UNIVERSITY OF CENTRAL OKLAHOMA

Edmond, Oklahoma

Jackson College of Graduate Studies

An Extraction Method Using Whole Insect Larvae to Isolate DNA from a Deceased Host

A THESIS

SUBMITED TO THE GRADUATE FACULTY

In partial fulfillment of the requirements

For the degree of

MASTER OF SCIENCE IN FORENSIC SCIENCE

By

William J. Christensen

Edmond, Oklahoma

2014

An Extraction Method Using Whole Insect Larvae to Isolate DNA from a Deceased Host

By: William J Christensen

A THESIS

APPROVED FOR THE W. ROGER WEBB FORENSIC SCIENCE INSTITUTE

September 2014

By _____

Dr. Wayne D. Lord Committee Chairperson

Rebecca Pace

Dr. Rebecca Pace Committee Member

Mr. James P. Creecy Committee Member

ACKNOWLEDGMENTS

I would like to thank my committee members, Dr. Wayne Lord, Mr. James Creecy, and Dr. Rebecca Pace. Dr. Lord and Mr. Creecy your support and expertise were invaluable to me while progressing through this research project. I would also like to thank, Janie Womble for your willingness to purchase and pursue the supplies needed to complete this research project.

I would like to send my gratitude towards Andrea Swiech and the employees of the Oklahoma State Bureau of Investigation's Forensic Science Center for the use of the facilities to analyze the data throughout the whole process during my research. The support was greatly appreciated from everyone at OSBI's Forensic Science Center and was significant factor in completing this research project. I would also like to send a specific thank you to all the members of the Forensic Biology Unit at the Forensic Science center. The support and willingness to bounce ideas and listen to concerns was very helpful throughout this whole process. The encouragement and cooperation was very much appreciative.

To all the faculty and staff at the Forensic Science Institute, your knowledge and assistance throughout my whole academic career at the University of Central Oklahoma was extremely valuable. I am extremely grateful to be able to learn from each of you.

Table of Contents

ABSTRACT

The analysis of entomological evidence continues to be a useful method for the identification of human remains from a crime scene, especially when the body is in a state of decomposition. During active decomposition the DNA within the organism may also degrade and could inhibit the ability to develop a genetic profile from traditional samples, such as blood and tissue from the body. Collection of entomological evidence such as larvae that developed on a body can provide a source of DNA to generate a genetic profile from a corpse. Typically DNA profiles obtained from larvae samples involved dissecting and removing crop contents. Dissection and removal of crop contents requires use of precise tools and entomological training, which most forensic laboratories do not have. This research assessed whole larvae DNA extraction, as a means to identify their food source. Two pigs, which were used as human analogs, were deposited in a secluded area and allowed to decompose. Pig #1 was unaltered, while pig #2 was deposited with commercially purchased human semen placed in several locations on the pig, to simulate the events of a sexual assault homicide. In total, forty-eight larvae extracts were analyzed with an Animaltype Pig PCR amplification kit. Four of these larvae extracts produced at least one callable allele at one locus with the Animaltype Pig PCR amplification kit. Fifty samples were amplified using an Applied Biosystems AmpFℓSTR® Y-Filer PCR amplification kit. Seven of these larvae samples produced at least one callable allele at one locus with the Applied Biosystems AmpFℓSTR® Y-Filer PCR amplification kit. Amplification of whole larvae extracts with the Animaltype Pig PCR amplification kit did not provide consistent results, but it did demonstrate the ability to develop a profile by extracting whole larvae. Whole larvae extracted from pig #2 indicated that a possible suspect's genetic profile could be obtained from whole larvae extraction; however, human commercial semen was consumed early in the

decomposition of the pig carcass. The results from this research serve as a template for future forensic molecular biology research.

List of Tables

List of Figures

1. INTRODUCTION

There are several factors involved in investigating crime scenes; however, when a crime scene involves a human body, the availability of entomological evidence is important. Collection of entomological evidence can be useful in identifying human remains at crime scenes, especially for decomposed corpses. As corpses decompose, DNA samples also degrade and genetic profiles may not be obtained from samples such as blood or tissue. Entomological evidence is collected to aid the investigators in determining the postmortem interval (PMI), which is an estimate of the time the corpse was deposited to when it was found by investigators (Goff & Lord, 2001). Typically, DNA analysis has been used with entomological evidence to identify the species of the insect; however, entomological evidence can be used to provide significant insight in determining what happened at crime scenes (Goff & Lord, 2001). Entomological evidence can provide additional information including movement of a corpse postmortem, differentiation of ante-mortem versus post-mortem injury, and sources on which to perform toxicological and DNA analysis (Catts & Goff, 1992). Past research using DNA obtained from fly larvae has been used to identify their food source (i.e. human remains) but this typically involved the dissection of the crop or gut of maggots (Goff & Lord, 2001).

1.1 Research Hypothesis

The objective of this research was to determine if viable DNA profiles could be obtained by extracting total DNA from a whole maggot, instead of the gut (crop) contents, to determine their food source. A viable DNA profile in a forensic laboratory is determined by the ability to enter a profile into the Combined DNA Index System (CODIS) database, which requires callable alleles at a minimum of eight CODIS core loci. Development of a whole larvae extraction method would provide a simplistic option for use in forensic laboratories with minimal

additional training and equipment needed. The proposed method would also provide an alternative method for identifying severely decomposed human remains. The following general hypotheses were tested:

H_1 = Extracting the entire larvae will provide a useful genetic profile (information obtained from eight loci or more) that can be used to determine the food source of the larvae.

H_0 = Extracting the entire larvae will not provide a useful genetic profile (information obtained from eight loci or more) that can be used to determine the food source of the larvae.

1.2 Uses of DNA Analysis in Forensic Entomology

Application of DNA analysis to forensic entomology was typically used to determine the species of the larvae samples to aid entomologists in determination of the postmortem interval (PMI) (Wellls & Stevens, 2008). Insect eggs and young larvae are extremely difficult to identify with a microscope and sometimes there are insects that are not as common to a specific geographic area that are found on corpses, which makes it difficult to identify the species in any stage of development (Wells, 2010). Identification of insect species with genotyping applications assists a forensic entomologist in accurately identifying the species, which is a crucial first step in entomological analysis (Wellls & Stevens, 2008). A majority of species identification is through mitochondrial DNA sequencing, which primarily focuses on the cytochrome c oxidase subunits one and two (COI & II) ((Wellls & Stevens, 2008). There is not a consensus on the locus that is used for the identification of species and some other targets include randomly amplified polymorphic DNA (RAPDs), the gene for 28S ribosomal RNA, the ribosomal internal transcribed spacer regions, and NADH dehydrogenase subunit 5 (Wellls & Stevens, 2008). Another application of DNA analysis to forensic entomology is dissecting crop

contents of larvae to determine their food source, which is the basis for the research that was performed in this study (Wells, Introna, Di Vella, Campobasso , Hayes, & Sperling, 2001).

1.3 Usefulness of Determining Larvae's Food Source

DNA analysis performed on evidentiary maggots can be used to link crime scenes together through the analysis of a maggot's food source. Assuming that there are primary and secondary crime scenes with maggots at both locations, analyzing DNA from the maggots could be used to identify the relationship between these locations. In addition, if maggots are found near or in a suspect's trash container, with no other biological evidence, the maggots could be analyzed to determine if they were associated with a human corpse or identify a previously unidentified corpse (Wells, 2010).

Development of a DNA profile of the host species from maggots would be useful during the course of death investigations. For example when investigators discover maggots, but no corpse, it would be useful to determine if maggots were feeding on a human or an animal carcass. If maggots were feeding on an animal carcass then no further investigation would be needed, but if they were feeding on human remains, then additional investigation would be warranted (Wells, et al., 2001). When maggots are not found directly on a corpse or there is another food source nearby, such as an animal carcass, it would be difficult to determine if larvae present actually came from a human corpse. Larvae are able to travel from alternate food sources, if physically disturbed, and in some way deposit themselves on a corpse at a crime scene (Wells, Introna, et al. 2001). Development of a genetic profile from the food source of larvae would aid investigators in determining if a crime was committed.

If a human corpse was discovered with indicators of a possible sexual assault, it may be possible to develop a suspect's profile using maggots collected from the corpse and crime scene.

Blowflies are attracted to a decaying body and will deposit their eggs in body orifices (Goff and Lord, 2001). In cases where there is trauma or wounds exposed, like those seen in violent sexual assaults, blowflies will be attracted to the trauma areas instead of normal body orifices such as the nose or mouth (Clery, 2001). Maggots found in genital areas in a more advanced state indicate the possibility of a sexual assault (Clery, 2001). When a body decomposes any evidence that a sexual assault has occurred may be difficult to collect or even find. Traditional methods of collecting swabs from genital areas or other areas on the body would not be useful for identifying suspect DNA. Decomposition of the body is likely to destroy any spermatozoa present on the body and therefore, the maggots may be the only source of DNA from a suspected perpetrator (Clery, 2001).

1.4 Research Problem

Most research involved in DNA analysis of maggot's gut content involves dissecting the crop of the maggot and extracting this portion (Figure 1). This requires the use of dissection tools and microscopes to locate and remove the crop of the maggot. In most forensic laboratories analysts do not have the training or the time for this tedious process. The ability to extract the entire maggot would decrease the time and training that it would take for analysts to perform this type of forensic DNA analysis. Shortening the process of extracting maggots would reduce reluctances of analysts to perform this type of DNA analysis, since it is outside normal evidence analyzed in forensic laboratories (Wells, 2010). Typically if an investigator needs this type of

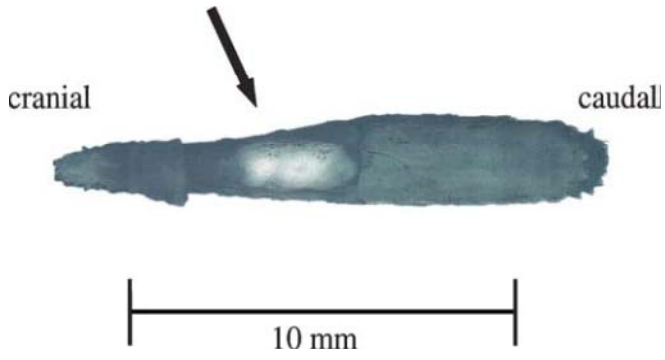


Figure 1. Visualization of the crop of a maggot ((Zehner, Amendt, & Kretteck, 2004). This figure depicts where the crop is located (arrow showing crop), typically the crops are dissected out with a scalpel and microscope.

analysis for an investigation they would send the maggots to academic laboratories for analysis. This could cause problems when introducing this type of evidence into a court of law, since often these laboratories are not accredited and may not be aware of the admissibility requirements, especially with respect to chain of custody (Wells, 2010). The ability to perform this analysis at forensic laboratories would solve these issues.

1.5 Research Goals

This research was conducted with the use of a pig as an analog for a human, because it is the closest in skin and bone composition to a human cadaver. The pig has been used as a major mammalian model for human biology research because of the similarities in size, physiology, and disease progression (Lunney, 2007). Pigs have also been used as models for skin and plastic surgery procedures for testing the toxicity of the skin (Swindle and Smith, 1998). Pigs also have high sequence and chromosome structure homology with humans, which make them an ideal model to correlate human behavior (Lunney, 2007). Using pigs as human analogs provides the researcher with the ability to obtain a wide variety of samples, which include detailed internal tissue samples. Since pig cell lines are well defined and represent a broad range of tissues; studies involving gene expression, drug susceptibility, etc. can be performed (Lunney, 2007).

The goals of the research were to: (1) evaluate the extraction of whole larvae collected and subsequent amplification of larvae extracts to develop a genetic DNA profile from the larvae's food source, (2) evaluate the ability to develop a male DNA profile from the larvae, and (3) determine how long a DNA profile can be obtained. Larvae samples collected from pig #1 were amplified with a Animaltype Pig PCR amplification kit (Biotype® Diagnostic GmbH, Dresden, Germany). The Animaltype Pig PCR kit amplifies short tandem repeats (STRs) from DNA of pigs to develop their genetic profiles. Larvae samples collected to evaluate the ability to

develop a male profile were amplified with the Applied Biosystems AmpFℓSTR® Y-Filer PCR amplification kit. This research could allow forensic laboratories to analyze larvae samples with ease and limited knowledge of entomology.

2. LITERATURE REVIEW

2.1 Forensic Entomology

Forensic entomology is defined as the application of the study of insects and other arthropods to legal issues, especially in a court of law (Catts & Goff, 1992). There are three broad categories of forensic entomology, including urban, stored-product, and medicolegal (Catts & Goff, 1992). Urban entomology deals with lawsuits that involve homeowners and landowners. Stored-product entomology involves infestations of insects in commercial products. Medicolegal forensic entomology involves the study of insects involving violent crimes, such as murder, suicide, and rape; the most common application of medicolegal forensic entomology is used in investigating deaths (Catts & Goff, 1992). Elements in investigations, such as time since death (i.e. the time between death and corpse discovery, which is generally referred to as the postmortem interval or PMI), movement of the corpse, manner and cause of death, and association of a suspect with the crime scene, may all relate to arthropod occurrence and activities (Goff & Lord, 2001).

Insects are very useful in death investigations because they are usually the first to find the decomposing corpse, typically within the first few hours following death (Catts & Goff, 1992; Sperling, Anderson, & Hickey, 1994). The process for determining PMI begins when blow flies lay their eggs. Succession of Arthropod fauna around the corpse is predictable, making collection of insects a useful tool to obtain information from crime scenes (Catts & Goff, 1992).

2.1.1 Estimation of PMI

Using entomological data to estimate PMI can be done in two ways. During early stages of decomposition, the estimate is made by calculating the amount of time needed for a species to develop into the life stage collected at the crime scene (Catts & Goff, 1992). Insects with the

longest period of development are used to determine the PMI. It is important to thoroughly evaluate all the assumptions made when determining the PMI. The second way to determine PMI relates to corpses that are in advanced stages of decomposition. PMI estimates in these cases are based on the composition of the arthropod community as it relates to expected patterns of the arthropods (Catts & Goff, 1992).

2.1.2 General Life Cycle of an Insect

The life cycle of insects can provide useful information in examining insects at crime scenes. In general, insects pass through an egg, larva, and pupa stage in their life cycle (Figure 2) (Lord & Goff, 1993). Usually the female will arrive at the body and deposit eggs, in natural body openings, that hatch into larvae or maggots that feed on decomposing tissues (Goff & Lord, 2001). There are three distinct stages to the development of maggots called instars. When maggots have reached maximum development, they will stop feeding on the corpse and move away from the decomposing body, entering the puparial stage. In the puparial stage the larval tissues are re-organized to produce the adult fly (Goff & Lord, 2001).

2.1.3 Blow Flies *(Calliphoridae)*

Blow files (Calliphoridae) are typically the first insects to arrive at the crime scene and arrive within minutes to a few hours after death (Lord & Goff, 1993). As described above, the blowfly will then deposit eggs in body openings and when the eggs hatch they will produce small, featureless, worm like insects that are called larvae or maggots. The larvae will pass through three instars before maturing into an adult fly. The blowfly larvae will secrete enzymes and bacteria that will aide in consuming soft tissues of the corpse (Lord & Goff, 1993). Once larvae reach the third instar stage, they will move away from the body and burrow into the ground beneath the corpse and enter the puparial stage. When blow flies become adult flies, a

pupal case is left behind which provides valuable information, even after the corpse has decomposed (Lord &Goff, 1993).

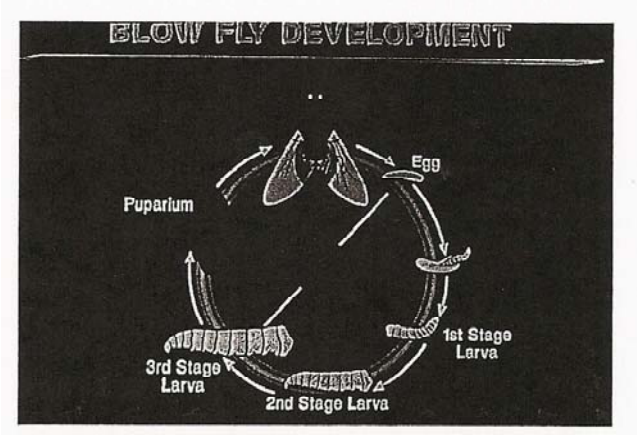


Figure 2. Life cycle of a fly in the family *Calliphoridae* ((Goff & Lord, 2001). Typical life cycle of a fly in the family

2.1.4 Stages of Decomposition

Decomposition is the breakdown of organic substances into simpler forms of matter. Generally there are five stages of decomposition that a body goes through (Goff & Lord, 2001). The first stage is called the fresh stage, which begins at the moment of death and ends when bloating becomes evident. The second stage is the bloated stage, where putrefaction begins and gases are produced by metabolic activities of bacteria that cause an inflation of the abdomen and eventually the entire corpse appears balloon-like. The temperature inside the body begins to rise and fluids from the body begin to penetrate the area beneath it. The third stage is the decay stage, where the skin is broken and the gases can escape and the body deflates. The fourth stage is the post-decay stage, in this stage remains are reduced to skin, cartilage, and bone. The fifth and final stage is the skeletal stage, in which only bone and hairs remain. There is no discernible end to the skeletal stage and in fact, valuable entomological evidence in the soil fauna can be detectable for months or years following death depending on the conditions (Goff & Lord, 2001).

It is important to be aware of the stages of decomposition when collecting evidence at crime scenes, to ensure all the evidence is collected.

2.2 Forensic DNA Analysis

2.2.1 Biology of DNA

Deoxyribonucleic acid (DNA) is commonly referred to as a genetic blueprint, because it contains information required for passing down genetic attributes to future generations. Half of the DNA comes from the father and the other half comes from the mother (Butler, 2005). DNA is contained in the nucleus of every cell in our body, with the exception of the red blood cells, which do not contain a nucleus. There are two primary purposes of DNA, to make copies of itself and pass that information on through cell division; the second is to carry out the instructions on making proteins (Butler, 2005). The entire DNA in a cell of the human body is referred to as a genome and contains the instructions for making an organism and determines an individual's physical features (Butler, 2005).

James Watson and Francis Crick were the first to describe the structure of the DNA molecule in 1953 (Watson, 1968). The DNA molecule consists of a nucleotide monomer, which contains a 2′-deoxyribose sugar, a phosphate group, and a nitrogenous base (Figure 3) (Watson & Crick, 1953). The sugar is a pentose and contains five carbons and a 3′ hydroxyl group that is specific to the DNA molecule. The

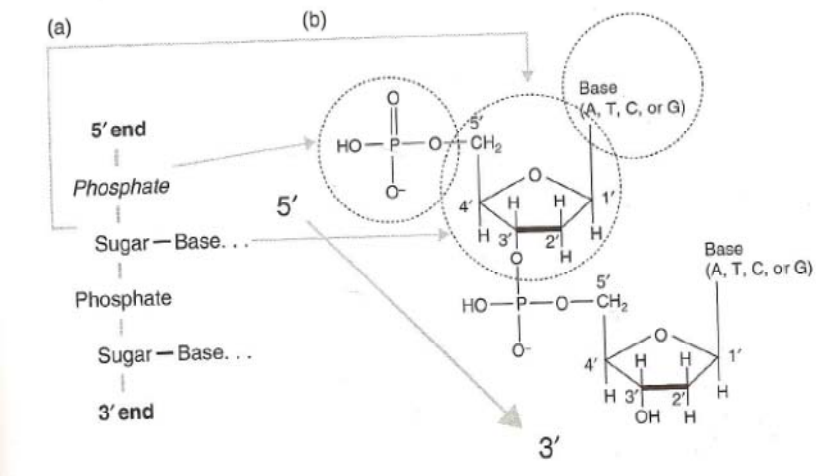


Figure 3. Nucleotide and Base Structure (Butler, 2010). The nucleotide consists of a 2′-deoxyribose sugar, a phosphate group, and a nitrogenous base. The nucleotides are composed of adenine (A), guanine (G), cytosine (C), and thymine (T).

phosphate group binds to the 5′ carbon of the 2′-deoxyribose sugar molecule with a phosphodiester bond. The phosphate group is a negatively charged molecule, which in turn makes the entire DNA molecule negatively charged. Nucleotides are identified as adenine (A), guanine (G), cytosine (C), and thymine (T). The nucleotides bind to the 1′ carbon of the 2′-deoxyribose by a glycosidic bond (Watson & Crick, 1953). Bases are paired together with hydrogen bonds, where adenine and thymine are bonded together with two hydrogen bonds and guanine and cytosine are paired together with three hydrogen bonds, which makes the bond of guanine and cytosine harder to break. The double helix structure of the DNA molecule is formed when the complementary and anti-parallel strands of DNA bond with one another as depicted in Figure 4 (Watson & Crick, 1953). The bases form the inner part of the double helix of the DNA molecule.

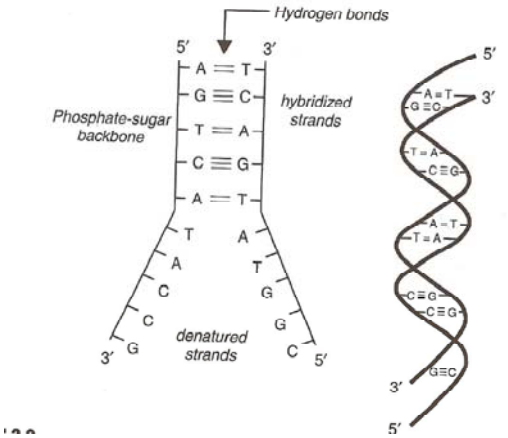


Figure 4. The Double Helix Structure of DNA (Butler, 2010). Depicts the double helix nature of the DNA molecule and the anti-parallel chains of the DNA molecule with complementary bases bonded by hydrogen bonds.

The human genome consists of 23 pairs of chromosomes, which are illustrated in Figure 5. Each of these chromosomes has a non-coding region and a coding region of DNA. The non-coding regions are referred to as introns and the coding regions are referred to as exons. The primary function of exons is to code for proteins, while the introns are commonly referred to as "junk" DNA because

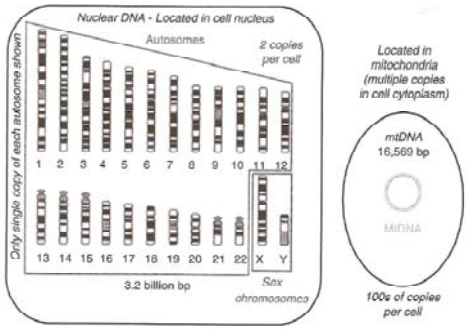


Figure 5. Representation of the Nuclear DNA Chromosomes (Butler, 2010). Displays a visual representation of the chromosomes and a visual representation of the mitochondrial

they do not code for proteins (Cantor & Smith, 1999). The markers that are used in Forensic DNA analysis are found in the introns and therefore they do not code for any genetic variation (Butler, 2010).

