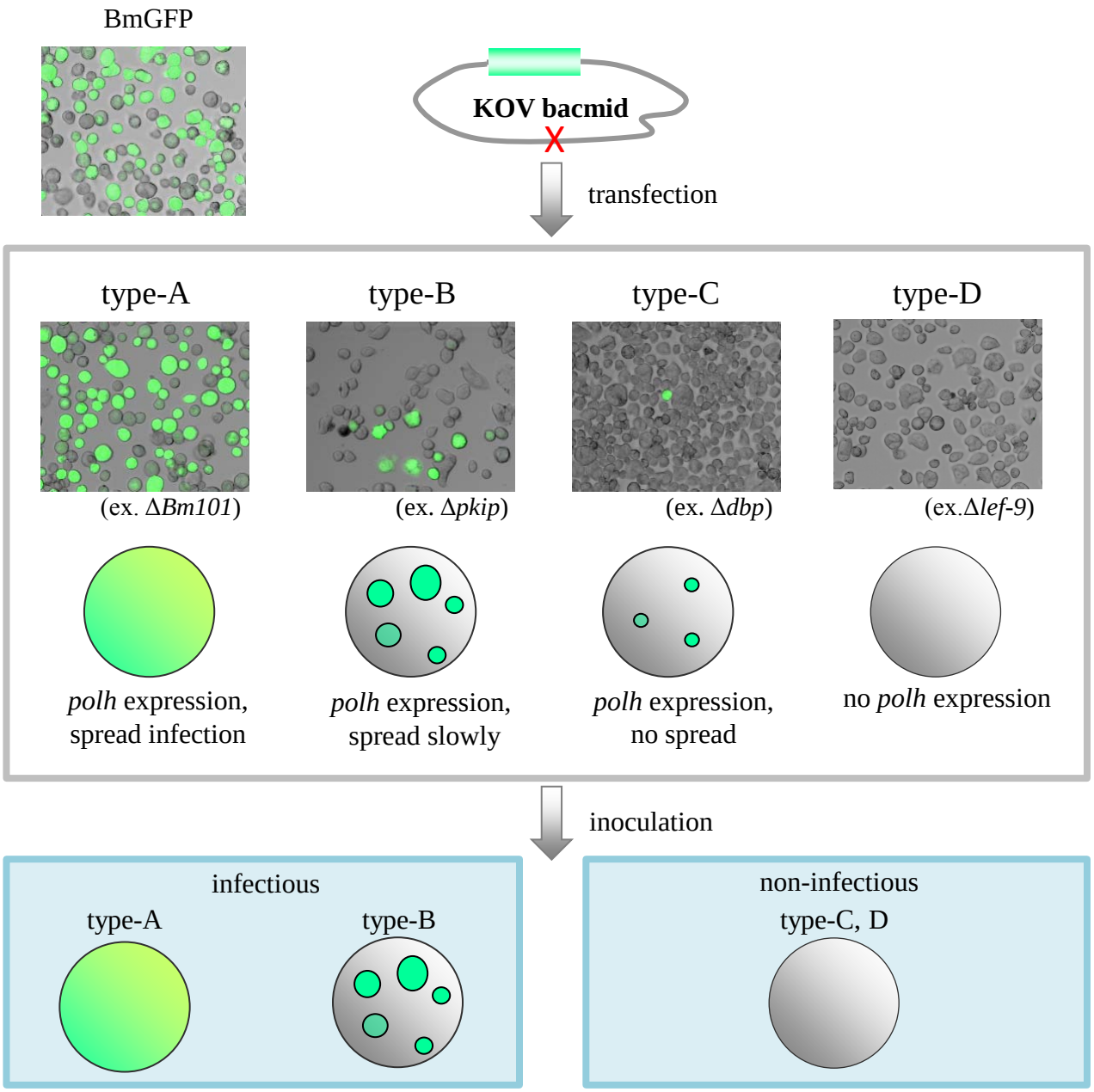
BmGFP
KOV bacmid
transfection
type-A
type-B
type-C
type-D
(ex. ΔBm101)
(ex. Δpkip)
(ex. Δdbp)
(ex.Δlef-9)
polh expression, spread infection
polh expression, spread slowly
polh expression, no spread
no polh expression
inoculation
infectious
type-A
type-B
non-infectious
type-C, D