2.2.2 History of DNA Analysis

Forensic DNA analysis is an important tool that aids investigators in solving criminal investigations. Forensic DNA analysis has changed drastically over the last three decades. The birth of forensic DNA analysis began in the early 1980's, when regions of the genome called variable number of tandem repeats (VNTR's) were first discovered (Nakamura et. al., 1987). Dr. Alec Jeffreys found that these regions contained several repeats of DNA sequences, which were different between two individuals (Jeffreys, Wilson, and Swee, 1985). Dr. Jeffreys developed a technique to examine the length differences of repeat sequences, which was the beginning of modern day forensic DNA analysis. The technique that Dr. Jeffreys developed became known as restriction fragment length polymorphism (RFLP) (Saki et.al., 1985). RFLP analysis required the use of a restriction enzyme to cut DNA surrounding the VNTR's in various locations called restriction sites. RFLP analysis was groundbreaking in its time, and was the beginning of various technological advancements which allowed the ability to analyze miniscule samples collected from crime scenes.

Advancements in DNA testing lead to the implementation and development of short tandem repeat (STR) analysis for analyzing samples in a forensic laboratory. STR analysis has a high power of discrimination and has rapid analysis speed. STR's are short and can be multiplexed together, giving the analyst the ability to amplify multiple markers at the same time (Moretti, Baumstark, Defenbaugh, Keys, Smerick, & Budowle, 2000). STR's can be amplified

with polymerase chain reaction (PCR), which allow millions of copies of a specific STR to be amplified.

Polymerase chain reaction (PCR) is an enzymatic process, which replicates a specific sequence of DNA in multiple cycles to yield millions of copies (Bloch, 1991). Kary Mullis first describes the PCR process in 1985, which is extremely important to forensic DNA analysis (Saiki, Scharf, Faloona, Mullis, Horn, and Erlich, 1985). Many samples collected from crime scenes are extremely limited in both quality and quantity and PCR gives the analyst the ability to analyze these samples by making millions of copies of the target sequence of DNA (Carracedo, 2005). PCR amplification requires the use of a DNA template strand, primers, deoxynucleodtide triphosphates (dNTP's) and *Taq* polymerase. *Taq* polymerase comes from the bacterium named *Thermus aquaticus* that inhabits a variety of thermal springs in Yellows Stone National Park and a spring in California (Brock and Freeze, 1969). Half of the primers are fluorescently labeled and bind to specific sequences on the DNA strand. The dNTP's provide the bases for elongation of the DNA strands. *Taq* polymerase adds the bases in the correct order based on the template DNA sequence (Carracedo, 2005). *Taq* polymerase is thermally stable and does not degrade near boiling temperatures (Holland, Abramson, Watson, and Gelfand, 1991). An example of a single PCR cycle is illustrated in figures 6 and 7. DNA is replicated through several cycles of heating and cooling with an instrument referred to as a thermal cycler. When the PCR process is

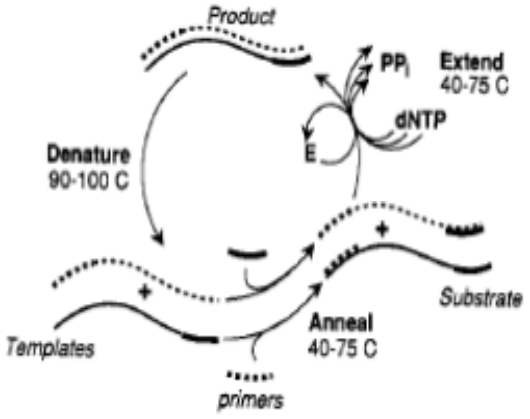


Figure 6. Model of Single PCR Cycle (Bloch, 1991). Model of a single PCR cycle, each cycle generates up to two molecules of product from the proceeding cycle and results in an exponential accumulation of product over a series of cycles.

complete there are millions of copies of the original DNA template, which allows a forensic scientist to analyze samples that are limited in both quality and quantity.

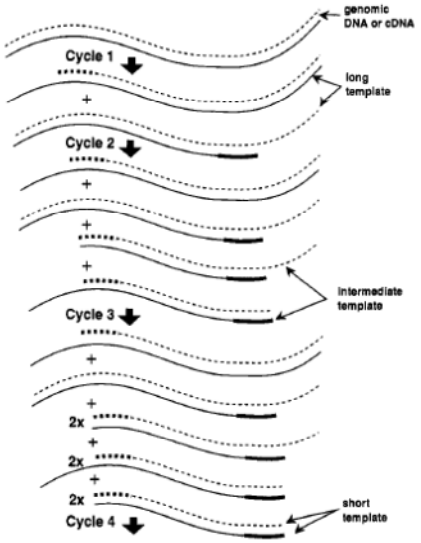


Figure 7. Illustration of the first few cycles of PCR amplification. (Bloch, 1991). The figure illustrates the PCR template size over the first few cycles of amplification of a DNA template strand. The ends of the DNA molecule presenting the target sequence in a PCR sample usually extend beyond the target sequence, defined by the two primers. The initial products of target replication still extend beyond the target sequence in one direction. Only in the third cycle can there begin to accumulate the relatively short duplexes generally described as PCR product.

2.2.3 DNA Analysis Process

Forensic DNA analysis consists of extraction, quantification, amplification, and genetic analysis. In the extraction process, DNA is isolated from biological materials. Isolated DNA is quantified to estimate the quantity of template DNA that was recovered in the extraction process. Extracted DNA is amplified based on quantification results and the resulting amplified DNA products are analyzed with a capillary electrophoresis genetic analyzer, which develops a genetic profile of the sample.

A standard organic extraction method was utilized for a portion of this research. Standard organic extraction method utilizes organic chemicals to isolate genomic DNA from biological samples. The organic extraction method can be summarized in four steps: (1) solubilization of the substance containing the DNA, (2) denaturation and hydrolysis of proteins,

(3) removal of denatured proteins, and (4) purification of DNA (Clark, NFTSC Science Serving Justice). Solubilization of stain components is accomplished by adding a lysis buffer, typically a stain extraction buffer (SEB). Stain extraction buffer (SEB) contains 1M Tris-HCl, 0.5 M ethylamine-diaminetetraacetate (EDTA), 0.39 M DTT, 5 M NaCl, 20% sodium dodecyl sulfate (SDS), and ultra-pure water (Oklahoma State Bureau of Investigation Organic Extraction Protocols). EDTA protects DNA from degrading and SDS breaks down the lipid bi-layer of cell membranes, producing permeability of a cell. Proteinase K will break down proteins and other macromolecules, which allows DNA to be released from cells. The addition of phenol chloroform isoamyl alcohol (PCI) promotes separation of non-polar (organic) and polar (aqueous) phases. DNA is negatively charged and will remain in the aqueous phase in its double stranded state. Proteins and other molecules are separated into the organic phase isolating the DNA. Phenol denatures proteins that were hydrolyzed with Proteinase K. The final step in organic extraction is purification of DNA, accomplished by using a centrifugal filter unit, which purifies and concentrates DNA. A centrifugal filter unit separates macromolecules by size through a series of washing and centrifugation steps (Clark, NFTSC Science Serving Justice). DNA will be retained in the filter, while other macromolecules and inhibitors that are smaller than the pore size of the filter will pass through..

The quantification step of the DNA analysis process estimates the amount of DNA in a sample. DNA can be quantified using a spectrophotometer, which consists of two instruments, a spectrometer and photometer (Caprette, 1996). A spectrometer produces light of a specific wavelength and the photometer measures the amount of light that is passed through the sample. Signal changes as the amount of light the sample absorbs changes and concentration can be measured by the extent of the absorption of light at the appropriate wavelength (Caprette, 1996).

The spectrophotometer used in this research was a NanoDrop UV-Vis Spectrophotometer, which quantifies nucleic acids in as little as a single microliter. When the sample is analyzed the software will display the DNA concentration, nucleic acid purity ratios, and spectra for each sample. A NanoDrop instrument utilizes fiber optic technology and surface tension which holds the sample in place between two optical surfaces at a defined path length (NanoDrop Products Termo Scientific Nucleic Acid Quantification Protocols, 2013). This feature minimizes contamination introduced, since a cuvette is not required. Samples are measured at two paths, 1-mm and 0.2 mm, providing a wide range of quantification values for each sample. The ability to analyze a wide range of concentrations minimizes the necessity for dilutions (NanoDrop Products Termo Scientific Nucleic Acid Quantification Protocols, 2013). The sample retention system for the NanaDrop instrument is illustrated in figure 8. A commercial quantification kit with a real time PCR instrument is routinely used in forensic laboratories to estimate the amount of DNA in a sample prior to the amplification process.

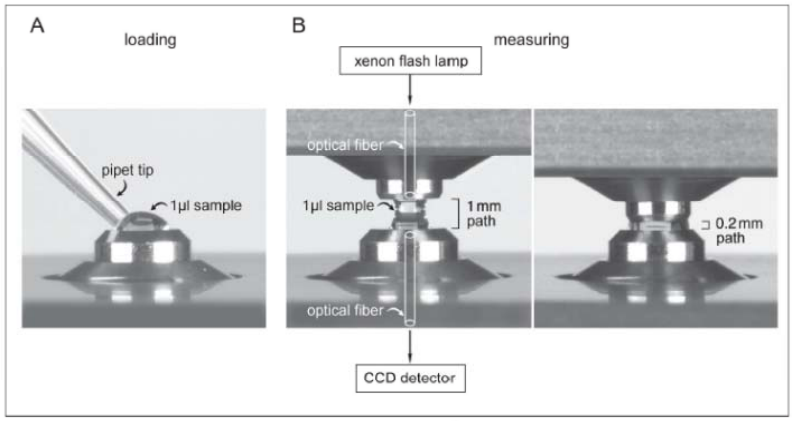


Figure 8. NanoDrop Sample Retention System ((NanoDrop Products Termo Scientific Nucleic Acid Quantification Protocols, 2013).This figure illustrates the sample retention system in the NanoDrop instrument. The sample volume is dispensed onto the lower optical surface and once the instrument lever arm is lowered the upper optical surfaces comes in contact with the liquid forming a defined path length.

A human quantification kit in conjunction with an ABI 7500 real time PCR instrument quantifies human DNA extracts. Applied Biosytstems Quantifiler® Y Human Male DNA

Quantification kit contains two 5' nuclease assays, a target-specific assay and an internal PCR (IPC) control assay. The target specific assay contains two primers for amplifying human male DNA and one TaqMan® MGB probe labeled with the FAM® dye for detecting the amplified sequence. An IPC assay contains an IPC template DNA which is synthetic and not found in nature, two primers for amplifying the IPC template DNA, and one TaqMan® MGB probe labeled with VIC® dye for detecting the amplified IPC DNA. The target gene on the Y-chromosome is the sex determining region (SRY), which is located at Yp11.3 and has a 64 base amplicon length, is haploid, and is located in the non-translated region (intron) (Applied Biosytems Quantifiler Kits User's Manual, 2012).

The TaqMan® MGB probe contains a reporter dye that is linked to the 5' end of the probe and a minor groove binder (MGB) at the 3' end of the probe. The MGB is modified allowing the melting temperature (Tm) to be increased without increasing the probe length, which allows for a shorter probe (Afonina, Zivarts, Kutyavin, Lukhtanov, Gamper, & Meyer, 1997; Kutyavin, Lukhtanov, Gamper, & Meyer, 1997). A non-fluorescent quencher is at the 3'end of the probe that does not fluoresce, allowing the sequence detection system to measure the reporter dye contributions more accurately (Applied Biosytems Quantifiler Kits User's Manual, 2012).

The 5' nuclease assay process takes place during PCR amplification and occurs every cycle and does not interfere with exponential accumulation of product. TaqMan® MGB probe anneals specifically to the complementary sequence between forward and reverse primer sites. When the probe is intact the quencher dye suppresses the reporter fluorescence primarily by Forster-type energy transfer (Figure 9) (Forster, 1948; Lakowicz, 1983). AmpliTaq Gold® DNA polymerase will cleave only the probes that are hybridized to the target sequence and cleavage separates the reporter dye from the quencher dye, which will cause fluorescence of the reporter

dye. The increase in the fluorescence signal will only occur if the target sequence is complementary to the probe and is amplified during the PCR process, which will not allow non-specific amplification to be detected (Applied Biosystems Allelic Discrimination Using the 5' Nuclease Assay, 2001). Polymerization of the strand will continue, but since the 3' end of the probe is blocked the strand will not extend during the PCR process.

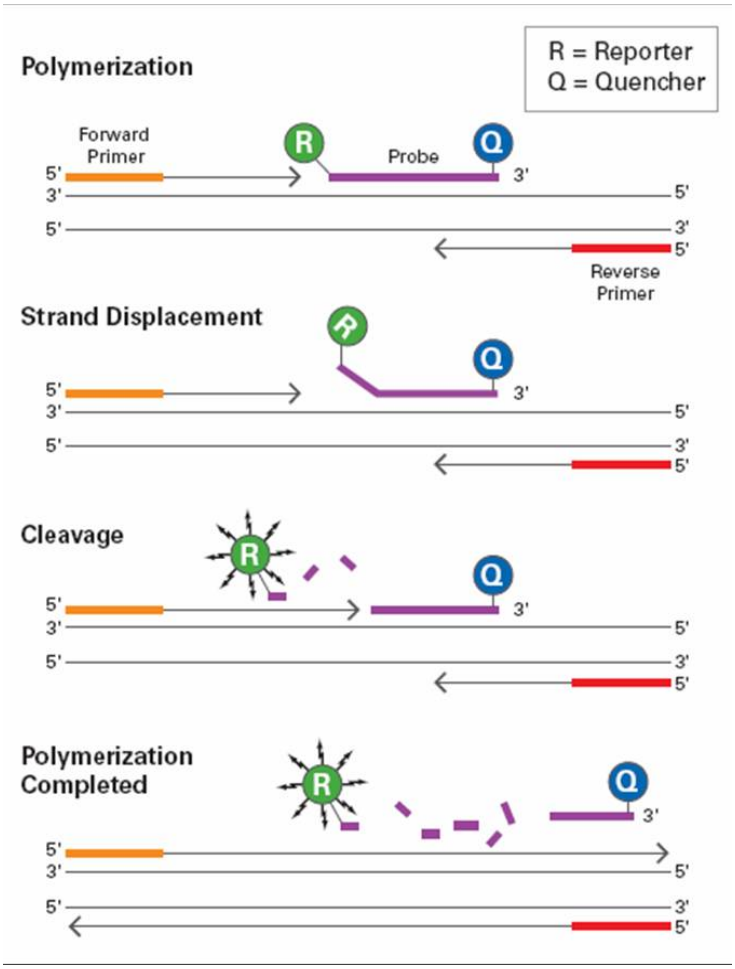


Figure 9: PCR amplification and detection with fluorogenic probes in the 5' nuclease assays (Applied Biosystems Allelic Discrimination Using the 5' Nuclease Assay, 2001). The main steps in the reaction sequence are polymerization, strand displacement, and cleavage. Two dyes, a fluorescent reporter (R) and a quencher (Q), are attatched to the fluorogenic probe. When both dyes are attatched to the probe, reporter dye emission is quenched. During each extension cycle, the DNA polymerase cleaves the reporter dye from the probe. Once seperated from the quencher, the reporter dye fluoresces.

Fluorescence is detected on the AB 7500 real time PCR instrument with the use of a tungsten-halogen lamp that directs the light to each well on a 96 well reaction plate. Light passes through the ABI prism optical adhesive cover and excites the fluorescent dyes in each well of the plate. A system of lenses, filters, and mirrors focuses the fluorescence emission into a charge-coupled device (CCD) camera. Filters separate light into a predictably spaced pattern. The sequence detection software (SDS) obtains data from the CCD camera and applies data analysis through algorithms ((Applied Biosytems Quantifiler Kits User's Manual, 2012).

Polymerase chain reaction (PCR) is an enzymatic process by which a specific region of DNA is replicated. PCR analysis occurs using repetitive cycles which consist of denaturing DNA strands, annealing primers to complementary sequences at a specific temperature, and extending DNA strands bound by primers using a DNA polymerase (Mullis, Falloona, Scharf, Saiki, Horn, & Erlich, 1986). The reaction is repeated for several cycles and results in the exponential accumulation of the specific DNA sequence that is bound by the primers. In theory, from as little as one molecule of DNA approximately one billion copies of the specific DNA segment can be generated after 30 cycles of PCR (Mullis K. B., 1985). Denaturation allows DNA to become single stranded and occurs at approximately 94 ° C. The temperature is lowered allowing annealing of primers to the template DNA, which typically occurs between 50º to 60 ºC. Elongation of DNA requires the temperature to be raised to approximately 72ºC, which is optimal *Taq* DNA polymerase. The elongation process

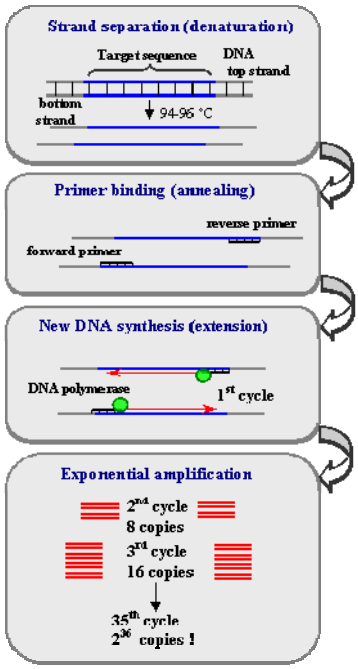


Figure 10. The PCR process (NCBI). The steps in the PCR process are shown in the figure, which include denaturation, annealing, and extension.

synthesizes new DNA nucleotides (Figure 10) (Mullis, Falloona, Scharf, Saiki, Horn, & Erlich, 1986).

The next phase of the DNA analysis process is analyzing the amplified DNA products with a capillary electrophoresis genetic analyzer. Capillary electrophoresis separates DNA fragments based on their molecular weight and shape. Electrophoresis is a process which separates charged molecules based on their movement through a medium, typically polymer, with an electric current applied (Roby & Figarelli, NFSTC Science Serving Justice). Separation of molecules depends on two elements; the charge and mass of the molecules. DNA is negatively charged and is constant throughout the DNA molecule; therefore separation is based on mass of the DNA molecule. DNA fragments are fluorescently labeled with dye markers, which allow the capillary electrophoresis instrument to detect DNA fragments as they migrate through the capillary (Butler, Ruitberg, & Vallone, 2001). The stationary phase of the capillary electrophoresis process is the polymer and typically in forensic DNA analysis is Performance Optimized Polymer 4 (POP-4). The mobile phase is the DNA fragments that migrate through the capillary (Roby & Figarelli, NFSTC Science Serving Justice). Amplified DNA samples are injected into a capillary electrophoresis instrument by electrokinetic injection, which transfers negatively charged ions as current flows from the cathode to the anode (Roby & Figarelli, NFSTC Science Serving Justice). POP-4 coats the capillary wall to control the flow during electrophoresis and is optimized to detect allele sizes from a single base pair up to 250 base pairs. A capillary electrophoresis instrument uses a laser to excite fluorescently labeled DNA fragments which are detected by a Charge Coupled Device (CCD) camera that measures relative fluorescence. This signal is converted to a peak which produces an electropherogram and when

all the peaks are present at all the loci analyzed a genetic profile from a sample is obtained (Butler, Ruitberg, & Vallone, 2001).

2.2.4 Additional Sources of DNA

Mitochondria of a cell are often referred to as the powerhouse of a cell because they are responsible for ATP synthesis and store energy from the breakdown of food. Performing mitochondrial DNA analysis in forensics is appropriate when there are charred remains, degraded samples, older skeletal remains, and hair shafts. In addition, performing mitochondrial DNA analysis from victims of a mass disaster is extremely helpful because quite often there is very little sample or fragments recovered ((Roby, NFSTC Science Serving Justice). Mitochondrial DNA is inherited from the maternal side; both males and females will inherit mitochondrial DNA from their mother (Dimauro and Davidzon, 2005).

Y-chromosome is specific to males and can be used to provide a male genetic profile. DNA from the Y-chromosome is passed from generation to generation through the paternal lineage and therefore all paternal males will have the same Y-STR profile (Ballantyne). Y-STR kits analyze loci on the SRY gene, which is the sex determining region of the Y-chromosome and results in a haplotype, which usually will only have one allele per loci as opposed to two that are normally obtained from autosomal DNA analysis (Jobling and Tyler-Smith, 1997). Y-STR analysis is particularly useful when there are high amounts of female DNA present and lower amounts of male DNA present, especially useful in sexual assault analysis. An example would be a vaginal swab taken from a victim, where there is a large amount of blood from the victim. Y-STR analysis is also useful in determining the number of semen donors in a case where there are multiple perpetrators, as well as paternity testing, missing persons, and several other types of cases (Ballantyne).

2.3 DNA Analysis of larvae

In forensic entomology, it is important to know the species of larvae encountered on corpses as soon as possible to provide an accurate estimate of time of death to aid investigations. Mitochondrial DNA (mtDNA) provides well-suited markers for identification of insect species (Sperling et al., 1994). Application of mtDNA to identify species allows identification within one day of receipt of the insects even, with dead insect material (Sperling et al., 1994).

In a study entitled "A DNA-Based Approach to the Identification of Insect Species Used for Postmortem Interval Estimation" (Felix et al., 1994) the authors studied several blowfly species in order to obtain their mitochondrial DNA profile. Thoraxes of adult flies were used for DNA extraction and the remaining parts of the flies were kept. Adult flies were stored frozen until used for DNA extraction. Flies were extracted to isolate DNA from the flies and were amplified using general insect mtDNA sequences that were developed. The authors concluded that the differences in DNA sequences obtained from different fly species were significant enough to clearly identify a species. Results obtained from this research conclude that testing can be extended to other species that are commonly found at a specific location. The authors indicate that DNA fingerprints for insects can easily be converted into a convenient storage system in a database, similar to CODIS (Combined DNA Indexing System).

Postmortem interval (PMI), a common use of insect evidence, is based on the age of the fly larvae. PMI is calculated by the determination of the species and then estimating the stage of development (Zehner, Amedndt, & Krettek, 2004). It is usually assumed that all of the maggot's development and feeding occurred on the victim; if it did not then the age estimate may be inaccurate (Wells et.al, 2001). In most cases this assumption can be made, because larvae were collected directly from or near a corpse. However, this assumption cannot always be made and

an alternative method for determining if a maggot came from a corpse would be useful (Wells et al., 2001). In certain cases it would be necessary to establish an association of larvae to a specific corpse, such as when maggots are found in the absence of a body. If maggots were determined to be from a human, then it would immediately trigger a criminal investigation (Zehner et.al, 2004). Maggots can also be used to determine the specific corpse of origin. "The question could arise when the maggots from one crime scene were divided, sent to different investigators and inconsistent conclusions were taken from the same evidence" (Zehner et al., 2004).

Human material that is ingested by the maggot might be suitable for DNA analysis and yield a genetic profile to determine the maggot's food source. The liquefied tissue of the host species is stored in the crop, which is a special region of the maggot's foregut (Zehner et al., 2004). With the examination of the crop contents, a genetic profile can be obtained.

In the article entitled "Human and Insect Mitochondrial DNA Analysis from Maggots" (Wells et al., 2001) the authors demonstrated that mitochondrial DNA can be obtained from the dissected gut contents of maggots that had fed upon human tissue. They also determined that, not only could you determine the maggot's food source, but also the species of the maggot. In this study, the authors obtained wild flies and placed them in a rearing jar containing human tissue (liver). Third instar larvae were taken and preserved in seventy percent ethanol and stored frozen. Larvae were dissected and crops removed for DNA analysis. The value of mitochondrial DNA for identifying human remains and fly species was demonstrated successfully.

With development of new technology, such as short tandem repeat (STR) analysis, it was necessary to determine if this technology could be used to analyze maggots to determine their

food source. In a study performed by Zehner, et al. (2004), crop contents of maggots collected from human corpses were analyzed using both STR analysis and mtDNA analysis. Authors collected feeding third instar larvae from thirteen human corpses during a autopsy. Maggots were killed in boiling water and then stored in seventy percent ethanol until they were analyzed for DNA. Corpses that these maggots were collected from showed marked signs of putrefaction, ranging from released rigor mortis to advanced and greenish color decay (Zehner, et.al, 2004). Ten of the cadavers that were used were recovered indoors and the remaining three were recovered outdoors. Maggot's crops were dissected and genetic analysis was attempted on the crops. If genetic analysis of the crop was unsuccessful, then up to four additional maggots were analyzed from the same corpse. DNA extraction was performed using a standard organic extraction method. DNA was amplified using the Applied Biosystems multiplex kit called Profiler Plus. The number of cycles of the thermal cycler was increased from 29 to 32, to increase the amount of template DNA available for genetic analysis. The amplified DNA products were analyzed using an ABI PRISM 3100 genetic analyzer using standard protocols. STR analysis of crop contents in this study produced seven cases with a complete STR profile and two cases where an incomplete profile was obtained. There were also four cases where an STR profile was not obtained. Profiles obtained from crop contents matched profiles obtained from the corpses. This study successfully demonstrated that STR analysis of crop contents of maggots could be used to link maggots to a specific corpse.

Several organic extraction methods have been utilized to isolate DNA from crop contents of fly larvae. The following are brief summaries of the various organic extraction methods utilized to isolate DNA from larvae samples.

In an experiment using lice, researchers washed lice with distilled water and sectioned them longitudinally along the dorsal midline and were suspended in tube with 300 µL of stain extraction buffer, which contained 10mM Tris, pH9.0, 100 mM NaCl, 39 mM DTT, and 10 mM EDTA. The suspension was vortexed for thirty seconds and incubated at 56 ° Celsius for two hours. Samples were extracted in 300 µL of a phenol/chloroform/isoamyl alcohol (24:24:1) solution. The PCI solution was centrifuged at 10,000 g for 2 minutes. The aqueous phase was removed and transferred to a Microcon concentrator and centrifuged at 5000 g for 5 minutes. Samples were washed with approximately 100 µL of TE^{-4} and the Microcon process was repeated. The DNA extract in the Microcon filter was inverted and DNA was collected (Lord, DiZinno, Wilson, Budowle, Taplin, & Meinking, 1998).

Extracted DNA from thoraces of adult flies was obtained by grinding flies into powder using a pestle and liquid nitrogen. A Lifton buffer, which contained 800 µL of 0.1 M Tris buffer, 0.2 M sucrose, 0.05 M EDTA, 0.5% sodium dodecyl sulfate (SDS), pH 9.0, was added to the powder to lyse the cell membranes. The solution was briefly vortexed and incubated at room temperature for fifteen minutes to two hours. Approximately 120 µL of 8 M potassium acetate was added to the solution mixed, and placed on ice for fifteen minutes to three hours. The resulting precipitate was centrifuged for fifteen minutes and the supernatant was added to a new tube. Supernatant was extracted with a phenol/chloroform/isoamyl alcohol solution. The pellet was washed with 500 µL of 70% ethanol solution and re-suspended in 200 µL of TE (Sperling, Anderson, & Hickey, 1993).

Zehner, Amendt, and Krettek (2004) isolated DNA crops of larvae by dissolving the crop in 25 µL of water. Extraction was performed using a standard phenol-chloroform extraction method and ethanol precipitation.

Another study was performed that used both mitochondrial DNA and STR analysis for analyzing the crops of maggots collected at crime scenes. Authors successfully recovered both human and animal DNA from host species, which served the dual purpose of identifying the insect species (Campobasso et al., 2005). The crop was dissected for DNA analysis and extracted to guarantee no exterior contamination. It is best to dissect the crop out for DNA analysis to ensure that the DNA extracted is from their food source, in addition to preserving taxonomically correct internal structures of the maggot (Campobasso et.al, 2005). This is important when there are minimal maggots available at the crime scene. The type of DNA analysis discussed in this section is mitochondrial DNA analysis. The authors removed external contaminants prior to extraction, by washing the maggots in twenty percent bleach solution. It was suggested that decontamination would significantly reduce external contaminants without disturbing DNA contained within the crop of larvae. This study analyzed maggots utilizing multiple time frames, to determine if it would affect the ability to recover DNA from the crop of the maggots. Maggots were collected at half-day intervals for six days. One group was immediately preserved and the other was kept alive, off the food source, for twenty-four and forty-eight hours. It was concluded that DNA could not be recovered from maggots which were kept off the food source for forty-eight hours. DNA was able to be recovered from maggots that were immediately preserved and analyzed with STR's within 2.5 to 4.5 days. The author's state that the length of time maggots are properly preserved and the postmortem interval do not influence the quality of DNA extracted (Zehner et.al, 2004). In conclusion, it is essential to properly preserve the maggots within twenty four hours of removal from the food source to ensure sufficient DNA is present within the crop of the larvae to obtain a useable DNA profile from the food source (Campobasso, Linville, et.al. 2005).

In the article "Application of DNA-Based Methods in Forensic Entomology" by Wells and Stevens (2008) common genotyping methods used are described. Identification of species of insects is the most common application of genotyping methods to forensic entomology. Other methods discussed are the identification of insect gut contents and characterization of population genetic structure of forensically important insect species. Proper applications of these procedures require an analyst to be an expert, but an analyst should also be aware of standards and expectations the legal system have imposed on forensic DNA analysis procedures. DNA analysis of gut contents from insects has several potential uses (Wells & Stevens, 2008). "A human genotype can be recovered from a fed mosquito, and this information could place a suspect at the location where the mosquito was found. Similarly, blood in a louse transferred during a sexual assault could identify the assailant" (Wells & Stevens, 2008). Carrion fly maggots are suitable for all types of genetic identification procedures and there are many questions that are answered by utilizing genotype application of gut contents of an insect. A crucial requirement for using larvae for DNA analysis is that the digestion process be halted as soon as possible after collection of the larvae. Killing and properly preserving the specimens will preserve DNA within the crop. When larvae have stopped feeding, crop contents will be emptied within twenty-four hours (Wells & Stevens, 2008).

The ability to obtain a human DNA profile from a maggot has potential to link a suspect to a victim, especially in sexual assaults. Maggots play an important role in preserving evidence that may have been otherwise lost. Liver samples were exposed to liquid semen and flies were allowed to deposit their eggs and feed upon the liver substrate (Clery, 2001). "Egg laying in the exposed genital area of victims before, during, or soon after death, in cases of sexual assault will create a situation where the maggots in the genital area are in a more advanced stage of

development than those found in other natural orifices" (Clery, 2001). Larvae will be deposited first in areas where there are wounds or trauma. In sexual assaults the vaginal and/or anal areas have a greater incidence of having received trauma; therefore larvae would be expected to be deposited it those areas first. A rape-homicide case is very difficult to solve, because they are usually a stranger-to-stranger crime (Clery, 2001). In violent rape-homicide cases, the body may not be located for a long period of time and larvae will be present on the corpse. The ability to detect seminal fluid and obtain a human genetic profile from potential suspects would aid the investigation and ultimately prosecuting a criminal case (Clery, 2001). It is concluded that dissection of crop versus extracting the whole larvae produce better results for both p30 and Y-STR testing (Clery, 2001). A one-twenty-fifth dilution was made for each sample used and a p30 enzyme linked immunosorbent assay (ELISA) was used to detect the p30 enzyme. Samples were extracted with a Chelex-based extraction method and quantified using quantiblot for determination of the amount DNA present extracts. Y-STR profiles were obtained using the polymerase chain reaction and analyzed with an ABI Prism 377 DNA sequencer (Clery, 2001).

A study involving liver samples was performed using varying amounts of seminal fluid. Eggs were placed on the liver samples and were allowed to hatch into first instar larvae and consume the liver substrate (Clery, 2001). Second instar larvae were removed approximately forty-eight hours after initial placement of eggs. Larvae were stored frozen until they were extracted. Larvae samples were also removed one hundred and forty-five hours after initial placement of eggs. Samples were extracted for both p30 testing and Y-STR genetic analysis. All larvae samples produced positive p30 results after forty-eight hours. A Y-STR profile was obtained from four of these larvae samples. A Y-STR profile was not obtained from all of the

larvae extracts; however, this study demonstrates the ability to detect both p30 and develop a Y-STR profile from extraction of larvae (Clery, 2001).

3. PILOT STUDY

3.1 Introduction

A pilot study was performed using pig liver samples to develop protocols for collection of larvae and develop an extraction technique for the larvae samples. A sample of a pig liver was placed outside and wild flies deposited their eggs on these liver samples. Liver samples were transported indoors where larvae and adult flies were collected and preserved. The goals of this study were to (1) develop protocols for the collection and preservation of the larvae samples, (2) develop protocols for the collection and preservation of the adult flies, (3) to develop an extraction technique to extract the larvae samples, (4) to assess two extraction methods, and (5) to develop an appropriate amplification target for the Animaltype Pig PCR amplification kit.

3.2 Materials and Methods

3.2.1 Sample Setup

A liver from a euthanized pig was obtained from the Swine Farm from Oklahoma State University in Stillwater, Oklahoma. The liver was divided up into eight equal portions; each portion of the liver was approximately 9.1 cm x 5.9 cm. Liver portions were placed into eight different rearing chambers (BioQuip® Small Berlese Funnel Trap, 32 oz, clear styrene collection container, Rancho Dominguez, CA). Vermiculite (Sta-Green Horticultural Vermiculite brand) was placed in the bottom of rearing chambers filling approximately ¼ of these compartments and liver samples were placed on top of the vermiculite. Four of the liver samples were treated with approximately one milliliter of commercial human semen (LEE Solutions). All eight of the rearing chambers, with their lids removed, were placed on the roof of Howell Hall at the University of Central Oklahoma campus, in Edmond, Oklahoma on October 15, 2013. The rearing chambers were monitored on a daily basis for indication of any larvae present on the liver

samples. On October 24, 2013 larvae were present on five of the eight chambers containing liver samples. The following day, the eight rearing chambers were removed from the roof and transported to the laboratory at the Oklahoma State Bureau of Investigation. Fresh liver samples were placed in the rearing chambers. The larvae samples were distributed to those rearing chambers that did not have an indication of larvae, ensuring that the treated ones were in the treated rearing chambers and the untreated ones were in the untreated chambers. Liver samples were kept moist by lightly spraying (approximately two sprays (Dynalon™ Quick Mist™ HDPE Sprayer Bottles (Catalog No. 03-438-12A, 16 oz, Thermo Fisher Scientific, Inc.))) with de-ionized water throughout the entire process. On October 31, 2013, an additional one-milliliter of commercial human semen was added to the treated liver samples. Table 1 illustrates the dates of the collection of larvae from the liver samples, as well as the dates adult flies were collected. Larvae samples were collected and placed in plastic conical tubes and frozen at -24^{0}C, until extracted. Adult flies were collected and persevered in the freezer at -24^{0}C, until they were taxonomically identified.

Table 1: Dates of Collection of Larvae from Liver Samples and Collection of Adult Flies

Date	Collection of Larvae		Collection of Adult Flies	
	Untreated	Treated	Untreated	Treated
10/24/13	Yes	Yes		
10/25/13	Yes	Yes		
10/28/13	Yes	Yes		
10/31/13	Yes	Yes		
11/11/13	No	Yes		
11/15/13	No	Yes		
11/5/13			Yes	Yes
11/7/13			Yes	Yes
11/11/13			Yes	Yes
11/13/13			Yes	Yes
11/17/13			Yes	Yes
11/20/13			Yes	Yes
11/22/13			Yes	Yes

3.2.2 Extraction of Larvae Samples

Larvae samples collected for the pilot study were extracted using two extraction methods. A standard organic extraction (Comey, et al., 1994) and extraction with the Qiagen DNeasy® Blood and Tissue Kit (Qiagen, Austin, Texas) were used to extract the larvae samples. The two extractions methods were compared to determine which extraction method provided better recovery of the pig DNA with minimal PCR inhibitors.

3.2.2.1 Organic Extraction Method Utilized for this Experiment

Larvae samples collected were extracted with a standard organic extraction (Comey, et al., 1994) method utilized by the Oklahoma State Bureau of Investigation Forensic Biology Unit. Larvae samples were placed in a microcentrifuge tube and crushed with a metal rod. Approximately 500 µL of a stain extraction buffer was added to the microcentrifuge tube. Stain extraction buffer (SEB) consisted of 1M Tris-HCl, 0.5 M EDTA, 0.39 M DTT, 5 M NaCl, 20% SDS, and ultra-pure water. Approximately 40 µL of Proteinase K was added to each microcentrifuge tube. Samples from the treated liver had an additional 20 µL of DTT added to them to break the disulfide bonds of the sperm cells. The solution was incubated at 57º C for approximately 4 to 24 hours on an Eppendorf Thermo Mixer at 750 RPM. The resulting solution was centrifuged at approximately 13,500 to 15,000 RPM for five minutes and 500 µL of phenol/chloroform/isoamyl (PCI) (24:24:1) solution (BioFisher Reagents) was added. DNA is contained in the aqueous layer which was collected and placed in a Microcon centrifugal filter device, the organic layer was discarded. The Microcon centrifugal filter device was centrifuged at ~ 2,300 RPM for twenty to thirty minutes. A wash step was performed by adding ~ 200 µL of TE^{-4} to the filter and centrifuging for an additional twenty to thirty minutes. If additional washes were necessary they were performed in the same manner. DNA was collected by inverting the

filter into a second tube and centrifuging at ~ 3,300 RPM for approximately five minutes. The resulting DNA extract was brought to a workable volume with TE^{-4}, if applicable.

3.2.2.2 Qiagen DNeasy® Blood and Tissue Extraction

DNeasy® Blood and Tissue kits (Qiagen part number 69506) are designed to extract and purify total DNA from a variety of samples. These samples include fresh or frozen animal tissues and cells, blood, and bacteria. Samples are free of contaminants and enzyme inhibitors which results in extracts that are highly suitable for polymerase chain reaction (PCR) procedures (Qiagen, 2006). Purification of DNA extracts does not require phenol or chloroform and involves minimal handling which makes the kit ideal to process multiple samples and does not require special handling precautions with hazardous chemicals. The buffer system in the kit is optimized to allow direct cell lysis followed by selective binding of the DNA to the DNeasy membrane (Qiagen, 2006). Simple centrifugation processing removes contaminants and *Taq* polymerase inhibitors, such as proteins and divalent cations which allow multiple samples to be processed in parallel (Qiagen, 2006).

According to the Qiagen DNeasy® Blood and Tissue Handbook (2006), the procedures are simple and straightforward. Samples are first lysed with Proteinase K and a buffer solution provided in the kit. Buffering conditions are optimized to provide efficient DNA binding to the DNeasy® membrane and the lysate is placed in the DNeasy® Mini Spin column. The spin column is centrifuged and the DNA is selectively bound to the DNeasy® membrane as contaminants pass through. Any remaining contaminants and enzyme inhibitors are removed through two wash steps and DNA is eluted with an elution buffer and results in extracted DNA that is ready to use (Qiagen, 2006). DNeasy® membranes combine binding properties of a silica-based membrane with simple micro-spin technology. DNA will adsorb into the DNeasy®

membrane in presence of high salt concentrations of a chaotropic salt, which will remove water from hydrated molecules in solutions (Qiagen, 2006). Buffer conditions of the blood and tissue kit are specifically designed to enable specific adsorption of DNA and removal of containments and enzyme inhibitors (Qiagen, 2006).

Larvae and known liver samples collected from the pilot study were extracted following the manufacturer's recommended protocol for extraction of animal tissues with spin columns. Samples were placed in a microcentrifuge tube and cut into small pieces or crushed with a metal rod, as appropriate. Approximately 180 µL of Buffer ATL and 20 µL of Proteinase K were added to each sample and mixed thoroughly. In addition, the liver samples treated with commercial human seminal fluid had ~ 20 µL of DTT added. Samples were incubated at 57° C on a Thermo Mixer (Eppendorf) at 750 RMP for approximately 4 to 24 hours to allow samples to completely lyse, which is a slight modification from the manufactures recommended protocol. Approximately 200 µL of Buffer AL was added to each sample followed by ~ 200 µL of ethanol; the samples were mixed thoroughly after each addition of chemicals. The mixture was added to the DNeasy® mini spin columns and centrifuged at approximately 8,000 RPM for one minute; flow through was discarded. DNA was washed by adding 500 µL of Buffer AW1 to the spin columns and centrifuging them at 8,000 RPM for one minute; flow through was discarded. A second wash was performed by adding 500 µL for Buffer AW2 to the spin columns and centrifuging them for three minutes at 14,000 RPM; flow through was discarded. DNA was eluted into a microcentrifuge tube by adding 200 µL of Buffer AE to the spin columns and centrifuging them for one minute at 8,000 RPM.

3.2.3 Quantification

The resulting DNA extracts that were obtained from the two extraction methods were quantified. This quantification was performed utilizing the Thermo Scientific NanoDrop 2000 UV-Vis Spectrophotometer. Each sample was quantified by placing approximately 2 µL of the sample on the pedestal, lowering the sampling arm and initiating the spectral measurement using the NanoDrop 2000 interface software (Thermo Fisher Scientific, Wilmington, DE) supplied with the instrument. After a sample was analyzed the sampling arm was raised and the pedestal was cleaned with a lint-free laboratory wipe between each subsequent analysis. Each sample was quantified in triplicate to obtain an average quantification value. The elution buffer, from the Qiagen DNeasy® Blood and Tissue Kit, was used as a blank for the Thermo Scientific NanoDrop 2000 UV-Vis Spectrophotometer. TE^{-4} was used as a blank for any dilutions and extracts processed with the standard organic extraction method.

Larvae obtained from the treated liver samples were quantified using the Applied Biosytstems Quantifiler® Y Human Male DNA Quantification kit using a AB 7500 real time PCR instrument. A set of eight standards of varying concentrations were analyzed concurrently with each quantification plate that was quantitated with the real time PCR quantification procedure. A serial dilution was used to achieve the following standard DNA concentrations: 50.000 ng/µL (Standard A), 16.700 ng/µL (Standard B), 5.560 ng/µL (Standard C), 1.850 ng/µL (Standard D), 0.620 ng/µL (Standard E), 0.210 ng/µL (Standard F), 0.068 ng/µL (Standard G), and 0.023 ng/µL (Standard H). The first standard was prepared by taking approximately 10 µL of the 200 ng/µL stock solution supplied with a Applied Biosystems Quantifiler® Y Human Male DNA Quantification kit and ~ 30 µL for TE^{-4}. A master mix was prepared for each well that contained a standard, DNA extract sample, or reagent blank. The master mix was prepared by

adding approximately 10.5 µL of the Quantifiler Y Human Male Primer Mix and approximately 12.5 µL of the Quantifiler PCR Reaction Mix to each reaction on a 96 well plate. For each quantification plate the appropriate volumes were placed in a 1.5 mL microcentrifuge tube, vortexed briefly, centrifuged and placed into the appropriate wells on the MicroAmp® Optical 96 well reaction plate. The plate was covered with a MicroAmp® Optical Adhesive film and placed on the AB 7500 real time PCR instrument.

3.2.4 Amplification

DNA extracted from larvae samples were amplified with an Animaltype Pig PCR Amplification Kit, which is a commercial kit for the fast and reliable genotyping of pig DNA. The Animaltype Pig PCR Amplification Kit uses eleven tetra-nucleotide short tandem repeat markers and a gender specific marker (SBH23), similar to the genotyping systems used for human identification. The Animaltype pig kit contains Nuclease-free water, Reaction Mix D, Primer Mix, DNA polymerase, Control DNA DL 157, DNA size standard 550, and an allelic ladder. This kit is designed for fast and reliable DNA genotyping of blood or tissue samples with one primer per locus fluorescently labeled with a three-dye system which provides well-balanced intensity of all signals (Animaltype Pig PCR Amplification Kit, December 2007). The detection limit of the Animaltype PCR amplification kit is less than approximately 1 ng of genomic DNA. The recommended range of input DNA is approximately 1 to 10 ng. The pig genotyping kit has been evaluated and validated for the use of a GeneAmp® 9700 thermal cycler, ABI Prism 3130 and 3100/3130 Genetic Analyzers (Animaltype Pig PCR Amplification Kit, December 2007).

A master mix was prepared by determining the number of reactions that was amplified with Animaltype Pig PCR amplification kit. The number of reactions included a positive amplification control, negative amplification control, reagent blanks, and larvae extracts. The

following ratios of the amplification kit components were used to prepare the master mix: 12.1 µL of the Nuclease free water, 5.0 µL of the reaction mix, 2.5 µL of the primer mix, and 0.4 µL of DNA *Taq* Polymerase for a total volume of 20 µL for the master mix. The master mix was pipetted into each amplification tube and the appropriate amount of DNA template was added for a total reaction volume of 25 µL. A positive control consisted of 5 µL of a 1:50 dilution of the control DNA DL 157 and the master mix. A negative amplification control consisted of 5 µL of TE^{-4} and the master mix. Reagent blanks were amplified by adding 5 µL of the reagent blank extract to the master mix. Amplification tubes were amplified by placing them on an Applied Biosystems GeneAmp® PCR System 9700 thermal cycler. The manufacture's recommendations (Figure 11) for the Animaltype Pig PCR kit were followed, which consisted of a four minute hot start for the activation of the Multi Taq2 DNA polymerase and 30 cycles to complete the polymerase chain reaction (PCR).

Standard Method

recommended for all DNA samples

Temperature	Time	
94°C	**4 min** (hot start for Activation of the Multi Taq2 DNA Polymerase)	
94°C	20 s	
60°C	40 s	30 Cycles
72°C	30 s	
70°C	60 min	
10°C	∞	hold

Figure 11. Protocol for the GeneAmp® PCR System 9700 thermal cycler for the Animaltype Pig PCR kit ((Animaltype Pig PCR Amplification Kit, December 2007). The thermal cycling parameters used for the amplification of DNA samples with the Animaltype Pig PCR amplification kit and the GeneAmp® PCR System 9700 thermal cycler.

3.2.5 Applied Biosystems 3130 Genetic Analysis

Amplified DNA products from extracted samples were analyzed using an Applied Biosystems 3130 Genetic Analyzer to obtain genetic profiles through capillary electrophoresis. A master mix was prepared by combining the Internal Lane Standard (ILS) ROX-550 and formamide in the following ratio: (0.2 µL of ROX-550 x # of samples) + (12.3 µL of formamide

x # of samples) (Animaltype Pig PCR Amplification Kit, December 2007). Twelve microliters of the resulting master mix was pipetted into each sample well of a 96 well plate. One microliter of the amplified DNA product or ladder was added to the appropriate wells on a 96 well plate. The 96 well plate was heat denatured at 95^0 C for 3 minutes and snaped cooled at approximately 0^0 C for an additional three minutes (Animaltype Pig PCR Amplification Kit, December 2007). The 96 well plate was loaded onto the AB 3130 Genetic Analyzer and the amplified DNA products were analyzed with the analysis parameters recommended by the Animaltype PCR amplification kit user manual (Figure 12).