Fig. 2

Table 1

ORF No.	Gene name	5' primer	3' primer
1	polyhedrin	-	-
2	p78/83	5'-GACGAATCGTAAATATGAATCTGTACAATCTTATTTATTCAATAATAGGAgtgtaggctggagctgcttc-3'	5'-AACACTATACATTGTTATTAGTACATTTATTAAGCGTTAGATTCTGTGCGcatatgaatatcctccttag-3'
3	Pk1	5'-ACCACCACCGCCACAAATGCTACGCTGCAAACGCTGGTACAATTTTACGAgtgtaggctggagctgcttc-3'	5'-AATAATTTTTATGATTTTTATTAACACCACTCACGACAAAAACTCATGTTcatatgaatatcctccttag-3'
4		5'-GCGTGCCTGTACATCAGTATTATTGAGCTTATCATTGCCTTACTGACAACgtgtaggctggagctgcttc-3'	5'-GTGGTGTTAATAAAAATCATAAAAATTATTGTAAATGTTTATTATTTAAAcatatgaatatcctccttag-3'
5		5'-ACGTCCAACAATGGTCGCACATCGTTAAATGGGATTCATTCAAATGCAACgtgtaggctggagctgcttc-3'	5'-TATTTATACGAAATAAATACATACTAATAACTATTATACATGTTTTTATTcatatgaatatcctccttag-3'
6	lef-1	5'-TTTTTTATAACCAAAAATTAACACAATAGAGGTCGAGTTCAAAGGGCAGC gtgtaggctggagctgcttc-3'	5'-TTTGTCTAGCAATTCCTTTGTTATACAACGAGAAAATTTAGTTCCCTTATcatatgaatatcctccttag-3'
7	egt	5'-GAAGCAAAAAATGACTATTCTTTGCTGGCTTGCACTGCTGTCTACGCTTACgtgtaggctggagctgcttc-3'	5'-CAAACCAAAAAAACCAGGTAAATAATGATGAATAACATTTATTGACGTAAcatatgaatatcctccttag-3'
7a		5'-ATTGTAATGAATCATATAGCACACTTTTAGTTACGTCAATAAATGTTATTgtgtaggctggagctgcttc-3'	5'-TTCGATGTTTTCGCCTTTGAACGTCCTGGCACAATAATGACTGCTCTGGTcatatgaatatcctccttag-3'
8	bv/odv-e26	5'-CAACCAGAGCAGTCATTATTGTGCCAGGACGTTCAAAGGCGAAAACATCGAAgtgtaggctggagctgcttc-3'	5'-ATTAAACGTTTGCCGCTAGCAATCTCGTCCGGATTTATAAACTCGAACCGcatatgaatatcctccttag-3'
9		5'-TTTGAAGGGTGAGGAAGAGCCCAATTGCGTTGAACGCATCACCATAATGCgtgtaggctggagctgcttc-3'	5'-AATTTAAAAATAAATAAAATAGTTAATAGCTGTCTACCCGTAAAATAATTcatatgaatatcctccttag-3'
10		5'-TATGGAACGTTTGTTAAATCAACTAAATCTTGGCGTTCTACCTTATATTAgtgtaggctggagctgcttc-3'	5'-ATGTATTGTTAGAAAGTTGTGTTGTTTTATTAGTATAACGAAAAAATACAcatatgaatatcctccttag-3'
11		5'-AATGAATAGCGGCGACGACGACGCAACACGACTACACTATTCAAACGCGCgtgtaggctggagctgcttc-3'	5'-TATTTATTTTTAATAATATTTTTCCAACAAAAAACTTAACACGTTGGTTAcatatgaatatcctccttag-3'
12	arif-1	5'-AGTATTCGATACATATTGCCATTACTTAGTTGTTGAATTAAATATTCAAAgtgtaggctggagctgcttc-3'	5'-ATTACATTAATACTTTATATAATCAAAAATAATACTTTATATAATATTTTcatatgaatatcctccttag-3'
13		5'-GTTTGTATTTCTTTATATAGTATACCAGCCCTTTTATCAGGCATACTTACgtgtaggctggagctgcttc-3'	5'-ACTAACTACTGACGTAACATTTAAACGACTTATTGTTTGTGTTATCATAAcatatgaatatcctccttag-3'
14		5'-ATAAGCCGTACATGTTGGCTTGCTAATTCAGTCAATATCAGACGTTTATCgtgtaggctggagctgcttc-3'	5'-ATTTTAATTATACATGTTTTATTTTATTCTTTCAATAATCATAGGATACAcatatgaatatcctccttag-3'
15	pkip	5'-AAAAATGTTGTTTTTTATTATAAGTCAAAATGTCTTGCATATTAACTGCGgtgtaggctggagctgcttc-3'	5'-TTTTATTTATATACTATTTTCTATTAAATATTCCAATGCACACAAATGTTcatatgaatatcctccttag-3'
16	dbp	5'-GAAACACTTCAATTAGACTTGAACCACAGCAGACAGCGCACGTCGGTAGCgtgtaggctggagctgcttc-3'	5'-ATGCAAGACATTTTGACTTATAATAAAAAACAACATTTTTATATATTCATcatatgaatatcctccttag-3'
17		5'-GGTTCAAGTCTAATTGAAGTGTTTCACAGAATATAAGATATATAATAAATgtgtaggctggagctgcttc-3'	5'-TTTAACTCGTTAAAGTTACGGTTAAAATGAGCACGTTTGGGTCGTTTTGGcatatgaatatcctccttag-3'
18	iap1	5'-AATGAACGAGGACACTCCTCCGTTTTATTTTATCAACACGCGCGACAACTgtgtaggctggagctgcttc-3'	5'-TTATAAAATCGGTGACGTCTTGACGACAGGTCGGACACTTTTGATCTAATcatatgaatatcctccttag-3'
19	lef-6	5'-TAAAATGGTGTTCGACGTGTACTACAACGGCTATTATGTGGAAAAAAAATgtgtaggctggagctgcttc-3'	5'-AATACATGTTTTATTGTTTTTCTAATACATTCAAGTCGTTTAGATGATTAcatatgaatatcctccttag-3'
20		5'-CTGTTTTACTTCATCTGTATATTTTTTCAAGATGTTCTCAAAGAATTACAgtgtaggctggagctgcttc-3'	5'-TTAAAAATTATTTATTCCGTAATTAAGAAAATTTTGTCTTGACTTCATAAcatatgaatatcctccttag-3'
21		5'-TAAAATACTTTATTCCATTTGATTTTTTTTATTTATGTGTATAACATATTgtgtaggctggagctgcttc-3'	5'-CATCTTTTTATCCAAATTAGGAGTTAATTATTATTCATTTGTATCGCGACcatatgaatatcctccttag-3'
22	bro-a	5'-AATAAATTTAATTTAATAAATTAAAATGGCTCAAGTTAAAATgtgtaggctggagctgcttc-3'	5'-TTAAAATTGTTATTCAATCGTGCAATCTGACGTTCCGTCATGCTCGACTGcatatgaatatcctccttag-3'

Table 1 Continued.

23	sod	5'-AGTAGGGTGACTGTTTGCTCAAAATTAAAATGGTGTAATTAAGAAACAACgtgtaggctggagctgcttc-3'	5'-AAAGCGATGACATCATTTACATATGGCAATTATTCCGCATCCCAAACGGCcatatgaatatcctccttag-3'
24	fgf	5'-CAAATTTTTATATAAAGGGGCCACTTTGCTATGGGGAATTAAAATTCGTCgtgtaggctggagctgcttc-3'	5'-ATAAAATAATTATTTAAATAAAAATGTTTTTATTGTAAAATACACATTGAcatatgaatatcctccttag-3'
25		5'-TTTTACACTATTACTTATAAATGACAACGGTTGCTGTGAATGTGCCCTTGgtgtaggctggagctgcttc-3'	5'-CTAGTTAATAATTTGTGTGATCAGATAACTATTAACGTCCACATGGTATTcatatgaatatcctccttag-3'
26	ubiquitin	5'-TTATAAGTAATAGTGTAAAAATGCAAATATTCATCAAAACATTGACGGGCgtgtaggctggagctgcttc-3'	5'-ATAAAAAACTTTGTACATTTAATGATTTTTATTATTATTATTATTATTATcatatgaatatcctccttag-3'
27	39k	5'-ACATGCCGGAGCAACAATCTTCTACAGAAACTGCGGCCGTGTGCAAAAATgtgtaggctggagctgcttc-3'	5'-ATAATAATAATAATAATAATAAAAATCATTAAATGTACAAAGTTTTTTATcatatgaatatcctccttag-3'
28	lef-11	5'-GCACGCACTTAGGCGGGTGTAATTCGGACTGCTTGACCCGAAGCGAAATAgtgtaggctggagctgcttc-3'	5'-TTTGTGATGAAGACACACCTTACGCTTAGAACATTTGTCGGTAATTACTGcatatgaatatcctccttag-3'
29		5'-TTAAACGATGGTGCTCGTGTGCGCAATTTTTTAAGTGTCAAATATGTAAAgtgtaggctggagctgcttc-3'	5'-AAATATTTGTTGAGCGGCACGATGGCCAGGCTGCGCGTTATTTCGCAATTcatatgaatatcctccttag-3'
30	p43	5'-GCCAATCATGGACAAACGTGCCAATTCTCGCAAACCCTTTCTGTTCTACAgtgtaggctggagctgcttc-3'	5'-TAAAAAATTGCGCACACGAGCACCATCGTTTAATACCTGATTGCAACTATcatatgaatatcctccttag-3'
31	p47	5'-CGCGTTATTTTCCATAATTATGTTTGTCACCCGGTTGGAGCACACCACAC gtgtaggctggagctgcttc-3'	5'-GATTGGCTCAGTATAACGACTGGTCAAAATTGACAACGCGTTTGCCGTAAcatatgaatatcctccttag-3'
32	lef-12	5'-ACACGTAGAATTCAACAGACGACTAGAGTACGTAGGCACAATCGCCACAA gtgtaggctggagctgcttc-3'	5'-ATTAAACTACGGTTATTGCGCTAAAATATCACATTTAACACGCACATTATcatatgaatatcctccttag-3'
33	gta	5'-TTTAGCGCAATAACCGTAGTTTAATCGAAGAGAATAGCCGTCTCGCCACAgtgtaggctggagctgcttc-3'	5'-GGCGACTTACCAGCTACAAGTTGAAAAATTTTTTAATGTCTTCGTAATTGcatatgaatatcctccttag-3'
34		5'-CTGGTAAGTCGCCATGAACACCCGATACGCTACTTGCTATGTTTGCGACGgtgtaggctggagctgcttc-3'	5'-GTTGTTGAGCGCGGCGGTTGGGGTAAATTGGAATCGAAATTCCGTTGGTAcatatgaatatcctccttag-3'
35		5'-GACGCAAGCGCTTCGAGTTTTGGCTCGCTCGCTACCTCCGCTGTACGACTgtgtaggctggagctgcttc-3'	5'-GTTACAAAGTTTTGTATTTCAATTTATATCTAATGCCGCTTTTACATTTAcatatgaatatcctccttag-3'
36		5'-CATGTTGCCCTACGAAATGGTGATTTCCGTGTTGGTTTACTTGTCGCCGGgtgtaggctggagctgcttc-3'	5'-GTCGTATGTTGCTTATTATTGATACAAAGTTTTCTTAATTGTAAACACACcatatgaatatcctccttag-3'
37	odv-e66	5'-TTATTGTCGTAGTTGTAATATTTTTAATATGTTTTTGGTGCCTATTAAATgtgtaggctggagctgcttc-3'	5'-CTTTTGTGTTAACAATCAGTCTTTTGAATATTACATTCTCCCTAAATGTAcatatgaatatcctccttag-3'
38	ets	5'-ATATATATGTATGCAACAACTTTTTAATATTCACATTAAAAAGTGCCAGGgtgtaggctggagctgcttc-3'	5'-AGACTGATATTGTTGTTGTTGTTGTTACAAAATAGAAAATAAAAAATATAcatatgaatatcctccttag-3'
39	lef-8	5'-AATTAGTGTGCAATAATCGCTTTACGATGACGGACGTAGTTCAAGATTTCgtgtaggctggagctgcttc-3'	5'-TTTTCGTGCACAGATATAATTTTATCATGTTTGCAATCGTGCAAGAGTTAcatatgaatatcctccttag-3'
40		5'-CGTAAAGCGATTATTGCACACTAATTATGTCTAAACGTGTTCGTGAAATGgtgtaggctggagctgcttc-3'	5'-GATTTTAAAGAGTTTTTTAAATCCTCAATGTTCTTGCTTATTATGTCAATcatatgaatatcctccttag-3'
41		5'-AATGACGTGGCCTTATTCCAATAGTTTTAAACTTATCAAAGCTTATCAAAgtgtaggctggagctgcttc-3'	5'-ATTAAAATGTCGAACAAAGGAAAAAAACAATTGTAACAAAAATAATTTACcatatgaatatcctccttag-3'
42	Bm42	5'-TATGTTCTGCACCGTTTGTTTAAAAGACAGAAATTACTATATGTTCAAACgtgtaggctggagctgcttc-3'	5'-CCTTTGGTTTCTCGAAGCATCGCGTTGCATTCCTCTAGCGTTTTTGGGGGcatatgaatatcctccttag-3'
42a	lef-10	5'-CCCTGGACATTGAACTCGATTTTAGGAATTTTTTTAAAATGCAATCATGAgtgtaggctggagctgcttc-3'	5'-TTCACCGAGGCACAGAGGTCTAACTTGATCGGTTTCTTGGTCGAACACATcatatgaatatcctccttag-3'
43	vp1054	5'-CAATTATTCAGATCCCGATAACGAAAACGACATGTTGCACATGACCGTGTgtgtaggctggagctgcttc-3'	5'-AATAAGAATGCTTGTTTAACAAATAGGTCAGCTGTTAAATATTGGAGATGcatatgaatatcctccttag-3'
44		5'-TTAAACAAGCATTCTTATTTCAATAATTGGAGACAGTTGTATCCACAATTgtgtaggctggagctgcttc-3'	5'-GTCAATATTTTTTCACCAATTTGTCAAATTTATTGTAATCTCGCTTGCCGcatatgaatatcctccttag-3'
45		5'-CATGTTGCGATCAATCATACGACGTTTCAAGAGTACAAACAATCTCAGCAgtgtaggctggagctgcttc-3'	5'-ATATCTAGCGCAATTAAATGAAACCATAAAAATACAAACAACAAACTTTAcatatgaatatcctccttag-3'
46		5'-CGGTTTATTTCACTCGAAGGTATTATCTCAGACAGTCGAACGTGCGCGCAgtgtaggctggagctgcttc-3'	5'-TAAATTAATTTTTTTTTTACTTGTACAGTTTTATTTCTAGTTCACGATCCcatatgaatatcctccttag-3'

Table 1 Continued.