Run Module (24min_50-500bp)

Run Modul Editor	Value
Oven Temperature [°C]	60
Poly Fill Volume	4840
Current Stability [µA]	5
PreRun Voltage [kV]	15
PreRun Time [s]	180
Injection Voltage [kV]	3.0
Injection Time [s]*	5
Voltage Number of Steps	40
Voltage Step Interval	15
Data Delay Time [s]	1
Run Voltage [kV]	15.0
Run Time [s]**	**1440**

* Apart from standard settings, the injection time may range between 1 and 20 s depending on the type of sample. If samples with very high signal intensities are to be recorded, a shorter injection time may be selected. For samples with low DNA content an injection time up to 20 s may be necessary.

** Depending on the analysis conditions, the Run Time for Animaltype **Pig** was modified in order to analyze lengths of fragments up to **500 bp**.

Figure 12. Run Module Analysis Parameters used for analyzing the amplified pig DNA products on AB 3130 Genetic Analyzer (Animaltype Pig PCR Amplification Kit, December 2007). The analysis parameters utilized to analyze the amplified pig PCR products on the AB 3130 Genetic Analyzer.

3.2.6 Data Analysis

Data obtained from the AB 3130 Genetic Analyzer was analyzed with Applied Biosystems GeneMapper® ID software version 3.2, which was used for fragment sizing and allele calling. Smoothing was set to light and used a 51 point baseline window. The size calling

method was the local southern method, which permitted the STR fragments to size accurately (Animaltype Pig PCR Amplification Kit, December 2007). Allele calls were made after DNA fragments were sized and compared to the allelic ladder. The peak amplitude threshold within the software was set at 100 RFU, so an allele call would only be assigned a peak that was equal to or greater than 100 RFU. Manufactures recommendations for thresholds are to be set between 50 to 200 RFU, which are determined individually by each laboratory (Animaltype Pig PCR Amplification Kit, December 2007).

3.3 Results

3.3.1 Results of Known Liver Samples

The first extracted DNA that was analyzed with the Animaltype Pig PCR amplification kit was a sample taken from the pig liver and a sample swab of pig liver blood, which were used as known samples. These samples were extracted using the Qiagen DNeasy® Blood and Tissue kit and a standard organic extraction method. The undiluted extracts from the known samples were quantified with the Thermo Scientific NanoDrop 2000 UV-Vis Spectrophotometer. Table 2 shows the concentration of the quantification of these samples.

Table 2: Quantification Results of Known Liver Sample and Known Blood Swab

Sample	Extraction Method	Concentration ng/uL	260/280	260/230
Pig Liver	Qiagen	461.3	1.32	0.55
Pig Blood Swab	Qiagen	50.2	2.03	0.98
RBK 1 012014	Qiagen	7.7	1.86	0.26
Pig Liver	Organic	9542.5	1.57	1.25
Pig Blood Swab	Organic	5588.7	1.51	1.33
RBK2 012014	Organic	7135	1.54	1.23

Based on the above quantification results a 10ng/µL concentration of the known samples was prepared. Known samples were used to prepare various amplification target concentrations, to determine an optimal DNA input target with the Animaltype Pig PCR amplification kit. A

series of amplification targets were prepared that ranged from 0.50 ng/5 µL to 3.0 ng/5 µL. All of the amplification target concentrations were amplified with the Animaltype Pig PCR amplification kit and analyzed with an AB 3130 Genetic Analyzer (Appendix A). The Qiagen DNeasy® Blood and Tissue extraction produced full profiles for liver samples at 2.0 ng and 3.0 ng input targets. The 0.5 ng and 1.0 ng input targets produced partial profiles. The Qiagen extraction for the blood swab did not produce full profiles for any amplification targets amplified, but did produce partial profiles on three of the four amplification targets tested. The standard organic extraction method used with liver samples did not produce any full profiles; however all of the profiles were partial. The standard organic extraction method utilized with the blood swab did not produce any profiles with all four of the amplification targets amplified. Results of all of the amplification targets amplified and the resulting number of loci producing results are illustrated in Table 3.

Table 3: Number of Loci Producing Results for Known Liver Samples (12 loci is a full profile)

Sample Name	Number of Loci Producing Results
1A (Pig Liver Qiagen) 0.5 ng	3
1B (Pig Liver Qiagen) 1.0 ng	11
1C (Pig Liver Qiagen) 2.0 ng	12
1D (Pig Liver Qiagen) 3.0 ng	12
2A (Pig Blood Swab Qiagen) 0.5 ng	0
2B (Pig Blood Swab Qiagen) 1.0 ng	2
2C (Pig Blood Swab Qiagen) 2.0 ng	6
2D (Pig Blood Swab Qiagen) 3.0 ng	10
3A (Pig Liver Organic) 0.5 ng	3
3B (Pig Liver Organic) 1.0 ng	4
3C (Pig Liver Organic) 2.0 ng	11
3D (Pig Liver Organic) 3.0 ng	10
4A (Pig Blood Swab Organic) 0.5 ng	0
4B (Pig Blood Swab Organic) 1.0 ng	0
4C (Pig Blood Swab Organic) 2.0 ng	0
4D (Pig Blood Swab Organic) 3.0 ng	0

3.3.2 Results of First Collection of Larvae from Liver Samples

Maggots collected from the pilot study were extracted from untreated and semen treated liver samples, as described above in section 3.2.1. These samples were extracted using the Qiagen DNeasy® Blood and Tissue and standard organic extraction methods. The samples were then quantified with the Thermo Scientific NanoDrop 2000 UV-Vis Spectrophotometer in triplicate (Table 4 and Table 5). Each sample was quantified in triplicate and the average was obtained. The average of the reagent black quantification value was subtracted from the average value to obtain the adjusted average quantification value.

Table 4: Quantification Results of larvae Collection #1 (Organic and Qiagen Extraction) Untreated 1= Qiagen Extraction, 2= Organic Extraction

Sample	Quant Value 1 (ng/µL)	Quant Value 2 (ng/ µL)	Quant Value 3 (ng/ µL)	Average (ng/ µL)	Adjusted Average Value (ng/ µL)	Standard Deviation (ng/ µL)
1A Neat	651.2	653.1	650.6	651.6	643.7	1.3
1B Neat	868.3	858.0	844.1	856.8	848.9	12.1
1C Neat	565.6	564.7	564.0	564.8	556.8	0.8
1D Neat	194.3	193.5	192.9	193.6	185.6	0.7
1E Neat	727.9	728.1	728.7	728.2	720.3	0.4
RB 1 031714	7.8	8.4	7.6	7.9	0.0	0.4
1A (1:50)	19.7	19.5	20.4	19.9	11.9	0.5
1B (1:50)	24.9	24.3	24.4	24.5	16.6	0.3
1C (1:50)	16.3	15.2	15.1	15.5	7.6	0.7
1D (1:50)	6.2	5.7	5.4	5.8	-2.2	0.4
1E (1:50)	19.9	20.3	20.1	20.1	12.2	0.2
2A Neat	8763.8	8524.9	5935.2	8741.3	7215.1	206.1
2B Neat	9952.0	10183.5	9940.8	10025.4	8499.3	137.0
2C Neat	10205.2	10110.6	10276.8	10197.5	8671.4	83.4
2D Neat	3574.0	3218.4	3269.4	3353.9	1827.8	192.3
2E Neat	8354.2	7160.8	7172.0	7562.3	6036.2	685.8
RB2 031714	1523.7	1522.4	1532.4	1526.2	0.0	5.4
2A (1:50)	224.0	223.0	222.3	223.1	223.1	0.9
2B (1:50)	311.3	309.5	310.2	310.3	310.3	0.9
2C (1:50)	295.1	298.0	297.0	296.7	296.7	1.5
2D (1:50)	334.2	332.4	330.5	332.4	332.4	1.9
2E (1:50)	191.5	199.3	191.2	194.0	194.0	4.6

The samples that are labeled with a 1 before the letter was extracted with the Qiagen DNeasy® Blood and Tissue kit and the samples that are labeled with a 2 are from the organic extraction

Table 5: Quantification Results of Larvae Collection #1 (Organic and Qiagen Extraction) Treated 1= Qiagen Extraction, 2= Organic Extraction

Sample	Quant Value 1 (ng/ µL)	Quant Value 2 (ng/ µL)	Quant Value 3 (ng/ µL)	Average (ng/ µL)	Adjusted Average Value (ng/ µL)	Standard Deviation (ng/ µL)
1AT Neat	644.1	642.7	646.8	644.5	636.8	2.08
1BT Neat	1070.4	1063.6	1085.3	1073.1	1065.4	11.10
1CT Neat	633.1	629.3	630.9	631.1	623.4	1.91
1DT Neat	502.5	506.1	506.8	505.1	497.4	2.31
1ET Neat	1049.7	1051.9	1055.8	1052.5	1044.7	3.09
RB 1 031714	7.7	7.5	8.0	7.7	0.0	0.25
1AT (1:50)	17.1	17.4	17.2	17.2	9.5	0.15
1BT (1:50)	23.8	23.2	24.2	23.7	16.0	0.50
1CT (1:50)	17.8	17.7	17.5	17.7	9.9	0.46
1DT (1:50)	42.0	41.3	41.5	41.6	33.9	0.15
1ET (1:50)	42.0	41.3	41.5	41.6	33.9	0.36
2AT Neat	239.0	235.7	236.2	237.0	0.0	1.78
2BT Neat	3417.1	3465.6	3430.6	3437.8	1386.2	25.03
2CT Neat	11356.9	11294.4	11285.0	11312.1	9260.5	39.08
2DT Neat	11321.8	11341.9	11501.5	11388.4	9336.8	98.46
2E T Neat	9084.6	9201.0	9267.0	9184.2	7132.6	92.35
RB2 031714	2072.8	2052.5	2029.5	2051.6	0.0	21.66
2AT (1:50)	4.5	5.3	6.8	5.5	5.5	1.17
2BT (1:50)	409.9	411.2	411.4	410.8	410.8	0.81
2CT (1:50)	358.5	357.7	361.3	359.2	359.2	1.89
2DT (1:50)	359.0	359.5	358.3	358.9	358.9	0.60
2ET (1:50)	256.4	258.0	258.2	257.5	257.5	0.99

The samples that are labeled with a 1 before the letter was extracted with the Qiagen DNeasy® Blood and Tissue kit and the samples that are labeled with a 2 are from the organic extraction

Larvae treated with commercial human seminal fluid were quantified with an Applied Biosystems Quantifiler® Y Human Male DNA Quantification kit. No quantification results were obtained for larvae samples; however, quantification results were obtained from the known semen swab (Table 6). Four larvae samples (2BT, 2CT, 2DT, and 2ET) extracted with the standard organic extraction method indicated the presences of a PCR inhibitor based on the IPC C_T values obtained. The remaining samples indicated no inhibition. Since, no quantification values were obtained from larvae extracts treated with human seminal fluid, these samples were not amplified.

Table 6: Quantification Results for Treated Larvae Samples with AB Quantifiler® Y Human Male DNA Quantification Kit
1= Qiagen Extraction, 2= Organic Extraction

Sample	IPC C_T	Sample C_T	Quantity (ng/uL)
1AT	27.11	Undet	0.00
1AT (1:50)	26.82	Undet	0.00
1BT	27.05	Undet	0.00
1BT (1:50)	26.65	Undet	0.00
1CT	26.86	Undet	0.00
1CT (1:50)	26.77	Undet	0.00
1DT	26.91	Undet	0.00
1DT (1:50)	26.92	Undet	0.00
1ET	27.35	Undet	0.00
1ET (1:50)	27.04	Undet	0.00
RB3 031714	27.00	Undet	0.00
2AT	26.90	Undet	0.00
2AT (1:50)	26.71	Undet	0.00
2BT	Undet	Undet	0.00
2BT (1:50)	27.01	Undet	0.00
2CT	Undet	Undet	0.00
2CT (1:50)	26.66	Undet	0.00
2DT	Undet	Undet	0.00
2DT (1:50)	26.97	Undet	0.00
2ET	Undet	Undet	0.00
2ET (1:50)	27.18	Undet	0.00
RB4 031714	27.28	Undet	0.00
Semen Swab	26.67	25.79	10.33
Semen Swab (1:50)	26.68	31.91	0.293
RBK 031914	26.58	Undet	0.00

The samples that are labeled with a 1 before the letter was extracted with the Qiagen DNeasy® Blood and Tissue kit and the samples that are labeled with a 2 are from the organic extraction

Using the above quantification results for the first collection of larvae from the untreated liver samples, DNA extracts were amplified with an Animaltype Pig PCR amplification kit. Known liver and blood swab extracts were also amplified at higher DNA input amounts to determine an optimal target range for amplification with the Animaltype Pig PCR kit (Appendix B and Table 7).

Table 7: Number of Loci Producing Results (12 loci is a full profile)

Sample Name	Number of Loci Producing Results
Pig Liver Qiagen 3.0 ng 5 seconds 1A	0
Pig Liver Qiagen 3.0 ng 10 seconds 1A	1
Pig Liver Qiagen 4.0 ng 5 seconds 1B	1
Pig Liver Qiagen 4.0 ng 10 seconds 1B	3
Pig Liver Qiagen 5.0 ng 5 seconds 1C	7
Pig Liver Qiagen 5.0 ng 10 seconds 1C	11
Pig Liver Qiagen 6.0 ng 5 seconds 1D	11
Pig Liver Qiagen 6.0 ng 10 seconds 1D	12
Pig Blood Swab Qiagen 5.0 ng 5 seconds 2A	0
Pig Blood Swab Qiagen 5.0 ng 10 seconds 2A	0
Pig Blood Swab Qiagen 6.0 ng 5 seconds 2B	0
Pig Blood Swab Qiagen 6.0 ng 10 seconds 2B	1
Pig Blood Swab Qiagen 7.0 ng 5 seconds 2C	1
Pig Blood Swab Qiagen 7.0 ng 10 seconds 2C	3
Pig Blood Swab Qiagen 8.0 ng 5 seconds 2D	8
Pig Blood Swab Qiagen 8.0 ng 10 seconds 2D	12
Pig Liver Organic 7.0 ng 5 seconds 3C	1
Pig Liver Organic 10.0 ng 10 seconds 3D	8
Blood Swab Organic 7.0 ng 5 seconds 4C	0
Blood Swab Organic 7.0 ng 10 seconds 4C	0
Blood Swab Organic 10.0 ng 5 seconds 4D	0

Larvae samples that were amplified from the first collection of untreated livers samples did not produce any results. No allele calls were obtained from the amplification of samples at an amplification target of 10 ng/5 µL (Appendix C). The maximum (5 µL) amount of DNA extract was amplified with an Animaltype Pig PCR kit and no allele calls were obtained from either extraction method (Appendix D). The maximum amounts of DNA extracts from 10 ng

concentration of pig liver and blood swab were also amplified (Appendix E). The number of loci producing results and the number of allele calls obtained from the amplification of the maximum (5 μL) amount of the 10 ng concentration of the known pig liver and blood swab extracts are depicted in Table 8.

Table 8: Number of loci (12) and Number of allele calls (21) for the Known Liver and Blood Samples

Sample	Number of loci producing results	Number of allele Calls
Pig Liver Qiagen 10 ng	12	21
Blood Swab Qiagen 10 ng	12	21
Pig Liver Organic 10 ng 5 seconds	12	20
Pig Liver Organic 10 ng 10 seconds	12	21
Blood Swab Organic 10 ng 5 seconds	8	10
Blood Swab Organic 10 ng 10 seconds	10	15

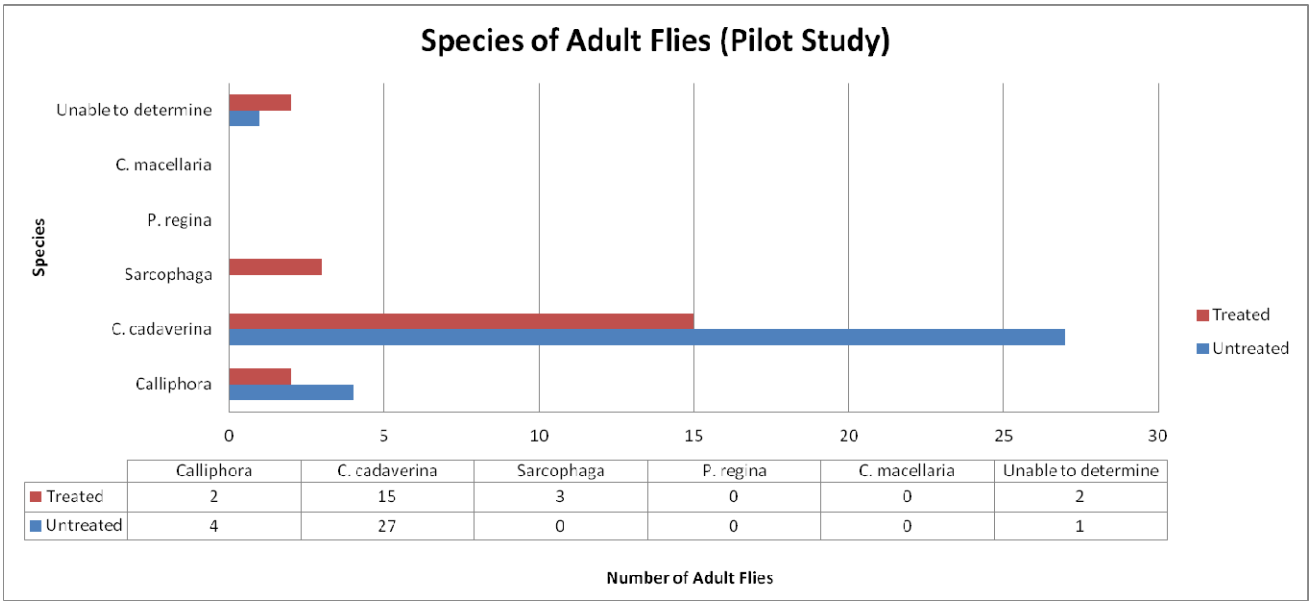
Further extractions were performed to determine if a different preservation method should be utilized. Three additional larvae samples from untreated liver samples and three samples of larvae that were placed in a water bath at approximately 77^0 C for 2-3 minutes, then stored in ethanol at room temperature were extracted using only the Qiagen DNeasy® Blood and Tissue kit (Lord & Burger, 1983). These extracts were not quantified and the maximum amount of extract and a dilution was amplified. One sample (3C and 4C) from each preservation method was concentrated to approximately 20 μL and the maximum amounts of these extracts were amplified. In addition, a sample was prepared to determine if PCR inhibition was present in the larvae extracts. This was accomplished by combining an equal amount of known liver extract with larvae extract in a microcentrifuge tube and the maximum amount of the resulting combined extract was amplified (Appendix F and Table 9).

Table 9: Number of Loci (12) and Number of Allele Calls (21) for Larvae Extraction

Sample	Number of loci producing results	Number of allele Calls
3A Neat (Frozen) 10 seconds	1 (OL allele called)	0
3B Neat (Frozen) 10 seconds	0	0
3C Neat (Frozen) 10 seconds	0	0
4A Neat (EtOH) 5 seconds	9	15
4A Neat (EtOH) 10 seconds	11	17
4A (1:10) 5 seconds	5	8
4A (1:10) 10 seconds	5	8
4B Neat (EtOH) 10 seconds	2	3
4C Neat (EtOH) 10 seconds	0	0
Known Liver Sample with Maggot Extract	12	21

3.3.3 Species Determination of Flies Collected from Liver Samples

Fifty-four adult flies were collected from both the untreated and treated liver samples. These flies were collected in a plastic conical tube and stored frozen at -24º C until they were taxonomically identified using a pictorial identification key (Pratt, Littig, & Scott, 1975). The taxonomical identifications were verified using "Keys to the Genera and Species of Blow Flies (Diptera*: Calliphoridae*) of America North of Mexico" by Whitworth (2006). There were thirty-two flies collected from untreated liver samples and twenty-two collected from treated liver samples. From the untreated liver samples four adult flies were identified as belonging to the family Calliphoridae *Calliphora.* These particular flies were not identified any further, as the taxonomical key did not depict the particular species. There were twenty-seven adult flies that were identified as belonging to the family Calliphoridae, *Cynomyposis cadaverina*. One fly was not intact and was not able to be identified. The treated liver samples had two adult flies that were identified as Calliphoridae *Calliphora*, fifteen identified as Calliphoridae *Cynomyposis cadaverina,* and thee that were identified as Sarcophagidae *Sarcophaga*. Two flies were not able to be identified due to lack of identifying features. Figure 13 illustrates the distribution of the identification of the adult flies and where they were collected.



	Calliphora	C. cadaverina	Sarcophaga	P. regina	C. macellaria	Unable to determine
Treated	2	15	3	0	0	2
Untreated	4	27	0	0	0	1

Figure 13: Distribution of species of adult flies identified in the pilot study and if they were collected from the untreated liver or the liver that was treated with commercial seminal fluid.

3.4 Discussion

Larvae collected from liver samples during the pilot study were stored at -24^0 C for approximately five months. None of these larvae samples produced results when amplified with the Animaltype Pig PCR amplification kit despite the fact that the extraction of the larvae samples produced a large amount of DNA according to the quantification with the Thermo Scientific NanoDrop 2000 UV-Vis Spectrophotometer. Since the entire larvae was extracted, there is a large amount of larvae DNA present in the sample and it cannot be determined if there was any actual pig DNA present. Liver samples treated with the commercial human semen were quantified with the Applied Biosystems Quantifiler® Y Human Male DNA Quantification kit. None of these samples produced any quantifiable results; however, low amounts of male DNA present in a sample may not always be detectable by the quantification kit and full profiles are commonly obtained with subsequent amplification of these samples. Ten samples were quantified with the Applied Biosystems Quantifiler® Y Human Male DNA Quantification kit and

four of these samples indicated a PCR inhibitor present based on the IPC C_T values obtained. A dilution (1:50) of the treated larvae samples was also quantified with the Quantifiler® Y Human Male DNA Quantification kit, which is typically performed when there are large concentrations of DNA and the dilution is also utilized to overcome inhibition. Quantification of these dilutions did not indicate any PCR inhibitors were present. Furthermore, all four of the samples that were inhibited were the extracts that were extracted using the standard organic extraction method, indicating that the Qiagen DNeasy® Blood and Tissue kit produced higher quality of DNA extracts. No useful information was obtained by quantifying the larvae samples with the Thermo Scientific NanoDrop 2000 UV-Vis Spectrophotometer. Subsequent samples collected for amplification with the Animaltype Pig PCR amplification were not quantified and the maximum amount of extract was used for the PCR amplification process. Larvae samples collected for determination of human seminal fluid will be quantified with the Applied Biosytstems Quantifiler® Y Human Male DNA Quantification kit.