47		5'-TTCTAATTACAATGTATCAAATTCCCGATATGTTATACAATGAAAAAATGgtgtaggctggagctgcttc-3'	5'-AAAAAAAATTAATTTATTAATAGTTGTAATAATTATCTTCGTCCTCATCTcatatgaatatcctccttag-3'
48		5'-AATAAAACACACACACACACAAAGTTTTTATTATATTGTCTTTTATTGATgtgtaggctggagctgcttc-3'	5'-TGTAATTAGAATTAAGCAATTTCGTCTTCGGTTTCTGTATCTGTAGTGGAcatatgaatatcctccttag-3'
49	fp	5'-TTACGCACCATATACGCATCGGGTTGATATAATTAATATGGATCAATTTGgtgtaggctggagctgcttc-3'	5'-TTCTTACATTACGAGATTCAACTTGATACTAAAATTAATTATCACTTAAAcatatgaatatcctccttag-3'
50	lef-9	5'-CTCTTATACTTTGCACTTTGCGTTAATACGTGTTCGTGTACAGACGTAATgtgtaggctggagctgcttc-3'	5'-AATTAATAAAACACTGATTGCATCTAATTAAATACGCTTTATTTATATTAcatatgaatatcctccttag-3'
51		5'-AAAGCGTATTTAATTAGATGCAATCAGTGTTTTATTAATTTTAAAGCAACgtgtaggctggagctgcttc-3'	5'-GGGCATTTTTTTTAATCCAGGATTTTAATAAAATAAAAACAAAATTTTAAcatatgaatatcctccttag-3'
52	gp37	5'-GTTACGACAGCGGTTATTATTCCGATTAGTGTTCTATAAGTATAATCATAgtgtaggctggagctgcttc-3'	5'-AATCCTGGATTAAAAAAAATGCCCCAATCCCAAGTTTTTGACACCTATGAcatatgaatatcctccttag-3'
53	dnapol	5'-ATTTAAAAATGAAAATATATTCTTACAATGAACTCAAAACGCGCTTTGCAgtgtaggctggagctgcttc-3'	5'-ATAACACCTTACAGTAACATACAATAAAACAACATAGTATCGTATATAATcatatgaatatcctccttag-3'
54		5'-CATAAATACCATGAGTGCTCGAATCAAAACTCTGGAGCGGTATGAGCACGgtgtaggctggagctgcttc-3'	5'-TTCTATTCGACGTTTGGTTGAACGCTGGCCGCTGTTGTTCGCGCCAACGTcatatgaatatcctccttag-3'
55	lef-3	5'-ATTGACAACAACAGCAATATGGCGACCAAAAGATTTTTTTCTGGAGAAAG gtgtaggctggagctgcttc-3'	5'-AATTACAAAAATGTATAATCATTTTCATCTTCGTCATACTTCAACAATCCcatatgaatatcctccttag-3'
56		5'-ATTGCTGTTGTTGTCAATATGTGGGAATCTATACGATGGCAAATACTGAAgtgtaggctggagctgcttc-3'	5'-GGTCGTTTGATCGTTAAATCGAGCGGGTCTGTTGAGAGCAGCTCTTATTCcatatgaatatcctccttag-3'
57		5'-ACTCAAAGATAATTTAAACACGTTCAGCAGCAAGTCGGTGGTTTGCGCTCgtgtaggctggagctgcttc-3'	5'-CATGTTGAATGATGCCTGATTTGAAGCCGCTGGCTTTTTATATACACACGcatatgaatatcctccttag-3'
58	iap2	5'-TCATTTACGATGTCGACAATTATTCTTTCAAAAATGATAGTGTGCAATAAgtgtaggctggagctgcttc-3'	5'-ATAATTATTAATAGCTTATTTTTAAAGAATGTCGTAGTCATTTGCAATGTcatatgaatatcctccttag-3'
58a		5'-AATGACTACGACATTCTTTAAAAATAAGCTATTAATAATTATTGCATTGTgtgtaggctggagctgcttc-3'	5'-CATTATGTACAATAATATGGGTTTTTATTTTACAACATTTATGTATATGAcatatgaatatcctccttag-3'
59		5'-ACCAATATCTAAACAGCGACACGCCCGATTACACCATAGAATCCTTAAACgtgtaggctggagctgcttc-3'	5'-ATAACAATTGTAATTTCATATACATAAATGTTGTAAAATAAAAACCCATAcatatgaatatcctccttag-3'
60		5'-GATAAATAAAAATGAAAATAAATTTGTGCAATATACAATTTCAGTCTTTAgtgtaggctggagctgcttc-3'	5'-ATCCAATTTCGTGTTCAAGAAATTGGTGATGATATCTTTGAACGTGCACGcatatgaatatcctccttag-3'
61		5'-TAATAAGTGCTCGTTAAAATGTCCAATTTAATGAAAAACTTTTTCACCGAgtgtaggctggagctgcttc-3'	5'-TTTTATTTATCTTACTATTAATACGCCGGCAATTGGTATGCTTCGTCCAGcatatgaatatcctccttag-3'
62		5'-AGTAAGGGAATAAAAATGAATTTATATTTGTTGTTGGGCGCACTGACCATgtgtaggctggagctgcttc-3'	5'-TTTAACGAGCACTTATTATCAATCTATTGAGCTGGTATTTTTGTTTAAAAcatatgaatatcctccttag-3'
63	vlf-1	5'-ACAATGAACGGTTTTAATGTTCGCAACGAAAACAATTTTAATTCTTGGAAgtgtaggctggagctgcttc-3'	5'-TTTTATTCCCTTACTCTATTCGTTGCGATAGTACAACAACGATTCTCCCGcatatgaatatcctccttag-3'
64		5'-ACATGAATTTGGACGTGCCATACTATCGGTTGGGCAACCACGAAAAGGTGgtgtaggctggagctgcttc-3'	5'-AGGAGAATCAGTGTCAATACTATCCGAATATTGTTGTTGTTCTCTTAATAcatatgaatatcctccttag-3'
65		5'-CGCACTGTATAATCATGGCGACGACTCTGTACACCAACAAGGTGTGGTGCgtgtaggctggagctgcttc-3'	5'-GTTTACAACTTATTTGCTAACAGAAATTTATGCAACAAAAGTGGTTTGGCcatatgaatatcctccttag-3'
66	gp41	5'-ACGTGGCAATTTTTATTACAACACCCCTCCGCCGCCGCTGAGGTATCCCTgtgtaggctggagctgcttc-3'	5'-GATTATACAGTGCGTCCTTTCGTGTTTGGCCGAGTGACGTTAGGCGCGGCcatatgaatatcctccttag-3'
67		5'-ACAACGCTGAACAAAATTAAATACGATAGTGAACTGTTGCTTCATTATTTgtgtaggctggagctgcttc-3'	5'-ACTTTTTATTTGTACTCTTATGATTACAAAACTCAATACACGGATTACTTcatatgaatatcctccttag-3'
68		5'-GGGCGTTGGCGCCCATATTATCAAGGTGGCTAGCTCGCAGCAACTTAGATgtgtaggctggagctgcttc-3'	5'-CTACGGCGCGTTTGGGCGACGACGTGTTTACAGCAGCGTCCGTCTTATTAcatatgaatatcctccttag-3'
69	p95	5'-TTTTCTTATAATAGCGTTTACTTTAATTTATTTGGCAATTTATTTTAAATgtgtaggctggagctgcttc-3'	5'-ATTTGTTTTCGACATAAAATGTTTATACAATGGAATCTTCTTGTAAATTAcatatgaatatcctccttag-3'
70	vp15	5'-TATATAAACGCTATATAACAGTTTTTGCTAGTGTTATTTTATACAACATTgtgtaggctggagctgcttc-3'	5'-TTAATTAATTGTTTACATAACATTCCACTTTAATGTAATAATATTCTTTAcatatgaatatcctccttag-3'

Table 1 Continued.

71	cg30	5'-AAATGGAGTTTGTCAAATTGCAATGCAACATTTGTTTTTCGGTTGCAGAAgtgtaggctggagctgcttc-3'	5'-TTAATTAATCTACATTTATTGTAACATTTGTGGTGATAGTAGTAGCGTTTcatatgaatatcctccttag-3'
72	vp39	5'-ATTTATAACGGCAACAATATGGCGCTAATGCCCGTGGGTATGGCGCCGCGgtgtaggctggagctgcttc-3'	5'-TTTTAGGCGGCTACACCTCCGCCTGCTTCGCCGAGAACAACACCAGGCGCcatatgaatatcctccttag-3'
73	lef-4	5'-ATTGTTGCCGTTATAAATATGGACCACGGCAATTTTATGATTGAGAAAGAgtgtaggctggagctgcttc-3'	5'-TTTGGCACGATTCGGTCGCGACGATGTTTCAACACGTTTATTGTCGTGTCcatatgaatatcctccttag-3'
74		5'-TACAAGTTTTTGTTTGATTCTTTTTATTAAGCAACATGTGGTACATTGTTgtgtaggctggagctgcttc-3'	5'-AGCACGTTAAGCGGATAGAACGGGCAGTCTGAGCTGTTAAAGTCAATACAcatatgaatatcctccttag-3'
75	p33	5'-TCTTCGCAAGCAACTTACTTATACCATTTTGCGTGTTTGATGAAATACAAgtgtaggctggagctgcttc-3'	5'-GTTGCTTAATAAAAAGAATCAAACAAAAACTTGTATTATTGCAAATTTAAcatatgaatatcctccttag-3'
76		5'-CGCCGACGAGCCTATTATTTATTTCGAAAATATTACAGAATGTCTTACGGgtgtaggctggagctgcttc-3'	5'-AATGTTTCAAAAGTACACTTAAATTATCGTTTTCCCAATTGACGGGCACGcatatgaatatcctccttag-3'
77	odv-e25	5'-AACAAATCATGTGGAAAATCGTGTTATTGATCGTTTTGCTCGTACTGATTgtgtaggctggagctgcttc-3'	5'-TAAATAATATATAAATAGACTTTTTTTGTTTTATTAATTCATTTCTCTGTcatatgaatatcctccttag-3'
78	dnahel	5'-TTTACAATTTTTTTTAAAAGACGTGCCTGAAGACAAAACGTACGAGATTAgtgtaggctggagctgcttc-3'	5'-ACAGAGAAATGAATTAATAAAACAAAAAAAGTCTATTTATATATTATTTAcatatgaatatcctccttag-3'
79		5'-TTTGGCTATCGTGTTTATATTTTTCGTGTTAATTTATTTAATAATTTCGAgtgtaggctggagctgcttc-3'	5'-TTAATAAACTTGGTCTGTAGATGAAACATTTTGGTTGTCCAAGTCCACGTcatatgaatatcctccttag-3'
80	bro-b	same as bro-a 5' primer	5'-TTACAACGACGCGTGGCGATTCGACGAAAGCGCGAAACAAACTGAACGGAcatatgaatatcctccttag-3'
81	bro-c	same as bro-a 5' primer	5'-CACTTTGCTGGTCACCCAATGCTTAAACGCTTGACGACATATAAAAATTTcatatgaatatcctccttag-3'
82	38k	5'-GTTCTCGTTTTAAGCGAGTACGCAGACCTCAAATACCTTGGCTTTGAAAAgtgtaggctggagctgcttc-3'	5'-AATGCATAATAAATAAAACATTTTGTAATTTAATACTGTTTTTTATTAACcatatgaatatcctccttag-3'
83	lef-5	5'-TTTTCAAAGCCAAGGTATTTGAGGTCTGCGTACTCGCTTAAAACGAGAACgtgtaggctggagctgcttc-3'	5'-CAGCCAGACATTCCACACATCCGACAGTAGCGAAGGAACGAAGCGATTTCcatatgaatatcctccttag-3'
84	p6.9	5'-TAAGGTAAAAACTACAGCTACATAAATTACACAATTTAAACATGGTTTATgtgtaggctggagctgcttc-3'	5'-TAGTAGCGTGTTCTGTAACTTCGGCGGCTTGTCAATGAACGGCTCCTGGAcatatgaatatcctccttag-3'
85	P40	5'-GTGTGCGTCGTCGGTCACGATGAGCGCTATCGCGTTGTATTTGGAAATAAgtgtaggctggagctgcttc-3'	5'-GTTTAAATTGTGTAATTTATGTAGCTGTAGTTTTTACCTTATTAATATTTcatatgaatatcctccttag-3'
86	P12	5'-TATCGATGATATGGACACTGGCAACAACATGTCGCAAACACGAAGAAGCCgtgtaggctggagctgcttc-3'	5'-CGTGACCGACGACGCACACTTACTCTGTAACTATCACTTGGATCGTGTTGcatatgaatatcctccttag-3'
87	P45	5'-TTATAAGGTTATATTGAATGCACCATGTGCGCTTACAGATTACAATACAGgtgtaggctggagctgcttc-3'	5'-TGATGTGCCTTATAAATGATTCGGGCACAATCATTTCTGTCATTAGCACGcatatgaatatcctccttag-3'
88	vp80	5'-GGTGCATTCAATATAACCTTATAATATGAACGATTCCAATTCACTGTTGAgtgtaggctggagctgcttc-3'	5'-TTTTTTATTATATAACATTGTAGTTTGCGTTCATTAACATTATTAGTCTTcatatgaatatcctccttag-3'
89	he65	5'-TTTGAGGCATATAAGGCTTGATACAGGCACAGTAAGCAACGCTGCTAACgtgtaggctggagctgcttc-3'	5'-ATTTTTAATTTTTATTTATATGCGACACAAATGGATAAATTTGATGGTACcatatgaatatcctccttag-3'
90		5'-CGTCAATGAAACAACGTGTAGTATTTTTTACAATATTTAAGTGAAACATTgtgtaggctggagctgcttc-3'	5'-TTACAAAGAAAACAAAGGCAATAAATGATTCTTTTTTTCAAGTTTACGCAcatatgaatatcctccttag-3'
91		5'-TTAAGAAGCACATTATGAAACCGACGGCGGCCGACATTATATCAAGAGTGgtgtaggctggagctgcttc-3'	5'-TTATGTTGTTGCATTTCTATTTCTAATATCATAATTTTCTAATAAAGTAGcatatgaatatcctccttag-3'
92	odv-ec43	5'-GTTCACAAATTAAAATCTAAAGTCTAATGAAGTAAGACATATACATCGCGgtgtaggctggagctgcttc-3'	5'-AATGTGCTTCTTAACTACAAATAATAGTTGTACTTGATGGGCGTCACCGTcatatgaatatcctccttag-3'
92a		5'-AAACGTATAAAAGTATTATGTGTTTTATTTAACTAGCATAAGATTTAAAGgtgtaggctggagctgcttc-3'	5'-CGCGATGTATATGTCTTACTTCATTAGACTTTAGATTTTAATTTGTGAACcatatgaatatcctccttag-3'
93		5'-ACAATAAAAAATTAAAACATATGCATCAGTGATATTGCCTGTGCTGCACTgtgtaggctggagctgcttc-3'	5'-ATCTTTAAATCTTATGCTAGTTAAATAAAACACATAATACTTTTATACGTcatatgaatatcctccttag-3'
94		5'-CCAATTTGTATAGACGCGTTTACAACTGAAATGTAAGAGATTCTGTCAACgtgtaggctggagctgcttc-3'	5'-GTAATTGATTAATATGTCGTACAGTTTGGCGGCGCTGTGTTTGCACACAAcatatgaatatcctccttag-3'