Since no results were obtained from the amplification of larvae with the Animaltype Pig PCR amplification kit, it could be concluded that the preservation method of the larvae, prior to extraction, was not adequate. Extraction of the known pig liver samples obtained full profiles, which suggest that the extraction and subsequent amplification with the Animaltype Pig PCR amplification kit performed as expected. In addition all controls associated with the amplification process performed as expected, indicating there was not a problem with the

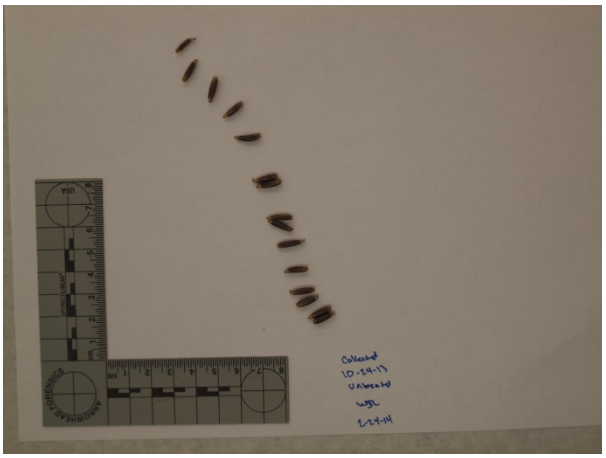


Figure 14: Representation of the larvae that were collected and extracted from the untreated liver sample.

amplification process. A visual observation of larvae prior to extraction would indicate possible degradation of the larvae based on their color (Figure 14). A different preservation method was utilized in the next phase of this research. Results obtained from previous research suggest that DNA recovery is optimal when larvae were stored at -70^0 C with no preservation fluid. In addition, research concluded that larvae stored in ethanol at 24^0 C and 4^0 C obtained better results than larvae that were stored without any preservation fluid (Linville, Hayes, & Wells, 2004). Larvae collected from the pigs, in the subsequent phase of the research, were preserved both at -70^0 C without any preservation fluid and placed in a water bath at approximately 77^0 C for 2 -3 minutes and stored in 95% ethanol until they were extracted (Lord & Burger, 1983). An additional preservation method was implemented, in which larvae were washed with de-ionized water and placed directly into a lysis buffer at time of collection. The lysis buffer , also referred to as stain extraction buffer (SEB), consisted of 1M Tris-HCl, 0.5 M EDTA, 0.39 M DTT, 5 M NaCl, 20% SDS, and ultra-pure water. The additional preservation method of adding the larvae directly to the lysis buffer was completed to ensure larvae samples would not degrade, prior to extraction. The larvae-lysis buffer mixture was stored at a 4^0 C in the refrigerator until the samples were extracted.

Extraction of known liver and blood swabs obtained better results from the Qiagen DNeasy® Blood and Tissue kit rather than from the standard organic extraction method. Overall, genetic profiles produced from the various amplification targets from the Qiagen DNeasy® Blood and Tissue kit extraction method produced a greater number of allele calls, as well as more loci obtaining results. The maximum amount of 10 ng of DNA from each known concentration extract was amplified with the Qiagen DNeasy® Blood and Tissue kit and produced profiles with no dropout for both liver and blood swab samples at = second injection times. The standard

organic extraction method had dropout of one allele from the liver sample at the 5 sec injection, but it was obtained through increasing the injection time to 10 seconds. The blood swab sample had complete dropout at four loci at 5 second injection and two loci at 10 second injection times. Overall, genetic profiles obtained from the Qiagen DNeasy® Blood and Tissue kit extraction method produced taller peak heights and obtained a full profile without increasing injection times on the genetic analyzer. The remainder of this research the larvae were only extracted using the Qiagen DNeasy® Blood and Tissue kit. Associated controls which include positive amplification controls, negative amplification controls, and reagent blanks performed as expected.

4. COLLECTION OF LARVAE FROM PIG CARCASSES (*SUS SCOFA)*

4.1 Introduction

The following study utilized two euthanized pig carcasses to represent a human body. The two pig carcasses were obtained from Oklahoma State University's Swine Farm, located in Stillwater, Oklahoma. Forensic entomologists view the domestic pig as a model species for research because of its similarity to humans (Swindle & Smith, 1998). One pig represented a decomposing body that was a victim to a violent crime and was identified as pig #1. The second pig was intended to model another decomposing body that was the victim of a sexual assault and homicide; this pig was identified as pig #2.

4.2 Materials and Methods

4.2.1 Deposition of pig carcass

Two pig carcasses were retrieved from a freezer and allowed to thaw in a refrigerator for two days prior to placing them in the field. The two pig carcasses, with approval from the Oklahoma Department of Wildlife Conservation, were placed in a grassy area at the Arcadia Conservation Education Area for decomposition on February 24, 2014. Appendix G is a map of the area, where the pig carcasses were located. The pig carcasses were placed in cages to prevent scavengers from removing the pig carcasses (Figure 15). Commercial human semen (LEE Biosoltions) was placed on

Figure 15: Depiction of the pig carcasses location at the Arcadia Lake Education Center. Pig #1 is located on the right and pig #2 is located on the left.

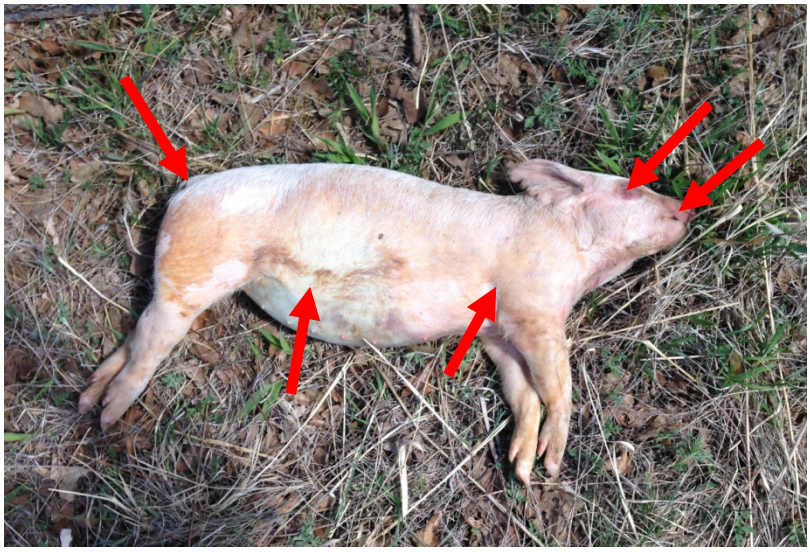


Figure 16: Depiction of the pig carcasses #2 showing the locations of the commercial seminal fluid deposited. 2 mL were deposited on the eye, mouth, back end, stomach, and shoulder. The red arrows represent the locations the commercial seminal fluid was deposited.

pig #2, to model the simulated sexual assault of the pig carcass. Approximately 2 mL of the commercial human semen was placed in five areas on the pig carcass. The commercial human semen was placed on the eye, mouth, back end, stomach, and shoulder of the pig carcass. Figure 16 is a depiction of the areas that the commercial semen was placed, with the red arrows indicating locations of the commercial semen. A cutting from the ear of each pig carcass was taken to use as a known sample for the pig carcasses.

4.2.2 Collection of the larvae from the carcasses

Crawling insects are typically collected from the surface and within a corpse and should be collected with forceps or fingers (Lord & Burger, 1983). Hands were protected with gloves at all times when larvae samples were collected (Lord & Goff, 1993). Larvae were collected from pig carcasses using forceps and were placed in plastic conical tubes. Larvae were collected directly from the pig carcasses or the area directly underneath them. Larvae collection from pig #2 was concentrated, as much as possible, from areas that were treated with commercial human seminal fluid. Three different collections were taken from each pig carcass, for the different preservation methods. The first preservation method involved placing larvae directly in a lysis buffer (SEB), where they were crushed with a metal rod. This was accomplished by collecting

larvae from the pig carcasses and placing them in a plastic conical tube. Larvae where washed with de-ionized water to remove dirt and debris. Larvae were then placed in microcentrifuge tubes, which contained SEB where larvae were crushed. The microcentrifuge tubes were stored in a refrigerator at 4^0C until they were extracted. The second preservation method was performed by collecting the larvae from the pig carcasses, where they were placed in plastic conical tubes and preserved frozen at -70^0 C in a freezer at Howell Hall Science Building at the University of Central Oklahoma. A final preservation method was obtained by removing larvae from pig carcasses and storing them in plastic conical tubes until they were transported to the OSBI Forensic Science Center laboratory. Once at the laboratory larvae were placed in a ~ 77^0 C water bath for two to three minutes and preserved in a 95% ethanol solution (Fisher Science Education Lot # 2AL65970) and stored in a refrigerator at 4^0 C until they extracted. Additional larvae were collected on three separate days and placed in rearing chambers (BioQuip® Small Berlese Funnel Trap, 32 oz, clear styrene collection container, Rancho Dominguez, CA) at the OSBI Forensic Science Center to develop into adult flies for taxonomic species identification. Vermiculite was placed in the bottom of the rearing chambers covering approximately ¼ of the chambers to allow larvae a place to burrow and absorb waste. A container of cat food (Fancy Feast Chicken and Liver brand) was placed in the rearing chambers as a food source for the larvae. Collected larvae were placed in the appropriate rearing chambers and were labeled with the appropriate pig and collection date. Cat food was replenished as necessary for all rearing chambers. When adult flies emerged, the entire rearing chamber was briefly placed in a -24^0 C freezer, to stun the adult flies, which were collected and stored in plastic conical tubes at ~ -24^0 C until taxonomically identified. Once the adult flies were collected the rearing chambers were

placed back at room temperature, where the larvae continued to develop into adult flies. Rearing chambers were monitored on daily until there were no longer adult flies observed.

Larvae were collected from the first indication of larvae present until pig carcasses were in the skeletal stage when larvae was no longer observed (Figure 17). The skeletal stage is when only bones and hair remain on the carcasses (Goff & Lord, 2001). The number of larvae collected was sufficient to ensure a representative sampling of larvae present from the pig carcasses (Lord and Goff, 1993). Table 10 lists the environmental conditions and the dates that larvae were collected from both pig carcasses.

Table 10: Collection Dates of Larvae and Environmental Conditions

Date	Time	Temperature (^{0}F)	Wind Speed (MPH)	Dew Point	Humidity	Pressure	Larvae Collected*	Collected for Rearing
4/24/14	1436	73	NW 21	---	32%	29.81 in	No €	No
4/25/14	1935	76	S 18	39^0	26%	1011.0 MB	No β	No
4/28/14	1616	71	W 17	36^0	28%	1002.5 MB	Yes	Yes
4/29/14	1554	54	NW 16	35^0	49%	1010.8 MB	Yes	No
4/30/14	1630	59	NW 13	27^0	30%	1019.9 MB	Yes	No
5/1/14	1640	67	NW 12	26^0	21%	1017.7 MB	Yes	Yes
5/2/14	1630	73	NW 6	26^0	17%	1014.3 MB	Yes	No
5/5/14	1515	96	SW 15	48^0	20%	1003.8 MB	Yes	Yes
5/6/14	1345	90	S 17	56^0	31%	1002.5 MB	Yes	No
5/7/14	1457	87	S 25	63^0	43%	1004.2 MB	Yes	No
5/8/14	1620	80	SW 14	61^0	53%	1006.6 MB	Yes	No
5/9/14	1508	76	E 4	50^0	40%	1011.5 MB	Yes ^	No
5/11/14	1435	85	S 26	65^0	52%	1004.6 MB	No £	No

* Larvae collected for the three preservation methods.
^ Only collected from pig #2, no indication of larvae present on pig #1.
£ No indication of any larvae present on either of the pig carcasses.
€ Initial deposition of the pig carcasses.
β No indication of any larvae present; however there were adult flies covering both pig caresses.

Figure 17: Depiction of the pig carcasses when the collection of the larvae was completed, as there were no longer larvae present on the pig carcasses. Pig #1 is on the left side and pig #2 is on the right side. Pictures taken on 5/11/14.

4.2.3 Extraction

Larvae collected from pig #1, pig #2, and known samples (collected from the ear) of each pig were extracted using the Qiagen DNeasy® Blood and Tissue kit following the manufacturer's recommended protocol for extracting animal tissues with spin columns, as described above in chapter 3.2.2.2. A modification of the protocol was to allow samples to incubate at 57º Celsius on a Thermo Mixer (Eppendorf) at 750 RMP for 4 to 24 hours. This modification was performed to ensure larvae samples were sufficiently lysed. An additional modification of approximately 40 µL of Pro K and 10 µL of DTT was added to each sample for pig #1 and 40 µL of Pro K and 40 µL of DTT was added to each sample for pig #2. The addition of Pro K and DTT was added to pig #1 to ensure that DNA would be released from the cells. The addition was added to pig#2 to ensure all of the DNA was released from the cells and the increase in DTT was added to ensure that the sperm cells would be completely lysed (DTT breaks down the disulfide bonds that make up the sperm cells and the sperm nuclei are impervious to extractions without DTT).

4.2.4 Quantification

Larvae collected from pig #1 were not quantified, based on the quantification results obtained from the pilot study (Chapter 3). The maximum amount of the DNA extract was used for amplification of larvae samples with the Animaltype Pig PCR amplification kit. Larvae collected from pig #2 were quantified with the Applied Biosytstems Quantifiler® Y Human Male DNA Quantification kit using the AB 7500 real time PCR instrument, as described in Chapter 3.

4.2.5 Amplification

Larvae collected from pig #1were amplified with an Animaltype Pig PCR amplification kit as described in Chapter 3. A master mix was prepared and added to each sample that was amplified with the Animaltype Pig PCR amplification kit. The number of reactions, which

included a positive amplification control, negative amplification control, reagent blanks, and larvae extracts, were determined. A master mix was prepared using the following ratios: 12.1 µL of the Nuclease free water, 5.0 µL of the reaction mix, 2.5 µL of the primer mix, and 0.4 µL of DNA *Taq* Polymerase for a total volume of 20 µL for the master mix. The master mix was pipetted into each amplification tube and the appropriate amount of DNA template was added for each amplification tube for a total reaction volume of 25 µL. A positive control consisted of 5 µL of a 1:50 dilution of the control DNA DL 157 and the master mix. A negative amplification control consisted of 5 µL of TE^{-4} and the master mix. Reagent blanks were amplified by adding 5 µL of the reagent blank extract to the master mix. Amplification tubes were placed on an Applied Biosystems GeneAmp® PCR System 9700 thermal cycler. Manufacture's recommendations for the Animaltype Pig PCR kit were followed, which consisted of a four minute hot start for activation of the Multi Taq2 DNA polymerase and 30 cycles to complete the polymerase chain reaction (PCR).

Based on results obtained from extraction of the first two larvae collections from pig #1, the master mix for the Animaltype Pig PCR amplification was altered slightly, to allow for more of the DNA *Taq* Polymerase to be added to the reaction. This was done to ensure there was enough of the DNA *Taq* Polymerase in the reaction to complete the amplification process. Altered ratios of the master mix were as follows: 6.7 µL of the Nuclease free water, 5.0 µL of the reaction mix, 2.5 µL of the primer mix, and 0.8 µL of DNA *Taq* Polymerase for a total volume of 15 µL for the master mix. The master mix was pipetted into each amplification tube and the appropriate amount of DNA template was added to each amplification tube for a total reaction volume of 25 µL. A negative amplification control and reagent blanks were adjusted to add 10 µL of the reagent blank or TE^{-4} (for the negative amplification control) to the master mix.

A positive control consisted of 10 µL of a 1:50 dilution of the control DNA DL 157 and the master mix. Samples were amplified using an Applied Biosystems GeneAmp® PCR System 9700 thermal cycler.

Larvae collected from pig #2 (treated with commercial semen) were amplified using an Applied Biosystems AmpFℓSTR® Y-Filer PCR amplification kit. A master mix was prepared and added to each sample that was amplified with the AmpFℓSTR® Y-Filer PCR amplification kit. A total number of reactions, which consisted of a positive amplification control, negative amplification control, reagent blanks, and larvae extracts, was determined. A master mix was prepared using the following volumes: 9.2 µL of PCR reaction mix, 5.0 µL of Y-Filer primer set, and 0.8 µL of AmpliTaq Gold® DNA polymerase for a total volume of 15 µL (Applied Biosystems AmpFlSTR Yfiler PCR Amplification Kit Users Guide, 2012). The master mix was pipetted into each amplification tube and the appropriate amount of DNA template was added for a total volume of 25 µL. A positive control consisted of 5 µL of the 007 DNA control, 5 µL of TE^{-4}, and the master mix. Negative amplification controls consisted of approximately 10 µL of TE^{-4} and the master mix. Reagent blanks were amplified by adding 10µL of the reagent blank extract to the master mix. Samples were amplified using an Applied Biosystems GeneAmp® PCR System 9700 thermal cycler. Samples were amplified following Applied Biosystems AmpFℓSTR® Y-Filer PCR Amplification Kit user's guide recommendations, which consisted of an eleven minute initial incubation step to activate the hot start DNA polymerase and a 30 cycle thermal cycling protocol (Figure 18).

Initial incubation step	Denature	Anneal	Extend	Final extension	Final hold
HOLD	CYCLE (30)			HOLD	HOLD
95°C 11 min	94°C 1 min	61°C 1min	72°C 1min	60°C 80 min	4°C ∞

Figure 18. Applied Biosystems AmpFlSTR® Y-Filer PCR Amplification Kit Thermal Cycling Parameters (Applied Biosystems AmpFlSTR Yfiler PCR Amplification Kit Users Guide, 2012). This figure depicts the thermal cycling parameters that were used when amplifying samples with the Applied Biosystems AmpFlSTR® Y-Filer PCR Amplification Kit using GeneAmp® PCR System 9700 thermal cycler.

4.2.6 Applied Biosystems 3130 Genetic Analysis

Amplified DNA products from pig #1 were analyzed using an Applied Biosystems 3130 Genetic Analyzer to obtain genetic profiles through capillary electrophoresis. A master mix was prepared by combining the Internal Lane Standard (ILS) ROX-550 and formamide in the following ratio: (0.2 µL of ROX-550 x # of samples) + (12.3 µL of formamide x # of samples) (Animaltype Pig PCR Amplification Kit, December 2007). After the first two collections of larvae samples were analyzed on an AB 3130 Genetic Analyzer the amount of the ROX-550 was increased to 0.5 µL and the formamide was decreased to 12.0 µL. Twelve microliters of the resulting master mix was pipetted into each sample well of an Applied Biosystems 96 well reaction plate. One microliter of the amplified DNA product or ladder was added to the appropriate wells on the reaction plate. The 96 well plate was heat denatured at 95^0 C for 3 minutes and snap cooled at approximately 0^0 C for an additional three minutes (Animaltype Pig PCR Amplification Kit, December 2007). The 96 well reaction plate was loaded onto the AB 3130 Genetic Analyzer. Amplified DNA products were injected both at 5 and 10 second injection times and the injection time that obtained the greater number of callable alleles was used for this research.

Amplified DNA products from pig #2 were analyzed using an Applied Biosytstems 3130 Genetic Analyzer to obtain the male (Y-Filer) genetic profiles through capillary electrophoresis.

A master mix was prepared by combining the ILS LIZ-600 (Applied Biosystems) and formamide in the following volumes: (0.5 µL of LIZ-600 x # of samples) + (8.5 µL x # of samples) (Applied Biosystems AmpFlSTR Yfiler PCR Amplification Kit Users Guide, 2012). Nine microliters of the resulting master mix was pipetted into each sample well of an Applied Biosystems 96 well reaction plate. One microliter of amplified DNA product or Y-Filer allelic ladder was added to the appropriate wells on a 96 well reaction plate. Amplified DNA products were heat denatured at 95^0 C for 3 minutes and snap cooled at approximately 0^0 C for an additional three minutes (Applied Biosystems AmpFlSTR Yfiler PCR Amplification Kit Users Guide, 2012). The 96 well reaction plate, which contained the amplified DNA products, was loaded onto the AB 3130 Genetic Analyzer. Amplified DNA products were injected both at 5 and 10 second injection times and the injection time that obtained the greater number of callable alleles was used for this research.

4.2.7 Data Analysis

Data obtained from analysis of samples from pig #1 using the AB 3130 Genetic Analyzer was analyzed with Applied Biosystems GeneMapper® ID software version 3.2 as described in Chapter 3. Data obtained from samples from pig #2 (Y-Filer) were analyzed with Applied Biosystems GeneMapper® ID-X version 1.4, which was used for fragment sizing and allele calling. The smoothing was set to light and used a 51 point baseline window. The sizing calling method was set to local southern method, which is how the STR fragments were sized (Applied Biosystems AmpFlSTR Yfiler PCR Amplification Kit Users Guide, 2012). Allele calls were made after DNA fragments were sized and compared to a Y-Filer allelic ladder. The peak amplitude threshold was set to 50 RFU for the minimum peak height that the software will assign an allele call to a peak.

4.3 Results

4.3.1 Results from the first two Collections and Known Samples

The first set of samples that were extracted, were the larvae samples collected from each preservation method for the first two collections from both pigs. Known samples (cuttings from ear cartilage) from each pig were also extracted. Samples were extracted with a Qiagen DNeasy® Blood and Tissue kit. DNA extracts from pig #1 were not quantified, but DNA extracts from pig #2 were quantified with the Applied Biosystems Quantifiler® Y Human Male DNA Quantification kit. The results of the quantification of these samples are tabulated in Table 11.

Quantification results obtained from larvae extracts from pig #2 resulted in concentration of larvae extracts and associated reagent blanks with a DNA SpeedVac Concentrator (Thermo Scientific Savant DNA 120 SpeedVac Concentrator) to approximately 15 µL. Quantification of the concentrated DNA extracts was tabulated in Table 12.

Table 11: Quantification Results for Larvae Samples from Pig #2

Sample	IPC C_T	Sample C_T	Quantity (ng/uL)
2A1	27.39	38.45	0.0101
2A2	26.89	Undet	0.0
2A3	26.91	39.83	0.00479
2A4	26.92	Undet	0.0
2A5	27.11	Undet	0.0
2B1	26.53	Undet	0.0
2B2	26.90	Undet	0.0
2B3	26.70	Undet	0.0
2B4	26.86	Undet	0.0
2B5	26.81	Undet	0.0
2C1	26.85	Undet	0.0
2C2	26.83	Undet	0.0
2C3	26.68	Undet	0.0
2C4	26.62	Undet	0.0
2C5	26.83	Undet	0.0
2A6	26.59	Undet	0.0
2A7	26.44	Undet	0.0
2A8	26.59	Undet	0.0
2A9	26.67	Undet	0.0
2A10	26.78	Undet	0.0
2B6	26.95	Undet	0.0
2B7	31.42	Undet	0.0

Table 11: Quantification Results for Larvae Samples from Pig #2

Sample	IPC C_T	Sample C_T	Quantity (ng/uL)
2B8	28.44	Undet	0.0
2B9	Undet	Undet	0.0
2B10	39.98	Undet	0.0
2C6	26.51	Undet	0.0
2C7	27.16	Undet	0.0
2C8	26.39	Undet	0.0
2C9	26.49	Undet	0.0
2C10	26.47	Undet	0.0
RBQ-2 051214	26.43	Undet	0.0
RBQ-4 051314	26.52	Undet	0.0

The samples that are labeled with a 1 before the letter are the samples from pig #1 and the samples that are labeled with a 2 before the letter are the samples from pig #2. The letter A represents the samples that were preserved in 95% ethanol and stored at 4^0 C, the letter B represents the samples that were immediately placed in the lysis buffer and stored at 4^0 C, and the letter C represents the samples that were stored at -70^0 C.