Table 1 Continued.

95		5'-TCTTCACACTCAATTAAGACGATCATGTTGAACTTTTGGCAAATACTTATgtgtaggctggagctgcttc-3'	5'-GTTGACAGAATCTCTTACATTTCAGTTGTAAACGCGTCTATACAAATTGGcatatgaatatcctccttag-3'
95a-96		5'-TAAACCTGGGTCTATATAACTCGGCGTCGGCCGAGTTTATTTTTAACATTgtgtaggctggagctgcttc-3'	5'-TCTTTAATTATTAAATAAAACAAAATGATATAATAAAATTTGGTATTTATcatatgaatatcctccttag-3'
96		5'-TTATAATACATATTTGAAAAATTACAGTGTAATTGAAGGTGCGATGTGTTgtgtaggctggagctgcttc-3'	5'-TCTTTAATTATTAAATAAAACAAAATGATATAATAAAATTTGGTATTTATcatatgaatatcctccttag-3'
97		5'-CAAAATATCTCGTACGTACAAAAATACTCGGACATTCTCCAATAAGTAAAgtgtaggctggagctgcttc-3'	5'-GTCTATTTGTTGCCCTTGGGTCATGGCGCCTGTAGTAATCGTTCTTATTGcatatgaatatcctccttag-3'
98		5'-TAACAACATGAGCATTTTAAACGTTGTAGAAGCGTGCGATTTGGCACACAgtgtaggctggagctgcttc-3'	5'-TATTTTTGCACACAAAATATTGAACTCGTTATGATTTCATCATTTGATAGcatatgaatatcctccttag-3'
98a		5'-CTATCAAATGATGAAATCATAACGAGTTCAATATTTTGTGTGCAAAAATAgtgtaggctggagctgcttc-3'	5'-ACACGAAGACAAATTGATTCAATGTAAAATTTATTTGTAATAAATTATATcatatgaatatcctccttag-3'
99		5'-AAGCGACCCATATATTGTCGAATATAGAACACCATGAAGCTGATTATCCTgtgtaggctggagctgcttc-3'	5'-TCAGCCCCTCGGGATGGCCAAAATGTGCCGTAGTTTTTAATGGATCTCTCcatatgaatatcctccttag-3'
100	Pk2	5'-TGGGAGCTCGTGTTTGAATTTAGTGACGCGTTAACACTTAATTGTATACCgtgtaggctggagctgcttc-3'	5'-GGTGTTCTATATTCGACAATATATGGGTCGCTTTTAAATCGCCTTGTCCCcatatgaatatcctccttag-3'
101		5'-AAGCATTTGTATATAATCAATTGAATTGTGCACTAATATAAGAATTAAAAgtgtaggctggagctgcttc-3'	5'-AAAACACATTTTTTTTATAAATTACTTTTTATTTTTTATGTCATCCATTAcatatgaatatcctccttag-3'
102	lef-7	5'-AAGCAAGTATCATTTGTGTGCAGTTGTTATTAGGATGTCGAGCGTTACAAgtgtaggctggagctgcttc-3'	5'-TAATGGATGACATAAAAAATAAAAAGTAATTTATAAAAAAAATGTGTTTTcatatgaatatcctccttag-3'
103	chitinase	5'-TAAACGTTTTGTGGTTGGTGGTCGCCGTTTCCAACGCGATTCCCGGCACGgtgtaggctggagctgcttc-3'	5'-CTCTAAGTTTAAACTGTGCGTTCATCGCGTTGAGCAAGTCGCCGTTATCGcatatgaatatcctccttag-3'
104	v-cath	5'-TTTAATTTGTCTTAATTTTAAGATGTAATTATTTTATGTAAAAAAAAAAATgtgtaggctggagctgcttc-3'	5'-TAATTACAAAATATCTTAGGCGATATGATTATGTTCCAAATAGCGAGAGAcatatgaatatcctccttag-3'
105	gp64/67	5'-GTGCCTTGTGTCACGTGGCCAAATAGCGGTCGGGTATATAAGATGCCTCAgtgtaggctggagctgcttc-3'	5'-TTTAATATTGTCTACTATTACGGTTTCTAACCATACAGTACAAAAATAAAcatatgaatatcctccttag-3'
106	p24	5'-GCATCTTATATACCCGACCGCTATTTGGCCACGTGACACAAGGCACGTTGgtgtaggctggagctgcttc-3'	5'-TGTCGAAATCGTACAAAACCCTTATTATTATTTATTCAGGCACATTAAATcatatgaatatcctccttag-3'
107	gp16	5'-TAATAAGGGTTTTGTACGATTTCGACAATGAACTTTTGGGCCACGTTTAGgtgtaggctggagctgcttc-3'	5'-ATTGAACGTTAAGCACGTTGTTAACTACCCCGTTTTTTGTTAATAGCACGcatatgaatatcctccttag-3'
108	pp34	5'-CACTAAGTTAATATACTAAAAATCATATAGTCGCTACAATATTTTGAAATgtgtaggctggagctgcttc-3'	5'-GCTTATTTTGTTGTCAACGTTCCCGTGAGTATATTTTTGATATCGTTAACcatatgaatatcctccttag-3'
109		5'-GCATGTCCGACAAAACGCCGACAAAAAAAAGCGGCGGCCATGCCATGACGgtgtaggctggagctgcttc-3'	5'-GATGTCGACGTGCTAACTTTACCAAATCTAAAGACCTAATGGTAAAATGGcatatgaatatcctccttag-3'
110	alk-exo	5'-TTTAACAATTATGTGAAAACGATCGAGTTGAGTCGAGCGCAGTTGTCTCGgtgtaggctggagctgcttc-3'	5'-GAATATAATTAAAGATTTATCATTTTATTTCCAAATAATATACACAATTGcatatgaatatcctccttag-3'
110a		5'-ACGCGGACGCTTTGCAACATTTGAAATTTTTGAAAGTAAATTATAACAAAgtgtaggctggagctgcttc-3'	5'-TAAAATGATAAATCTTTAATTATATTCTTTTATTTATACATTAATTTTATcatatgaatatcctccttag-3'
111		5'-CAATCCACTCCACAGCGTCCAAAACGGACATATTTTTATATACACGAGCGgtgtaggctggagctgcttc-3'	5'-TTTATAATATAAACGTTCACGTTACGACGCGCGTGTAATTTTCGGGACCAcatatgaatatcctccttag-3'
112	p35	5'-ATTGCAAAATGTGTGTAATTTTTCCGGTAGAAATCGACGTGTCCCAGACGgtgtaggctggagctgcttc-3'	5'-ACACAATCAAGCAATGACAAAGAATAATATTAGGCAATAAATTTTAACATcatatgaatatcctccttag-3'
113	p26	5'-GATAATTAAATAATTTATTTGCAAGCTATACGTTGAATCGAACGGACGTTgtgtaggctggagctgcttc-3'	5'-TATAAGATTTTAATTAATATACAAATAATTTAATAATAATTCTTATTTAGcatatgaatatcctccttag-3'
114	p10	5'-TTGTATATTAATTAAAATCTTATACTGTAAATTACATTTTATTTACTATCgtgtaggctggagctgcttc-3'	5'-ACGACGAGCGTCTGAATCGAATTCAAAAGCTTGGATTTTCAGCGTAGGCT catatgaatatcctccttag-3'
115	p74	5'-TCTAATAAATATTTCATTATTATCGTTGTTTAATTTAAGTCATATATAACgtgtaggctggagctgcttc-3'	5'-CAGACGCTCGTCGTGGTAAACGCAGTTCCAAGTAAATAAATCGTTTTTTTcatatgaatatcctccttag-3'
116	me53	5'-TGAACGTGCACAGTATTGTGTTGATTTTTGAGTGCTAACCAACAGTTACAgtgtaggctggagctgcttc-3'	5'-CAATAACGATACATTTTATTTAGACATTGTTGTTTACAATATTAATTAACcatatgaatatcctccttag-3'

Table 1 Continued.

117	ie-0	5'-GCTTGCTTGCGCGCTGGATAGTATAAGTGATTGATAACGAGCAACGCAACgtgtaggctggagctgcttc-3'	5'-TGGACACTTTGCGTGCGTGGTGGCCGTTTTCCACATGGTAACGCAGCACGcatatgaatatcctccttag-3'
118	p49	5'-GCACGCAACGCAAAATGAGTGGCGGCGGCAACTTGTTGACTCTGGAAAGAgtgtaggctggagctgcttc-3'	5'-ATTATTATTATACCGAGTCGGGGATCAATAATTTGAGAAGCGTGTTCTGAcatatgaatatcctccttag-3'
119	odv-e18	5'-TAATAATATGATTTACACAGATCCCACTACTGGCGCTACGACTAGCACAGgtgtaggctggagctgcttc-3'	5'-TTTTATACACTTATCTACAACATTTGCCTTTGAGGCGTGTTCATAAAGGGcatatgaatatcctccttag-3'
120	odv-ec27	5'-ATAAGTGTATAAAAATGAAACGTGTCAAATGCAACAAAGTTCGAACGGTCgtgtaggctggagctgcttc-3'	5'-GAATTTATTTTGTCAAAATCTTCAATGGTAAATTTGAATTTGCTTAACAGcatatgaatatcctccttag-3'
121		5'-ACATCAGGCTTTAATATATATAATTTTAATAAATAATTAAATAATATACAgtgtaggctggagctgcttc-3'	5'-TAACAAGTTTCTATACATGCGAGCCGCACACCCGCTGCCCGTGCGATCGTcatatgaatatcctccttag-3'
122		5'-GCAACTGAAACAATATTCAACATGAACGTCAATTTATACTGCCCTAATGGgtgtaggctggagctgcttc-3'	5'-AGAGTGGCTTTCTAGTTGCACAACACTATTATCGATTTGCAGTTCGGGACcatatgaatatcctccttag-3'
123	ie-1	5'-AGTTGCAAGTTGACATTGGCGGCGACACGATCGTGAACAACCAAACGACTgtgtaggctggagctgcttc-3'	5'-TTTTTTTATATTTACAATTTAGTTTTTGTTCCGCAAACGTTATAGCGTCGcatatgaatatcctccttag-3'
124	odv-e56	5'-TATATTTATTAAATAAGTTTGCTTTAAAATGAGTTTTTTTACAAATCTTCgtgtaggctggagctgcttc-3'	5'-ATATTTTATATTATTTTTGTCTTTTATTATCGACGAGGGGCCGTTGTTGAcatatgaatatcctccttag-3'
125		5'-ATTTATTGAAACGGTATATAACTTTAGCGATCATACAATGGAGAGATATCgtgtaggctggagctgcttc-3'	5'-TTTAAAGCAAACTTATTTAATAAATATATCACAGTAAAGGTTTTGCAAAAcatatgaatatcctccttag-3'
126		5'-AATAGTTTATTTGTTTTTTATAATTATTTTTATTGTTTTGAAATCTAAAAgtgtaggctggagctgcttc-3'	5'-ATAAATTTTATTTTTATTTTTGTTTTGGTTAGCAGTACATCCATAATCTGATcatatgaatatcctccttag-3'
127	ie-2	5'-GCACAGTTCAAGCCTCACAGCCTAACGAACAGTATCGCTACCAGCCAGCCgtgtaggctggagctgcttc-3'	5'-TATTTATATTATTTACAACACTTAAGGTTTAGACATCTCAATAGTGTATA catatgaatatcctccttag-3'
128	pe38	5'-TTGTCAACTCGTAAGCACAGTTTGTTGCGGAGAGCCTGCCAATAAGCAAA gtgtaggctggagctgcttc-3'	5'-TACATACATATCACATTAATTTTCAAACCCAAAATTAACAGTAACATTTAcatatgaatatcctccttag-3'
129		5'-AACGTTTTTTATTTGTTTTTTTTATTTTATGTGATTAAGAAACTTTTAACgtgtaggctggagctgcttc-3'	5'-ATTGTTATTTTAAATTATAATATCATTTAAATTTTTATGATGCAAGAATTcatatgaatatcctccttag-3'
130	ptp	5'-GAGTACATATTTAGTTACGTTTCTGAGATAAGATTGAAAGCACGTGTAAAgtgtaggctggagctgcttc-3'	5'-ATTAATAAATCTTGAACGTAATTTTGTCTTTCAATTTTGTGACCTCTGGCcatatgaatatcctccttag-3'
131	bro-d	same as bro-a 5' primer	5'-TTAATATTATTTGCATTTTTTAACAAATACTTTATTCTATTTTCAAATTGcatatgaatatcctccttag-3'
132	bro-e	5'-GTTTGTTTTGCGCTTTCGTCGAATCTCTACGCGTAGTTTGTAAATAAAAAgtgtaggctggagctgcttc-3'	5'-TTACAACGACGCGTGACGATTCGACGAAAGCGCGAAACAAACTGAACGGAcatatgaatatcctccttag-3'
133		5'-AATATATTATTGGATAAAGTTGCATTAAATGAAACTAACTTACAAGATGGgtgtaggctggagctgcttc-3'	5'-AACTGAGAACAACTAGTAGTGGTGTTGCTACAAATTCCCTCCGGCGTTGAcatatgaatatcctccttag-3'
134		5'-TTAATGCAACTTTATCCAATAATATATTATGTATAGCACGTCAAAAATTAgtgtaggctggagctgcttc-3'	5'-TCTTGCACCAGCGGTCCCGATTCGACTAACGGTCGATTGGATGGCTTAATcatatgaatatcctccttag-3'
135	lef-2	5'-CGTGTGGAGTCCTCTCATTAGCGCGTCATGTTTAGACAAGAAAGCTACAT gtgtaggctggagctgcttc-3'	5'-TAATTACAAATAGGATTGAGGCCCTTGCAGTTGCCAGCAAACGGACAGAGcatatgaatatcctccttag-3'