Table 12: Quantification Results for Larvae Samples from Pig #2 after Concentration

Sample	IPC C_T	Sample C_T	Quantity (ng/uL)
2A1	Undet	Undet	0.0
2A2	38.96	Undet	0.0
2A3	Undet	Undet	0.0
2A4	Undet	Undet	0.0
2A5	Undet	Undet	0.0
2B1	39.26	Undet	0.0
2B2	Undet	Undet	0.0
2B3	Undet	Undet	0.0
2B4	Undet	Undet	0.0
2B5	27.77	Undet	0.0
2C1	39.62	Undet	0.0
2C2	39.98	Undet	0.0
2C3	Undet	Undet	0.0
2C4	39.53	Undet	0.0
2C5	Undet	Undet	0.0
2A6	38.26	Undet	0.0
2A7	30.12	Undet	0.0
2A8	35.00	Undet	0.0
2A9	37.70	Undet	0.0
2A10	34.10	Undet	0.0
2B6	Undet	Undet	0.0
2B7	Undet	Undet	0.0
2B8	Undet	Undet	0.0
2B9	Undet	Undet	0.0
2B10	Undet	Undet	0.0
2C6	Undet	Undet	0.0
2C7	Undet	Undet	0.0
2C8	Undet	Undet	0.0
2C9	Undet	Undet	0.0
2C10	Undet	Undet	0.0
RBQ-2 051214	27.12	Undet	0.0
RBQ-4 051314	27.26	Undet	0.0

Quantification of larvae samples extracted from pig #2 indicated that two samples (2B7 and 2B9) were inhibited based on IPC C_T values. Quantification of larvae samples from pig #2 after concentration to 15 µL resulted in every sample, expect one, indicating inhibition. Based on results obtained from quantification of these concentrated extracts, TE^{-4} was added to the extracts to increase the volume to approximately 100 µL. Addition of TE^{-4} was completed prior to amplification with the Applied Biosystems AmpFℓSTR® Y-Filer PCR amplification kit, to reduce the amount of PCR inhibition in the extracts.

Extracts obtained from extraction of larvae from pig #1 and known samples from both pig #1 and #2 were amplified using an Animaltype Pig PCR amplification kit. The maximum amount (~ 5 µL) of DNA extract was amplified for all of the larvae samples. Known (ear cutting) pig samples were amplified using the maximum amount (~ 5 µL) of a 1:50 dilution of known pig sample extracts. Amplification of both known pig samples obtained complete profiles; while amplification of larvae samples from pig #1 obtained results from one (1A2) of thirty samples amplified (Appendix H and Table 13). Amplification of thirty larvae samples from pig #2 obtained callable alleles from nine (2A1, 2A2, 2A3, 2A4, 2A5, 2A7, 2A8, 2C9, and 2C10) samples; amplification of the known seminal fluid swab obtained a full male profile (Appendix I and Table 14).

Table 13: Number of loci (12) and Number of Allele Calls (20) for the Collection 1 and 2 of Pig #1

Sample	Number of loci producing results	Number of allele Calls
1A1	0	0
1A2	12	20
1A3	0	0
1A4	0	0
1A5	0	0
1B1	0	0
1B2	0	0
1B3	0	0
1B4	0	0
1B5	0	0
1C1	0	0
1C2	0	0

Table 13: Number of loci (12) and Number of Allele Calls (20) for the Collection 1 and 2 of Pig #1

Sample	Number of loci producing results	Number of allele Calls
1C3	0	0
1C4	0	0
1C5	0	0
1A6	0	0
1A7	0	0
1A8	0	0
1A9	0	0
1A10	0	0
1B6	0	0
1B7	0	0
1B8	0	0
1B9	0	0
1B10	0	0
1C6	0	0
1C7	0	0
1C8	0	0
1C9	0	0
1C10	0	0
Pig #1 Known	12	20
Pig#2 Known	12	21

Table 14: Number of Loci (16) and Number of Allele Calls (17) for the Collection 1 and 2 of Pig #2

Sample	Number of loci producing results	Number of allele Calls
2A1	10	10
2A2	3	3
2A3	9	9
2A4	7	7
2A5	6	6
2B1	0	0
2B2	0	0
2B3	0	0
2B4	0	0
2B5	0	0
2C1	0	0
2C2	0	0
2C3	0	0
2C4	0	0
2C5	0	0
2A6	0	0
2A7	2	2
2A8	2	2
2A9	0	0
2A10	0	0
2B6	0	0
2B7	0	0
2B8	0	0
2B9	0	0
2B10	0	0
2C6	0	0
2C7	0	0

Table 14: Number of Loci (16) and Number of Allele Calls (17) for the Collection 1 and 2 of Pig #2

Sample	Number of loci producing results	Number of allele Calls
2C8	0	0
2C9	1	1
2C10	1	1
Known Semen Swab	16	17

4.3.2 Results of Troubleshooting

Several troubleshooting steps were conducted based on results obtained from extraction of collections 1 and 2 of larvae samples from pig #1. Three extracts that were preserved in 95% ethanol and stored at 4^0 C and three extracts that were preserved at -70^0 C were concentrated using a DNA SpeedVac Concentrator to a volume of approximately 50 µL. The maximum amount of DNA extract amplified was also increased for these samples from 5 µL to 10 µL. DNA *Taq* polymerase was increased from 0.4 µL per sample to 0.8 µL per sample and the amount of Nuclease free water was decreased to 6.7 µL per sample. An additional three samples, from both preservation methods described earlier, were also processed through a Microcon filtration device and washed with TE^{-4}. The resulting extract was collected and a maximum amount of 15 µL of these extracts was amplified, by reducing the amount of nuclease free water in the master mix to 1.7 µL per sample. Results of these troubleshooting steps for pig #1 are indicated in Appendix J. Out of the twelve samples that were amplified only one (1A7) of these samples, which was processed through a Microcon filtration device, produced any results, which was one callable allele at one locus.

Several troubleshooting steps were also performed on larvae samples obtained from pig #2. Three extracts preserved in 95% ethanol and stored at 4^0 C and three extracts that were preserved at -70^0 C were concentrated using a DNA SpeedVac Concentrator to approximately 50 µL. Approximately 10 µL of these concentrated extracts were amplified with an Applied Biosystems Y-Filer amplification kit. Three additional samples from both preservation methods

were processed with a Microcon filtration device and washed with TE^{-4}. Approximately 10 µL of samples processed with a Microcon filtration device were amplified. Results of these troubleshooting steps for pig #2 are depicted in Appendix K. Twelve samples were amplified from these troubleshooting steps and two of these samples produced results; which were samples processed with a Microcon filtration device (2A8 and 2A9). One of the samples (2A8) produced results at three loci, with three allele calls, while the other sample (2A9) produced one allele call at one locus.

4.3.3 Results of Collections 3 through 10

It was determined, based on results obtained from larvae extracts from collections 1 and 2 from both pigs, that larvae samples preserved in 95% ethanol and stored at 4^0 C provided the best results. In addition, based on quantification results of samples from pig #2, all larvae extracts (both pig#1 and #2) were concentrated to approximately 100 µL with a DNA SpeedVac Concentrator prior to quantification. Results of quantification of larvae samples from collections 3 through 10 from pig #2 are tabulated in Table 15.

Table 15: Quantification Results for Larvae Samples from Pig #2 for Collections 3 through 10

Sample	IPC C_T	Sample C_T	Quantity (ng/uL)
2A11	26.98	Undet	0.0
2A12	26.63	Undet	0.0
2A13	26.15	Undet	0.0
2A14	26.49	Undet	0.0
2A15	26.56	Undet	0.0
2A16	26.73	Undet	0.0
2A17	26.48	Undet	0.0
2A18	26.32	Undet	0.0
2A19	26.04	Undet	0.0
2A20	26.35	Undet	0.0
2A21	26.55	Undet	0.0
2A22	26.83	Undet	0.0
2A23	26.41	Undet	0.0
2A24	26.16	Undet	0.0
2A25	26.22	Undet	0.0
2A26	26.46	Undet	0.0
2A27	26.45	Undet	0.0
2A28	26.39	Undet	0.0
2A29	26.53	Undet	0.0

Table 15: Quantification Results for Larvae Samples from Pig #2 for Collections 3 through 10

Sample	IPC C_T	Sample C_T	Quantity (ng/uL)
2A30	26.78	Undet	0.0
2A31	26.94	Undet	0.0
2A32	26.85	Undet	0.0
2A33	26.79	Undet	0.0
2A34	26.53	Undet	0.0
2A35	26.40	Undet	0.0
2A36	26.39	Undet	0.0
2A37	26.90	Undet	0.0
2A38	26.39	Undet	0.0
2A39	26.29	Undet	0.0
2A40	26.33	Undet	0.0
2A41	26.45	Undet	0.0
2A42	26.50	Undet	0.0
2A43	26.78	Undet	0.0
2A44	26.68	Undet	0.0
2A45	26.66	Undet	0.0
2A46	28.76	Undet	0.0
2A47	26.34	Undet	0.0
2A48	30.56	Undet	0.0
2A49	27.25	Undet	0.0
2A50	27.08	Undet	0.0
RBQ-2 060914	26.16	Undet	0.0

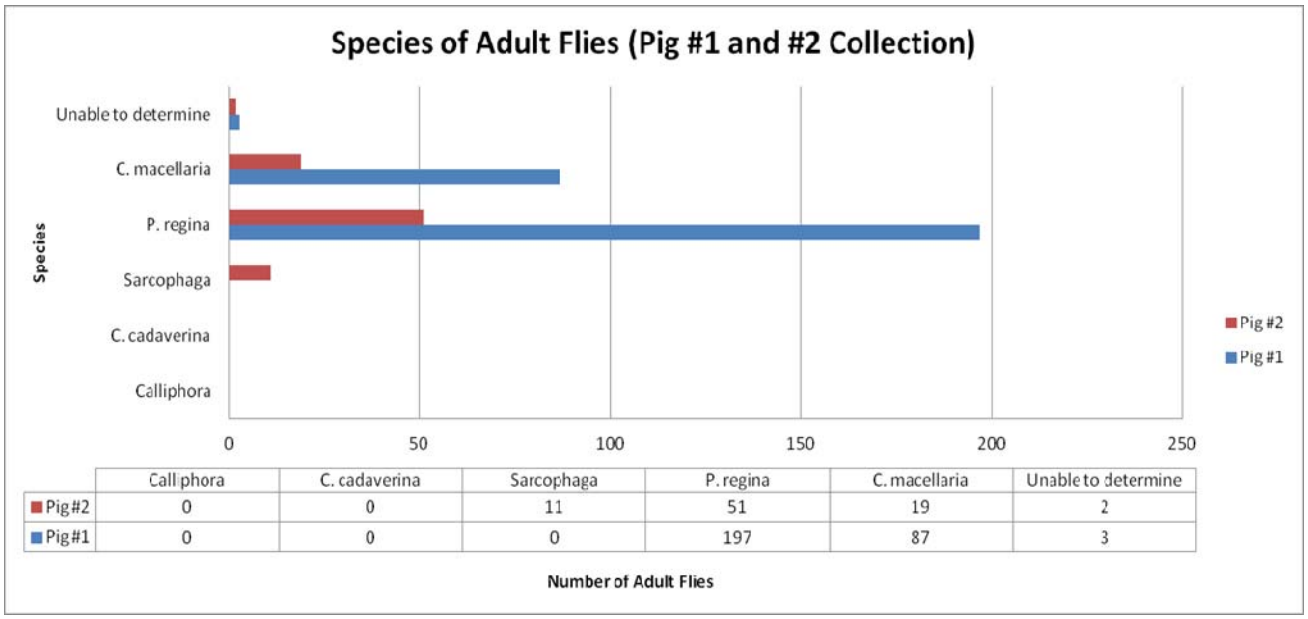
Larvae samples collected from pig#1 were amplified using a maximum volume of 10 µL of DNA extract with an Animaltype Pig PCR amplification kit. Amplification of larvae samples from collections 3 through 10 from pig #1 are depicted in Appendix L, with only one sample (1A17) that produced a callable allele at one locus. The remaining samples did not obtain any callable alleles at any of the loci tested.

Larvae samples collected from pig #2 were amplified with an Applied Biosystems AmpFℓSTR® Y-Filer PCR amplification kit. Amplification of larvae samples from collections 3 through 10 from pig #2 are depicted in Appendix M. The samples did not obtain any callable alleles at any of the loci tested.

4.3.4 Species Determination of Flies Collected from Pig #1 and Pig #2

Larvae were collected from both pigs on three days to rear to adult flies (Table 10). Adult flies were collected and stored in plastic conical tubes at -24º C, until they were

taxonomically identified. There were 370 adult flies collected from both pigs. There were 287 adult flies collected from pig #1 and 83 adult flies collected from pig #2. Using a pictorial key, entitled Pictorial Key to Common Domestic Flies in the U.S. (Pratt, Littig, & Scott, 1975), 87 adult flies from pig #1 were identified as Calliphoridae *Cochiliomyia macellaria*. There were 197 adult flies from pig #1 identified as Calliphoridae *Phormia regina* and three that were not identified due to lack of identifiable characteristics. There were 11 adult flies collected from pig #2 identified as Sarcophagidae *Sarcophaga*, 51 identified as Calliphoridae *Phormia regina*, and 19 identified as Calliphoridae *Cochiliomyia macellaria*. There were 2 adult flies from pig #2 that were not identified due to lack of identifiable characteristics. Figure 19 depicts the number of the different species collected from both pigs.



	Calliphora	C. cadaverina	Sarcophaga	P. regina	C. macellaria	Unable to determine
Pig #2	0	0	11	51	19	2
Pig #1	0	0	0	197	87	3

Figure 19: Distribution of Species Adult Flies Identified from Pig #1 and Pig #2

4.4 Discussion

Five replicates from collections 1 and 2 of each preservation method were extracted with the Qiagen DNeasy® Blood and Tissue kit. Extracted larvae samples from pig #2 were quantified with an Applied Biosystems Quantifiler® Y Human Male DNA Quantification kit using an AB 7500 real time PCR instrument. Two samples stored in ethanol at 4^0 C produced low-level quantification results. Two samples out of thirty gave an indication of slight inhibition. Based on these results, DNA extracts and associated reagent blanks were concentrated to approximately15 µL using a DNA SpeedVac concentrator and were quantified again. Quantification resulted in twenty-nine of thirty samples displaying inhibition based on the IPC values, which indicated there was a PCR inhibitor which would interfere with the PCR process. TE^{-4} was added to DNA extracts and associated reagent blanks to bring the extract volume to ~ 100 µL, which removed the PCR inhibitor. Samples were amplified with an Applied Biosystems AmpFℓSTR® Y-Filer PCR amplification kit for human male DNA. Samples extracted from pig#1 were not concentrated or quantified, and the maximum amount of each extract was used for amplification. One genetic profile from a larva extract, that was preserved in 95% ethanol (sample 1A2), was obtained from extracts from pig #1 out of thirty samples which were amplified. A full genetic profile was obtained from sample 1A2 and was concordant with the genetic profile from the known sample from pig #1. Seven samples preserved in 95% ethanol (2A1, 2A2, 2A3, 2A4, 2A5, 2A7, and 2A8) and two preserved at -70°C (2C9 and 2C10) out of thirty samples amplified from pig #2 produced callable alleles. Full profiles were not obtained from any of these samples. Four samples (2A1, 2A3, 2A4, and 2A5) produced an allele call at five or more of the sixteen loci tested. The remaining five samples (2A2, 2A7, 2A8, 2C9, and 2C10) produced allele calls at one to three loci. It can be concluded that DNA was being

detected from the host species by extracting a whole larvae; however, the results were not consistent. Results for pig #1 indicated that the Animaltype Pig PCR amplification kit may not have been sensitive enough to detect pig DNA in a sample. This amplification kit was designed for samples that contain significantly more DNA than those normally encountered in forensic casework. Another factor that should be considered is the amount of pig DNA present in a crop of larvae and whether there was sufficient material to obtain a genetic profile from the pig.

Twelve samples from pig #1 were subjected to various troubleshooting methods to try an increase the likelihood of obtaining a genetic profile. First, three samples that were stored in ethanol and three that were preserved at -70^0 C, were concentrated to approximately 50 µL to determine if better results could be obtained, since there were indicators of inhibition with the samples concentrated to approximately 15 µL. Each of these six samples was amplified with an Animaltype Pig PCR kit, using approximately 10 µL of the extract. A Microcon filtration device was used to attempt to purify and remove additional inhibitors from extracts from each preservation method. The resulting collection from the Microcon filtration was amplified by using approximately 15 µL of the extract. The amount of the DNA extract obtained after the use of the Microcon filtration devices was approximately 150 µL for the extract, due to the large amount of DNA present in these samples. The same parameters were used for twelve extracts from both preservation methods from pig #2, though the maximum amount of extract used in the amplification of these samples was not increased; it remained at 10 µL for the Applied Biosystems AmpFℓSTR® Y-Filer PCR amplification kit. Out of twelve samples subjected to additional steps and amplified with the Animaltype Pig PCR kit, only one sample (1A7) produced one allele call at one locus. This sample was one which was filtered with a Microcon filtration device and approximately 15 µL was amplified. No significant results were obtained

from these troubleshooting steps with the Animaltype PCR amplification kit. It can be concluded that there is insufficient pig DNA in these samples to achieve more callable alleles or obtain better genetic profiles. Two samples produced partial results out of twelve samples that were subjected to troubleshooting and amplified with an Applied Biosystems AmpFℓSTR® Y-Filer PCR amplification kit. One sample (2A8) produce allele calls at two loci, while the other sample (2A9) produced one allele call at one locus. Both of these samples were purified with a Microcon filtration device. There was no significant increase in the amount of sample that produced allele calls and, in fact, there were better results with the samples before these troubleshooting steps were employed.

The sample that produced a full profile from pig #1 and five of the samples that produced the most callable alleles from pig #2 were from the preservation method in which the larvae were stored in 95% ethanol at 4^0 C. Since the preservation method where the larvae were preserved in 95% ethanol at 4^0 C obtained the best results, only larvae collected from this preservation method were extracted for the remainder of the collections (3 through 10). DNA extracts and associated reagent blanks from both pig #1 and pig #2 were concentrated to approximately 100 µL, prior to amplification. In addition, the maximum amount of DNA extract amplified was increased from 5 µL to 10 µL in the Animaltype PCR amplification kit to increase the amount of starting DNA template for amplification. The maximum amount of DNA extract used for the Applied Biosystems AmpFℓSTR® Y-Filer PCR amplification kit remained at approximately 10 µL. These steps were all done to increase the amount of DNA in the sample, in an attempt to produce better amplification results.

Larvae extracts from pig #2 for collections 3 through 10 produced no quantification results with the Applied Biosystems Quantifiler® Y Human Male DNA Quantification kit. Only

one sample (2A48) out of forty indicated slight inhibition. One sample (1A17) out of thirty-five samples that were amplified with the Animaltype Pig PCR amplification kit for pig #1 produced one allele call at one locus. Samples from pig #2 did not produce any results with the Applied Biosystems AmpFℓSTR® Y-Filer PCR amplification kit. There does not appear to be any inhibition present in these extracts, which indicates that there is not sufficient DNA present to obtain a genetic profile from these samples. Again, a factor for the samples amplified with the Animaltype Pig PCR amplification kit could be the sensitivity of the kit. Samples from pig #2 that were collected within the first few days of decomposition obtained more callable alleles than samples that were collected later in the decomposition process. It can be concluded, based on these results, that the commercial human semen was completely consumed within the first few days of decomposition, resulting in seminal fluid not being present on the pig carcass for larvae to consume. Positive amplification controls, negative amplification controls, and reagent blanks performed as expected with all samples analyzed with the AB 3130 Genetic Analyzer.

5. CONCLUSION

Extraction of whole carrion fly larvae would provide a forensic laboratory the ability to use larvae to identify severely decomposed bodies or identify suspects in some cases. This research demonstrated that it was possible to develop a genetic profile from a larvae's food source by extracting a whole larvae; however, results were not consistent and further research is need before this technique could be implemented into a forensic DNA laboratory. This study also demonstrated the ability to develop a male genetic profile from extraction of whole larvae, where its food source contained seminal fluid. This research demonstrated that it is possible to develop a suspect profile from the extraction of whole fly larvae; however, limited results were obtained from this extraction method and additional research is needed before this method could be utilized in a traditional forensic laboratory.

Overall, from both the pilot study and samples collected from pig #1, forty-eight samples were amplified using an Animaltype Pig PCR amplification kit. Four samples produced at least one allele call at a locus amplified using this kit. The following hypotheses were tested using a hypothesis test statistic from data obtained from amplified DNA products that were extracted from whole larvae and amplified with an Animaltype Pig PCR amplification kit (D'Agostino, Sullivan, & Beiser, 2006).

H_1 = The average result of extracting entire larvae, which were collected between the time they were first observed to the point of total decomposition, using the Qiagen DNeasy® Blood and Tissue kit extraction kit will provide genetic information that is concordant with the food source of a larvae at one locus, at a minimum with the Animaltype Pig PCR amplification kit using a AB 3130 Genetic Analyzer.

H_0 = The average result of extracting entire larvae, which were collected between the time they were first observed to the point of total decomposition, using the Qiagen DNeasy® Blood and Tissue kit extraction kit will not provide genetic information that is concordant with what the food source of a larvae at one locus, at a minimum with the Animaltype Pig PCR amplification kit using a AB 3130 Genetic Analyzer.

Figure 20 depicts the equation that was used to calculate this test statistic (D'Agostino, Sullivan, & Beiser, 2006). A one tailed test with α of 0.05 (confidence level of 95%) was used.

$$t = \frac{\bar{X} - \mu_o}{\frac{s}{\sqrt{n}}}, df = n - 1$$

Figure 20: Test Statistic Equation used to test Hypothesis, where $\bar{X}$ = Sample mean, μ_o = population mean, s = sample standard deviation, n = sample size, and df = degrees of freedom.

The resulting calculation of the test statistic resulted in a t critical value of 2.019, with a t statistic value of 1.609, which would conclude that the null hypothesis (H_0) would not be rejected. The p value was 0.0572, which is greater than α of 0.0500, which would also result in the null hypothesis (H_0) not being rejected (Appendix Q). Therefore, the average result of extracting entire larvae, which were collected between the time they were first observed to the point of total decomposition, using the Qiagen DNeasy® Blood and Tissue kit extraction kit will not provide any genetic information at any loci that is concordant with what the larvae consumed with the Animaltype Pig PCR amplification kit using a AB 3130 genetic analyzer.

One sample extracted and amplified with the Animaltype PCR Pig amplification kit produced a full profile, which was concordant with the known sample obtained from pig #1. This indicates that it is possible to develop a genetic profile from extracting of whole larvae to

determine its food source. However, the remaining larvae samples did not produce consistent callable alleles with the remaining larvae extracts that were amplified, concluding that this extraction method will not provide reliable and consistent results. An Animaltype Pig PCR amplification kit is designed for kinship testing and determination of gender of a pig sample, not for forensic DNA testing application. The amplification kit is preferentially designed for fast and reliable DNA genotyping of blood or tissue samples from pigs (Animaltype Pig PCR Amplification Kit, December 2007). This kit is a four dye kit and its sensitivity is not comparable to current human forensic amplification kits available in forensic laboratories currently. If this research was conducted with amplification kits that were more sensitive, similar to amplification kits that are currently used in forensic DNA laboratories, results could possibly be different and the extraction method may provide consistent and reliable results. In addition, a Thermo Scientific NanoDrop 2000 UV-Vis Spectrophotometer only quantifies total DNA in a sample and the amount of pig DNA in each extraction was unable to be determined. Development of a quantification method which is specific to estimating the quantity of actual pig DNA from extraction of whole larvae would be beneficial for future research in this area.

Overall, fifty samples were amplified with an Applied Biosystems AmpFℓSTR® Y-Filer PCR amplification kit. Seven of these fifty samples produced at least one callable allele at one locus that was amplified. The following hypotheses were tested using a hypothesis test statistic from data obtained from amplified DNA products that were extracted from whole larvae and amplified with an Applied Biosystems AmpFℓSTR® Y-Filer PCR amplification kit (D'Agostino, Sullivan, & Beiser, 2006).

H_1 = The average result of extracting whole larvae, which were collected between the time they were first observed to the point of total decomposition, using the Qiagen

DNeasy® Blood and Tissue extraction kit will provide genetic information that is concordant with the food source of a larvae at one locus, at a minimum with the Applied Biosystems AmpFℓSTR® Y-Filer PCR amplification kit using an AB 3130 Genetic Analyzer.

H_0 = The average result of extracting whole larvae, which were collected between the time they were first observed to the point of total decomposition, using the Qiagen DNeasy® Blood and Tissue kit extraction kit will not provide genetic information that is concordant with the food source of a larvae at one locus, at a minimum with the Applied Biosystems AmpFℓSTR® Y-Filer PCR amplification kit using an AB 3130 Genetic Analyzer.

Using the equation depicted in Figure 20 and the same confidence level the calculated t critical value was 2.012 and the calculated t statistic value of 2.434, which would conclude that the null hypothesis (H_0) would be rejected. The calculated p value was 0.0094, which is less than α of 0.0500, which would also conclude that the null hypothesis (H_0) would be rejected (Appendix R). Therefore, the average result of extracting whole larvae, which were collected from the time they were first observed to the point of total decomposition, using the Qiagen DNeasy® Blood and Tissue kit extraction kit will provide genetic information that is concordant with what the larvae consumed at one locus, at a minimum with the Applied Biosystems AmpFℓSTR® Y-Filer PCR amplification kit using an AB 3130 Genetic Analyzer.

Data obtained from amplified DNA products of extracted whole larvae samples with an Applied Biosystems AmpFℓSTR® Y-Filer PCR amplification kit concluded that a useable, male genetic profile could be obtained. These results were obtained from larvae samples that were collected and extracted from the first two collections (Figure 21). Subsequent collections of

larvae samples did not provide any callable alleles when extracted and amplified with an AmpFℓSTR® Y-Filer PCR amplification kit. Results obtained from amplified DNA products from these subsequent collections indicated that the commercial human semen was consumed by the larvae. Whole larvae extracts indicated PCR inhibition, based on IPC C_T values, when concentrated to ~ 15 µL, and PCR inhibitors were no longer present when the extracted larvae samples volume was increased to ~ 100 µL. Since PCR inhibition was no longer present at a volume of 100 µL, it can be concluded that the PCR process was not inhibited, which suggests that the commercial human semen was consumed in latter collections of larvae.

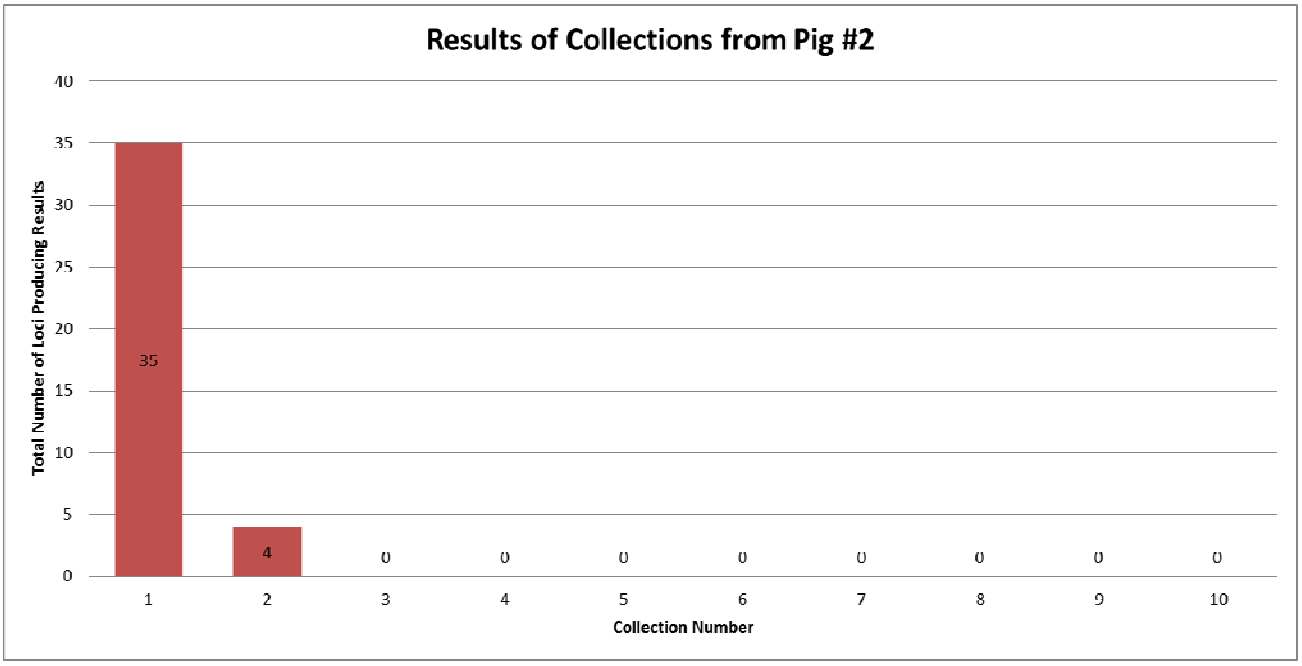


Figure 21: Depiction of the number of loci producing results based on when the larvae were collected from pig #2. The number of loci producing results decreases with subsequent collections, where no results were obtained after collection 2.

Results obtained from this research provide a foundation for future research into the extraction of whole larvae for determination of the larvae's food source. If research was continued using pigs as human analogs, then development of more sensitive STR amplification kits for genotyping of pig DNA would be beneficial to develop pig genetic profiles; which would

accurately reflect capabilities of current amplification kits used in forensic laboratories. In addition, development of a specific quantification kit to determine the amount of pig DNA present in a whole larvae extracts, would be beneficial to future research. Extracts amplified with an Applied Biosystems AmpFℓSTR® Y-Filer PCR amplification kit provided more genetic information than amplification with an Animaltype Pig PCR amplification kit. These results suggest that an experiment utilizing human samples, would provide a better determination of the feasibility of this extraction method for use in forensic laboratories. Performing this study with current human amplification kits would be beneficial to determining if there would be an increase in the number of whole larvae extracts which produced results. Research into extraction of whole larvae samples for a determination of their food source, obtained from human remains, would be beneficial to the forensic science community.

Overall, results obtained from this research provided a foundation for the ability to extract whole larvae samples for determination of their food source, by development of a genetic profile. Extraction of whole larvae samples did not produce reliable and consistent results, but the ability to obtain a genetic profile from extraction of whole larvae was shown. Amplification of whole larvae extracts with more sensitive kits could provide results that are reliable and consistent. Whole larvae extracts amplified with an Applied Biosystems AmpFℓSTR® Y-Filer PCR amplification kit did produce results which were more consistent; however, the commercial human semen probably was consumed within the first two collections. It can be concluded that by extracting whole larvae, a suspect profile could be developed, provided that larvae samples were collected early enough in the decomposition process. Overall, results obtained in this research provide information which can be used in development of research and case analysis protocols for extracting whole larvae in a forensic DNA laboratory.

LITERATURE CITED

(2012). *Applied Biosystems AmpFlSTR Yfiler PCR Amplification Kit Users Guide*.

Afonina, I., Zivarts, M., Kutyavin, I., Lukhtanov, E., Gamper, H., & Meyer, R. B. (1997). Efficient Priming of PCR with short oligonucleotides Conjugated to a Minor Groove Binder. *Nucleic Acids Res, 25*, 2657-2660.

Animaltype Pig PCR Amplification Kit. (December 2007).

Applied Biosystems Allelic Discrimination Using the 5' Nuclease Assay. (2001). Foster City, CA.

Applied Biosytems Quantifiler Kits User's Manual. (2012).

Ballantyne, J. (n.d.). *Other Nuclear DNA Markers and Technologies, Y-STR's*. Retrieved August 21, 2013, from NFSTC Sciene Serving Justice: http://www.nfstc.org/pdi/Subject09/pdi_s09_m01_02.htm

Bloch, W. (1991). A Biochemical Perspective of the Polymerase Chain Reaction. *Biochemistry 30 (11)*, 2735-27457.

Brock, T. D., & Freeze, H. (Aug 1969). Thermus Acquaticus gen. n. and sp. n., a Non-Sporulating Extreme Thermophile. *Journal of Bacteriology*, 289-297.

Bucheli, S., Bytheway, J., & Gangitano, D. (2010). Necrophagous Caterpillars Provide Human mtDNA Evidence. *Journal of Forensic Sciences, 55*(4), 1130-1132.

Butler , J. M. (2005). *Forensic DNA Analysis: Biology, Technology, and Genetics of STR Markers* (2nd ed.). Burlington, MA, USA: Elsevier Academic Press.

Butler, J., Ruitberg, C. M., & Vallone, P. M. (2001). Capillary Electrophoresis as a Tool for Optimization of Multiplex PCR Reactions. *Anal Chem: 369*, 200-205.

Campobasso, C., Linville, J., Wells, J., & Introna , F. (2005). Forensic Genetic Analysis of Insect Gut Contents. *The American Journal of Forensic Medicine and Pathology, 26*, 161-166.

Cantor, C. R., & Smith, C. L. (1999). *Genomics: The Science and Technology Behind the Human Genome Project.* New York: John Wiley & Sons.

Caprette, D. (1996, September 16). *Experimental Biosciences*. Retrieved August 7, 2013, from Principles of Spectrophotometry: http://www.ruf.rice.edu/~bioslabs/methods/protein/spectrophotometer.html

Carracedo, A. (2005). *Methods in Molecular Biology Forensic DNA Typing Protocols.* Totowa, New Jersey: Humana Press Inc.

Catts, E. P., & Goff, M. L. (1992). Forensic Entomology in Criminal Investigations. *Annual Review Entomology, 37*, 253-72.

Clark, L. (n.d.). *NFSTC Science Serving Justice*. Retrieved August 7, 2013, from DNA Analyst Training: http://www.nfstc.org/pdi/Subject03/pdi_s03_m02_00.htm

Clery, J. M. (2001). Stability of Prostate Specific Antigen (PSA), and Subsequent Y-STR Typing of Lucilia (Phaenicia) Sericata (Meigen) (Diptera: Calliphoridae) Maggots Reared from a Simulated Postmortem Sexual Assault. *Forensic Science International, 120 (1-20*, 72-78.

Comey, C. T., Koons, B. W., Presley, K. W., Smerick, J. B., Sobieralski, C. A., Stanley, D. M., et al. (1994). DNA Extraction Strategies for Amplified Fragment Length Polymorphism Analysis. *ASTM International*, 1254-1269.

D'Agostino, S. R., Sullivan, L. M., & Beiser, A. S. (2006). *Introductory Applied Biostatistics.* Belmont, CA, USA: Brooks/Cole CENGAGE Learning.

Dimauro, S., & Davidzon, G. (2005). Mithochondrial DNA and Disease. *Annals of Medicine*, 37: 222-232.

DiZinno, J. A., Lord, W. D., Collins-Morton, M. B., Wilson, M. R., & Goff, M. (2002). Mitochondrial DNA Sequencing of Beetle Larvae (Nitdulidae: Omosita) Recovered from Human Bone. *Journal of Forensic Science 47(6)*, 1337-1339.

Dodge, H. R. (1953). Identifying Common Flies. *Public Health Reports, 68*(3), 345-350.

Forster, V. T. (1948). Zwischenmolekulare Energiewanderung und fluoresenz. *Annals of Physics*, 2:55-75.

Goff, M., & Lord, W. D. (2001). Forensic Entomology. *Human Identification*, 1-19.

Harvey, M. L. (2005). An Alternative for the Extraction and Storage of DNA from Insects in Forensic Entomolgy. *Journal of Forensic Science, 50*(3), 627-629.

Haskell, N. H. (2010). How Insect Evidence is Used in Estimating the Postmortem Interval and Geographical Association of the Crime Scene. *American Academy of Forensic Science.* Seattle.

Holland, P. M., Abramson, R. D., Watson, R., & Gelfand, D. H. (1991). Detection of Specific Polymerase Chain Reaction Product by Utilizing the 5' to 3' Exonuclease Activity of Thermus Aquaticus DNA Polymerase. *Proc. Natl. Acad. Sci. USA, Vol. 88*, 7276-7280.

Jeffreys, A. J., Wilson, V., & Thein, S. L. (1985). Hypervariable 'mimisatellite' Regions in Human DNA. *Nature Vol .314*, 67-73.

Jobling, M. A., Pandya, A., & Tyler-Smith, C. (1997). The Y Chromosome in Forensic Analysis and Paternity Testing. *International Journal of Legal Medcine*, 110: 118-124.

Kester, K. M., Toothman, M. H., Brown, B. L., Street, W. S., & Cruz, T. D. (2010). Recovery of Enviormental Human DNA by Insects. *Journal of Forensic Sciences*, doi: 10.1111/j.1556-4029.2010.01500.x.

Kutyavin, I. V., Lukhtanov, E. A., Gamper, H. B., & Meyer, R. B. (1997). Oligonucleotides with Conjugated Dihydropyrroloindole Tripeptides: Base Composition and Backbone Effects on Hybridization. *Nucleic Acids Res, 25*, 3718-3723.

Lakowicz, J. R. (1983). *Principles of Fluorescence Spectroscopy.* New York: Springer Science + Business Mediam LLC.

Linville, J. G., Hayes, J., & Wells, J. D. (2004, March). Mitochondrial DNA and STR Analyses of Maggot Crop Contents: Effect of Specimen Preservation Technique. *Journal of Forensic Science, 49*(2), 1-4.

Lord, W. D., & Burger, J. F. (1983). Collection and Preservation of Forensically Important Entomological Materials. *Journal of Forensic Sciences, JFSCA, Vol. 28, No. 4*, 936-944.

Lord, W. D., & Goff, M. (1993). Forensic Entomology: Insects in the Investigation of Violent Crime. *In: Proceeding of the International Symposium on Mass Disasters and Crime Scene Reconstruction, U.S. Government Printing Office, Washigton DC*, 163-171.

Lord, W. D., Catts, E. P., Scarboro, D. A., & Hadfield, D. B. (1986). The Green Blow Fly, Lucilia Illustris (Meigen), As An Indicator of Human Post-Mortem Interval: A Case of Homicide from Fort Lewis, Washington. *Bull. Soc. Vector Ecol., 11(2)*, 271-275.

Lord, W. D., DiZinno, J. A., Wilson, M. R., Budowle, B., Taplin, D., & Meinking, T. L. (1998). Isolation, Amplification, and Sequencing of Human Mitochondrial DNA Obtained from Human Crab Louse, Pthirus Pubis (L.), Blood Meals. *Journal of Forensic Sciences 43 (5)*, 1097-1100.

Lord, W. D., Johnson, R. W., & Johnston, F. (1986). The Blue Bottle Fly, Calliphora Vicina (=Erythrocephala) As an Indicator of Human Post-Mortem Interval: A Case of Homicide from Suburban Washigton, D.C. *Bull. Soc. Vector Ecol., 11(2)*, 276-280.

Lunney, J. K. (2007). Advances in Swine Biomedical Model Genomics. *International Journal of Biological Sciences*, 3(3): 179-184.

Moretti, T. R., Baumstark, A. L., Defenbaugh, D. A., Keys, K. M., Smerick, J. B., & Budowle, B. (2000). Validation of Short Tandem Repeats (STRS) for Forensic Usage: Performance Testing of Fluorescent Multiplex STR Systems fand Analysis of Authentic and Simulated Forensic Samples. *ASTM International*, 647-660.

Mullis, K. B. (1985). *Patent No. 4,683,202.* USA.

Mullis, K., Falloona, F., Scharf, S., Saiki, R., Horn, G., & Erlich, H. (1986). Specific Enzymatic Amplification of DNA In Vitro: The Polymerase Chain Reaction. *Cold Springs Harbor Symposia on Quantitative Bioloyg*, 263-273.

Nakamura, Y., Leppert, M., O'Connell, P., Wolff, R., Holm, T., Culver, M., et al. (1987). Variable Number of Tandem Repat (VNTR) Markers for Human Gene Mapping. *Science 235 (4796)*, 1616-1622.

NanoDrop Products Termo Scientific Nucleic Acid Quantification Protocols. (2013). Retrieved from Current Protocols in Molecular Biology; Quantitation of DNA and RNA with Absorption and Fluorescene Spectroscopy: http://nanodrop.com/Library/CPMB-1st.pdf

NCBI. (n.d.). *PCR*. Retrieved August 19, 2013, from NCBI: http://www.ncbi.nlm.nih.gov/projects/genome/probe/doc/TechPCR.shtml

Nolte, K. B., Pinder, R. D., & Lord, W. D. (1992). Insect Larvae used to Detect Cocaine Poisoning in a Decomposed Body. *Journal of Forensic Sciences*, 1179-1185.

Pratt, H. D., Littig, K. S., & Scott, H. G. (1975). *Flies of Public Health Importance and Their Control.* Atlanta, Georgia, USA: U.S. Department of Health, Education, and Welfare (CDC).

Qiagen. (2006). DNeasy Blood and Tissue Handbook.

Roby, R. (n.d.). *NFSTC Science Serving Justice*. Retrieved August 21, 2013, from Non-Nuclear DNA Markers Mithochondrial DNA: http://www.nfstc.org/pdi/Subject09/pdi_s09_m02_01.htm

Roby, R., & Figarelli, D. (n.d.). *NFSTC Science Serving Justice*. Retrieved August 19, 2013, from DNA Analyst Training Capillary Electrophoresis: http://www.nfstc.org/pdi/Subject05/pdi_s05_m02.htm

Saiki, R. K., Scharf, S., Faloon, F., Mullis, K. B., Horn, G. T., Erlich, H. A., et al. (1985). Enzymatic Amplification of B-Globin Genomic Sequences and Restriction Site Analysis for Diagnosis of Sickle Cell Anemia. *Science 230 (4732)*, 1350-1354.

Saiki, R. K., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A., et al. (1952). Enzymatic Amplification of B-Globin Genomic Sequences and Restriction Site Analysis for Diagnosis of Sickel Cell Anemia. *Science, Vol 230, No. 4732*, 1350-1354.

Schoenly, K. G., Haskell, N. H., Mills, D. K., Bieme-Ndi, C., Larsen, K., & Lee, Y. (2006). Using Pig Carcasses as Model Corpses. *The American Biology Teacher, Volume 68, No. 7*, 402-410.

Smith, A. C., & Swindle, M. M. (1998). Compartive Anatomy and Physiology of the Pig. *Scand. J. Lab: Anim. Sci. Supp. 1 Vol 25*, 11-21.

Sperling, F., Anderson, G., & Hickey, D. (1994). A DNA-Based Approach to the Identification of Insect Species Used for Postmortem Interval Estimation. *Journal of Forensic Sciences, JFSA 29 (2)*, 148-427.

Swindle, M., & Smith, A. C. (1998). Compartive Anatomy and Physiology of the Pig. *Scand. J. Lab. Anim. Sci. Suppl 25*, 11-21.

Watson, J. D. (1968). *The Double Helix: A Personal Account of the Discovery of the Structure of DNA.* New York: Simoan & Schuster.

Watson, J. D., & Crick, F. H. (1953). The Structure of DNA. *Cold Spring Harb Symp Quant Biol 18*, 123-131.

Wellls, J., & Stevens, J. (2008). Application of DNA-Based Methods in Forensic Entomology. *Annual Review of Entomology, 53*, 103-120.

Wells, J. D. (2010). Using Insects for Forensic DNA Analysis: A Brief Practial Guide. *American Academy of Forensic Sciences.* Seattle.

Wells, J., Introna, F., Di Vella, G., Campobasso , C., Hayes, J., & Sperling, F. (2001). Human and Insect Mitochondrial DNA Analysis from Maggots. *Journal of Fornensic Science*, 685-687.

Whitworth, T. (2006). Keys to the Genera and Species of Blow Flies (Diptera: Calliphoridae) of America North of Mexico. *Proceedings of the Entomological Society of Washington*, 689-725.

Zehner, R., Amendt, J., & Kretteck, R. (2004). STR Typing of Human DNA from Fly Larvae Fed on Decomposing Bodies. *Journal of Forensic Sciences, 49*, 1-4.