ORF No.	Gene name	type
1 (8)	polyhedrin	A
2 (9)	p78/83	C
3 (10)	Pk1	C
4 (11) *		A
5 (13)		A
6 (14) *	lef-1	C
7 (15)	egt	A
7a (-) *		A
8 (16)	bv/odv-e26	A
9 (17)		A
10 (18)		A
11 (19) *		A
12 (20/21)	arif-1	A
13 (22)	pif-2	A
14 (23)	f protein	A
15 (24)	pkip	B
16 (25)	dbp	C
17 (26) *		B
18 (27)	iap1	A
19 (28)	lef-6	A
20 (29) *		A
21 (30)		A
22 (-)	bro-a	A
23 (31)	sod	A
24 (32)	fgf	A
25 (34) *		C
26 (35)	ubiquitin	A
27 (36)	39k	A
28 (37)	lef-11	D
29 (38)	nudix	A
30 (39)	p43	A
31 (40) *	p47	D
32 (41)	lef-12	A
33 (42)	gta	A
34 (43)		A
35 (44) *		A
36 (45) *		A
37 (46)	odv-e66	A
38 (47) *	ets	A
39 (50)	lef-8	D
40 (51) *	dnaJ domain	C

Table 2

ORF No.	Gene name	type
41 (52)		A
42 (53)		C
42a (53a) *	lef-10	D
43 (54)	vp1054	C
44 (55) *		A
45 (56) *		A
46 (57) *		A
47 (58/59)	chaB-like	A
48 (60) *	chaB-like	A
49 (61)	fp	A
50 (62) *	lef-9	D
51 (63) *		A
52 (64)	gp37	A
53 (65)	dnapol	C
54 (66)		C
55 (67)	lef-3	D
56 (68)		A
57 (69)	mtase	A
58 (71)	iap2	A
58a (72) *		A
59 (73) *		C
60 (74)		A
61 (75) *		C
62 (76)		C
63 (77)	vlf-1	C
64 (78) *		C
65 (79) *		B
66 (80)	gp41	B
67 (81)		C
68 (82)	tlp	A
69 (83) *	p95	C
70 (87) *	p15	A
71 (88)	cg30	A
72 (89) *	vp39	C
73 (90)	lef-4	D
74 (91)		A
75 (92)	p33	C
76 (93) *		C
77 (94) *	odv-e25	C
78 (95)	dnahel	D
79 (96)	pif-4	C

Table 2 Continued.

ORF No.	Gene name	type
80 (-)	bro-b	A
81 (-)	bro-c	A
82 (98)	38k	C
83 (99)	lef-5	D
84 (100)	p6.9	C
85 (101)	bv/odv-c42	C
86 (102) *	p12	C
87 (103)	p45	C
88 (104)	vp80	C
89 (105)	he65	A
90 (106/107)		C
91 (108) *		A
92 (109)	odv-ec43	C
92a (110) *		A
93 (111) *		A
94 (114)		A
95 (115)	pif-3	A
95a-96 (116-117) *		A
96 (117)		A
97 (119)	pif-1	A
98 (120) *		A
98a (121) *		A
99 (122) *		A
100 (123)	Pk2	A
101 (124) *		A
102 (125)	lef-7	A
103 (126)	chitinase	A
104 (127)	v-cath	A
105 (128)	gp64/67	C
106 (129)	p24	A
107 (130)	gp16	A
108 (131)	pp34	A
109 (132) *		C
110 (133)	an	C
110a (-) *		A
111 (134)		A
112 (135)	p35	A
113 (136)	p26	A
114 (137)	p10	A
115 (138)	p74	A
116 (139)	me53	B

Table 2 Continued.

ORF No.	Gene name	type
117 (141)	ie-0	B
118 (142)	p49	C
119 (143)	odv-e18	B
120 (144)	odv-ec27	C
121 (145)		A
122 (146)		C
123 (147)	ie-1	D
124 (148)	odv-e56	A
125 (149) *		A
126 (150)		A
127 (151)	ie-2	A
128 (153)	pe38	A
129 (154) *		A
130 (1)	ptp	A
131 (2) *	bro-d	B
132 (-)	bro-e	A
133 (4) *		A
134 (5) *		A
135 (6)	lef-2	C

Table 2 Continued.