Appendix A: DNA Table Depicting Results of Varying Input DNA Amplification Targets

Genetic Loci / Item Number Name	SBH2	SBH18	SBH4	SO655	SBH23	SBH20	SBH1	SBH10	SBH13	387A12F	SBH22	SBH19
1A (Pig Liver Qiagen) 0.5 ng	6, 24	NR	NR	NR	X	36	NR	NR	NR	NR	NR	NR
1B (Pig Liver Qiagen) 1.0 ng	6, 24	12	49.1, 57	NR	X	30, 36	10, 14	48, 49	9, 15	15.1	18, 23	14
1C (Pig Liver Qiagen) 2.0 ng	6, 24	12	49.1, 57	5, 10	X	30, 36	10, 14	47, 48, 49	9, 15	15.1	18, 23	14
1D (Pig Liver Qiagen) 3.0 ng	6, 24	12	49.1, 57	5, 10	X	30, 36	10, 14	47, 48, 49	9, 15	15.1	18, 23	14
2A (Pig Blood Swab Qiagen) 0.5 ng	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2B (Pig Blood Swab Qiagen) 1.0 ng	6	12	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2C (Pig Blood Swab Qiagen) 2.0 ng	6, 24	12	57	NR	X	NR	NR	48, 49	15	NR	NR	NR
2D (Pig Blood Swab Qiagen) 3.0 ng	6, 24	12	49.1, 57	NR	X	30	10	48	9	15.1	NR	14
3A (Pig Liver Organic) 0.5 ng	6, 24	NR	NR	NR	X	NR	NR	NR	9	NR	NR	NR
3B (Pig Liver Organic) 1.0 ng	6, 24	NR	NR	NR	X	36	NR	NR	9, 15	NR	NR	NR
3C (Pig Liver Organic) 2.0 ng	6, 24	12	49.1, 57	NR	X	30, 36	10	NR	9, 15	15.1	18	14
3D (Pig Liver Organic) 3.0 ng	6, 24	12	49.1	5	X	30, 36	NR	NR	9, 15	15.1	18	14
4A (Pig Blood Swab Organic) 0.5 ng	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
4B (Pig Blood Swab Organic) 1.0 ng	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
4C (Pig Blood Swab Organic) 2.0 ng	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
4D (Pig Blood Swab Organic) 3.0 ng	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

Appendix B: DNA Table depicting results of Higher Amplification Targets of Known liver and Blood Sample

Genetic Loci → / Item Number Name ↓	SBH2	SBH18	SBH4	SO655	SBH23	SBH20	SBH1	SBH10	SBH13	387A12F	SBH22	SBH19
Pig Liver Qiagen 3.0 ng 5 seconds 1A	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Pig Liver Qiagen 3.0 ng 10 seconds 1A	6	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Pig Liver Qiagen 4.0 ng 5 seconds 1B	6	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Pig Liver Qiagen 4.0 ng 10 seconds 1B	6, 24	NR	NR	NR	X	NR	NR	NR	9	NR	NR	NR
Pig Liver Qiagen 5.0 ng 5 seconds 1C	6, 24	NR	NR	NR	X	30, 36	NR	48	9	15.1	NR	14
Pig Liver Qiagen 5.0 ng 10 seconds 1C	6, 24	12	55.1	NR	X	30, 36	10, 14	48, 49	9, 15	15.1	18	14
Pig Liver Qiagen 6.0 ng 5 seconds 1D	6, 24	12	49.1, 55.1	NR	X	30, 36	10, 14	48, 49	9, 15	15.1	18, 23	14
Pig Liver Qiagen 6.0 ng 10 seconds 1D	6, 24	12	49.1, 55.1	5, 10	X	30, 36	10, 14	48, 49	9, 15	15.1	18, 23	14
Pig Blood Swab Qiagen 5.0 ng 5 seconds 2A	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Pig Blood Swab Qiagen 5.0 ng 10 seconds 2A	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Pig Blood Swab Qiagen 6.0 ng 5 seconds 2B	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Pig Blood Swab Qiagen 6.0 ng 10 seconds 2B	6, 24	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Pig Blood Swab Qiagen 7.0 ng 5 seconds 2C	6	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Pig Blood Swab Qiagen 7.0 ng 10 seconds 2C	6	12	NR	NR	X	NR	NR	NR	NR	NR	NR	NR
Pig Blood Swab Qiagen 8.0 ng 5 seconds 2D	6, 24	12	49.1, 55.1	NR	X	NR	10, 14	NR	9	15.1	NR	14

Genetic Loci / Item Number Name	SBH2	SBH18	SBH4	SO655	SBH23	SBH20	SBH1	SBH10	SBH13	387A12F	SBH22	SBH19
Pig Blood Swab Qiagen 8.0 ng 10 seconds 2D	6, 24	12	49.1, 55.1	5, 10	X	30, 36	10, 14	48, 49	9, 15	15.1	18, 23	14
Pig Liver Organic 7.0 ng 5 seconds 3C	6, 24	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Pig Liver Organic 10.0 ng 10 seconds 3D	6, 24	12	NR	NR	X	30, 36	10, 14	NR	9, 15	15.1	18	NR
Blood Swab Organic 7.0 ng 5 seconds 4C	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Blood Swab Organic 7.0 ng 10 seconds 4C	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Blood Swab Organic 10.0 ng 5 seconds 4D	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

Appendix C: DNA Table for the First Larvae Collection from the Untreated Liver Samples at 10 ng/5 uL Amplification

Target

Genetic Loci / Item Number Name	SBH2	SBH18	SBH4	SO655	SBH23	SBH20	SBH1	SBH10	SBH13	387A12F	SBH22	SBH19
1A	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1B	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1C	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1D	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1E	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2B	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2C	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2D	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2E	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

Appendix D: DNA Table for the First Larvae Collection from Untreated Liver Samples Maximum Amount of DNA Extract Amplified

Genetic Loci → / Item Number Name ↓	SBH2	SBH18	SBH4	SO655	SBH23	SBH20	SBH1	SBH10	SBH13	387A12F	SBH22	SBH19
1A	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1B	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1C	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1D	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1E	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2B	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2C	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2D	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2E	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

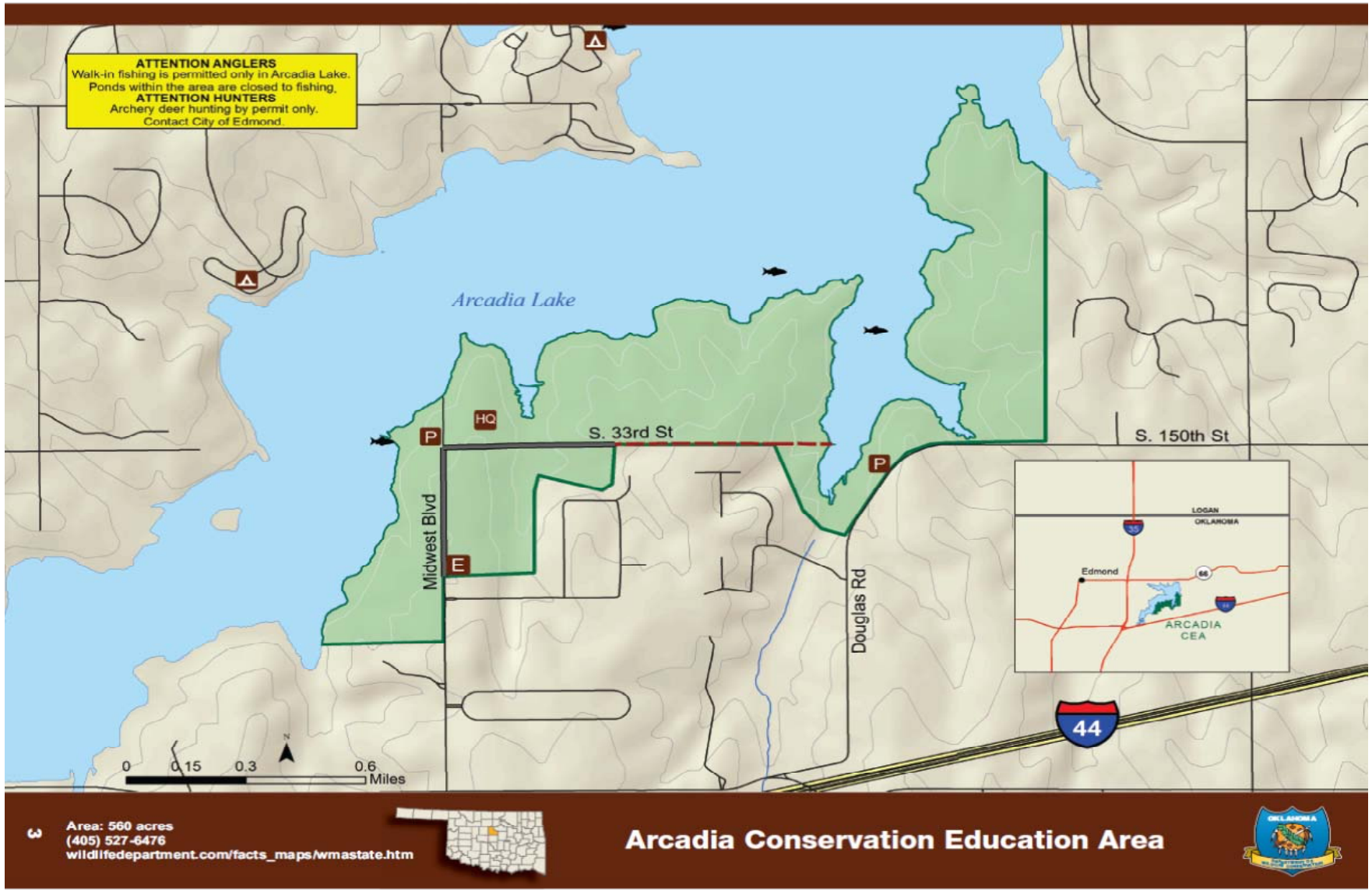
Appendix E: DNA Table for Maximum Amount of 10 ng Concentration of the Known Liver and Blood Samples

Genetic Loci → / Item Number Name ↓	SBH2	SBH18	SBH4	SO655	SBH23	SBH20	SBH1	SBH10	SBH13	387A12F	SBH22	SBH19
Pig Liver Qiagen 10 ng	6, 24	12	49.1, 56	5, 10	X	30, 36	10, 14	47, 48, 49	9, 15	15.1	18, 23	14
Blood Swab Qiagen 10 ng	6, 24	12	49.1, 56	5, 10	X	30, 36	10, 14	47, 48, 49	9, 15	15.1	18, 23	14
Pig Liver Organic 10 ng 5 seconds	6, 24	12	49.1, 56	5, 10	X	30, 36	10, 14	48, 49	9, 15	15.1	18, 23	14
Pig Liver Organic 10 ng 10 seconds	6, 24	12	49.1, 56	5, 10	X	30, 36	10, 14	47, 48, 49	9, 15	15.1	18, 23	14
Blood Swab Organic 10 ng 5 seconds	6, 24	12	56	NR	X	30, 36	14	48	9	NR	NR	NR
Blood Swab Organic 10 ng 10 seconds	6, 24	12	49.1, 56	NR	X	30, 36	10, 14	48	9, 15	15.1	18	NR

Appendix F: DNA Table for Extraction of Additional Frozen Larvae and Larvae Stored in Ethanol

Genetic Loci → / Item Number Name ↓	SBH2	SBH18	SBH4	SO655	SBH23	SBH20	SBH1	SBH10	SBH13	387A12F	SBH22	SBH19
3A Neat (Frozen) 10 seconds	OL	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
3B Neat (Frozen) 10 seconds	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
3C Neat (Frozen) 10 seconds	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
4A Neat (EtOH) 5 seconds	6, 24	12	49.1, 56	NR	X	30, 36	10, 14	NR	9, 15	15.1	18, 23	NR
4A Neat (EtOH) 10 seconds	6, 24	12	49.1, 56	NR	X	30, 36	10, 14	48	9, 15	15.1	18, 23	14
4A (1:10) 5 seconds	6, 24	NR	NR	NR	X	30, 36	NR	NR	9, 15	15.1	NR	NR
4A (1:10) 10 seconds	6, 24	NR	NR	NR	X	30, 36	NR	NR	9, 15	15.1	NR	NR
4B Neat (EtOH) 10 seconds	6, 24	NR	NR	NR	X	NR	NR	NR	NR	NR	NR	NR
4C Neat (EtOH) 10 seconds	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Known Liver Sample with Maggot Extract (Rule out Inhibition from maggot extract)	6, 24	12	49.1, 56	5, 10	X	30, 36	10, 14	47, 48, 49	9, 15	15.1	18, 23	14

Appendix G: Map of the Arcadia Conservation Education Area



Appendix H: DNA Table Depicting Results of Larvae Extraction from Pig #1 (Collections 1 and 2)

Genetic Loci / Item Number Name	SBH2	SBH18	SBH4	SO655	SBH23	SBH20	SBH1	SBH10	SBH13	387A12F	SBH22	SBH19
1A1	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A2	6	15	57, 65.3	5, 12	Y, X	19, 23	13	49	15, 16	13, 15.1	22, 23	11, 15
1A3	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A4	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A5	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1B1	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1B2	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1B3	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1B4	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1B5	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1C1	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1C2	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1C3	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1C4	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1C5	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

Genetic Loci / Item Number Name	SBH2	SBH18	SBH4	SO655	SBH23	SBH20	SBH1	SBH10	SBH13	387A12F	SBH22	SBH19
1A6	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A7	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A8	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A9	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A10	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1B6	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1B7	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1B8	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1B9	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1B10	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1C6	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1C7	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1C8	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1C9	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1C10	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Pig #1 Known	6	15	57, 65.3	5, 12	Y, X	19, 23	13	49	15, 16	13, 15.1	22, 23	11, 15
Pig#2 Known	24, 26	12, 13	62	11	X	30, 36	11, 14	48	9, 15	9, 15.1	20, 23	14, 16

Appendix I: DNA Table Depicting Results of Larvae Extraction from Pig #2 (Collections 1 and 2)

Genetic Loci / Item Number Name	B_DYS456	B_DYS389I	B_DYS390	B_DYS389II	G_DYS458	G_DYS19	G_DYS385	Y_DYS393	Y_DYS391	Y_DYS439	Y_DYS635	Y_DYS392	R_Y_GATA H4	R-DYS437	R-DYS438	R_DYS448
2A1	13	12	22	NR	15	14	NR	14	10	NR	NR	NR	11	16	10	NR
2A2	13	12	NR	NR	NR	NR	NR	NR	10	NR	NR	NR	NR	NR	NR	NR
2A3	13	12	NR	NR	15	NR	NR	14	10	NR	22	NR	11	16	10	NR
2A4	13	12	NR	NR	15	NR	NR	14	10	NR	NR	NR	11	16	NR	NR
2A5	13	12	NR	NR	15	NR	14	10	NR	NR	NR	NR	NR	16	NR	NR
2B1	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2B2	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2B3	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2B4	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2B5	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2C1	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2C2	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2C3	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2C4	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2C5	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

Genetic Loci / Item Number Name	B_DYS456	B_DYS389I	B_DYS390	B_DYS389II	G_DYS458	G_DYS19	G_DYS385	Y_DYS393	Y_DYS391	Y_DYS439	Y_DYS635	Y_DYS392	R_Y_GATA H4	R-DYS437	R-DYS438	R_DYS448
2A6	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A7	13	12	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A8	13	NR	NR	NR	NR	NR	NR	NR	10	NR	NR	NR	NR	NR	NR	NR
2A9	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A10	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2B6	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2B7	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2B8	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2B9	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2B10	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2C6	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2C7	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2C8	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2C9	NR	12	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2C10	NR	NR	NR	NR	NR	NR	NR	NR	10	NR	NR	NR	NR	NR	NR	NR
Known Semen Swab	13	12	22	28	15	14	13-14	14	10	12	22	11	11	16	10	20

Appendix J: DNA Table Depicting Results of Troubleshooting from Larvae Extraction of Pig #1

Genetic Loci / Item Number Name	SBH2	SBH18	SBH4	SO655	SBH23	SBH20	SBH1	SBH10	SBH13	387A12F	SBH22	SBH19
1A1 (Dried down to ~ 50 uL with SpeedVac and amped ~ 10 uL)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A3 (Dried down to ~ 50 uL with SpeedVac and amped ~ 10 uL)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A4 (Dried down to ~ 50 uL with SpeedVac and amped ~ 10 uL)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1C1 (Dried down to ~ 50 uL with SpeedVac and amped ~ 10 uL)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1C2 (Dried down to ~ 50 uL with SpeedVac and amped ~ 10 uL)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1C3 (Dried down to ~ 50 uL with SpeedVac and amped ~ 10 uL)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A6 (Ran through Microcon wash and amped ~ 15 uL of resulting extract)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A7 (Ran through Microcon wash and amped ~ 15 uL of resulting extract)	6	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A8 (Ran through Microcon wash and amped ~ 15 uL of resulting extract)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1C6 (Ran through Microcon wash and amped ~ 15 uL of resulting extract)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1C7 (Ran through Microcon wash and amped ~ 15 uL of resulting extract)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1C8 (Ran through Microcon wash and amped ~ 15 uL of resulting extract)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Pig #1 Known	6	15	57, 65.3	5, 12	Y, X	19, 23	13	49	15, 16	13, 15.1	22, 23	11, 15
Pig#2 Known	24, 26	12, 13	62	11	X	30, 36	11, 14	48	9, 15	9, 15.1	20, 23	14, 16

Appendix K: DNA Table Depicting Results of Troubleshooting from Larvae Extraction of Pig #2

Genetic Loci → / Item Number Name ↓	B_DYS456	B_DYS389I	B_DYS390	B_DYS389II	G_DYS458	G_DYS19	G_DYS385	Y_DYS393	Y_DYS391	Y_DYS439	Y_DYS635	Y_DYS392	R_Y_GATA H4	R-DYS437	R-DYS438	R_DYS448
2A2 (Dried down to ~ 50uL with Speedvac)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A3 (Dried down to ~ 50uL with Speedvac)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A4 (Dried down to ~ 50uL with Speedvac)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2C1 (Dried down to ~ 50uL with Speedvac)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2C2 (Dried down to ~ 50uL with Speedvac)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2C3 (Dried down to ~ 50uL with Speedvac)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A8 (Ran through Microcon Wash, Amped ~ 10 uL of resulting extract)	13	NR	NR	NR	15	NR	NR	NR	NR	NR	NR	NR	NR	16	NR	NR
2A9 (Ran through Microcon Wash, Amped ~ 10 uL of resulting extract)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	16	NR	NR
2A10 (Ran through Microcon Wash, Amped ~ 10 uL of resulting extract)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2C8 (Ran through Microcon Wash, Amped ~ 10 uL of resulting extract)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2C9 (Ran through Microcon Wash, Amped ~ 10 uL of resulting extract)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2C10 (Ran through Microcon Wash, Amped ~ 10 uL of resulting extract)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Known Semen Swab	13	12	22	28	15	14	13-14	14	10	12	22	11	11	16	10	20

Appendix L: DNA Table Depicting Results of Larvae Extraction from Pig #1 (Collections 3 through 10)

Genetic Loci → / Item Number Name ↓	SBH2	SBH18	SBH4	SO655	SBH23	SBH20	SBH1	SBH10	SBH13	387A12F	SBH22	SBH19
1A11	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A12	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A13	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A14	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A15	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A16 (Bad Injection)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A17	6	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A18	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A19	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A20	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A21	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A22	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A23	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A24	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A25	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

Genetic Loci / Item Number Name	SBH2	SBH18	SBH4	SO655	SBH23	SBH20	SBH1	SBH10	SBH13	387A12F	SBH22	SBH19
1A26	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A27	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A28	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A29	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A30	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A31	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A32	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A33	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A34	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A35	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A36	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A37	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A38	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A39	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A40	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A41	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A42	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

Genetic Loci / Item Number Name	SBH2	SBH18	SBH4	SO655	SBH23	SBH20	SBH1	SBH10	SBH13	387A12F	SBH22	SBH19
1A43	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A44	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1A45	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Pig #1 Known	6	15	57, 65.3	5, 12	Y, X	19, 23	13	49	15, 16	13, 15.1	22, 23	11, 15
Pig#2 Known	24, 26	12, 13	62	11	X	30, 36	11, 14	48	9, 15	9, 15.1	20, 23	14, 16

Appendix M: DNA Table Depicting Results of Larvae Extraction from Pig #2 (Collections 3 through 10)

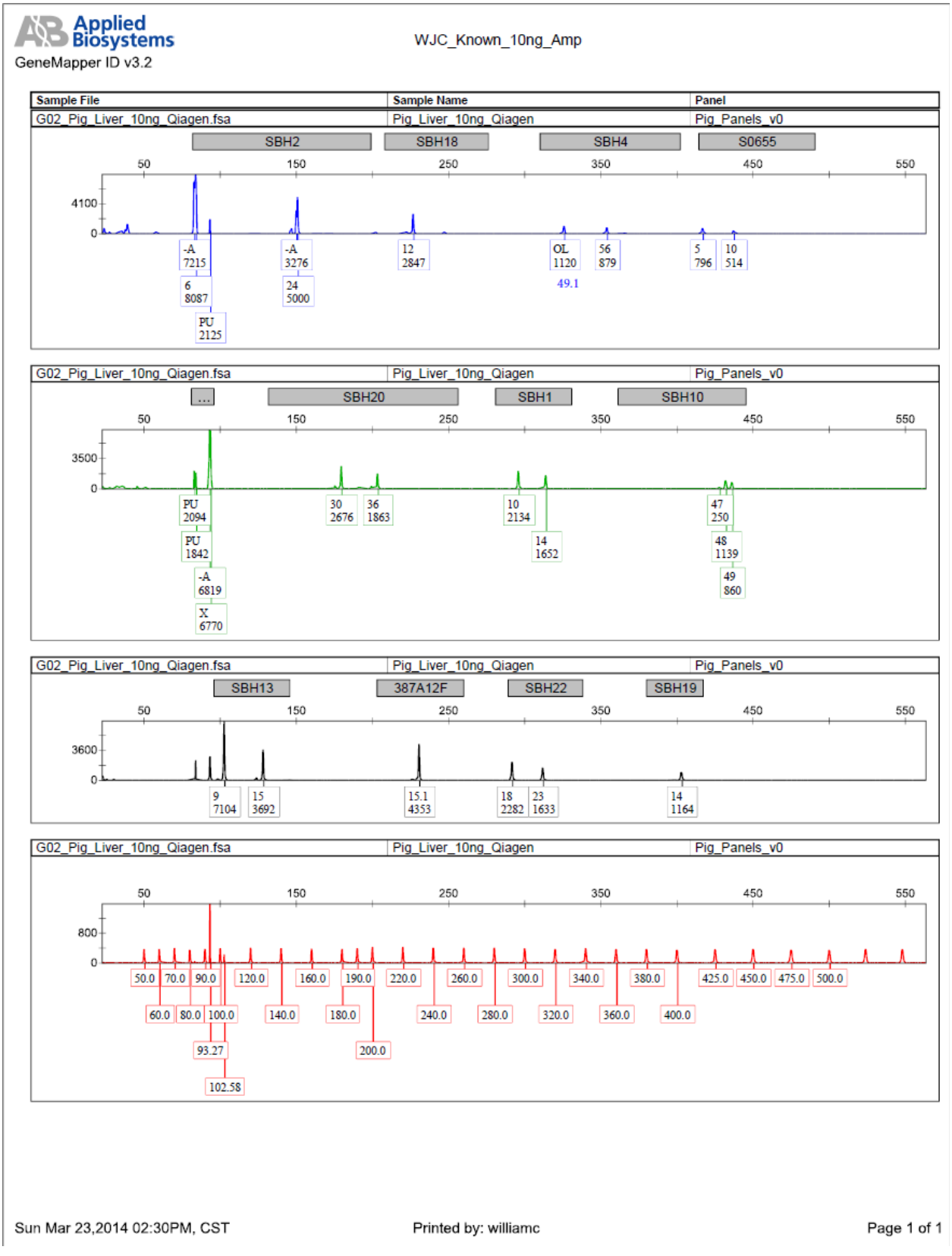
Genetic Loci → / Item Number Name ↓	B_DYS456	B_DYS389I	B_DYS390	B_DYS389II	G_DYS458	G_DYS19	G_DYS385	Y_DYS393	Y_DYS391	Y_DYS439	Y_DYS635	Y_DYS392	R_Y_GATA H4	R-DYS437	R-DYS438	R_DYS448
2A11 (Bad Injection)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A12	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A13	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A14	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A15	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A16 (Bad Injection)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A17	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A18	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A19 (Bad Injection)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A20	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A21	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A22	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A23	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A24	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A25	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

Genetic Loci / Item Number Name	B_DYS456	B_DYS389I	B_DYS390	B_DYS389II	G_DYS458	G_DYS19	G_DYS385	Y_DYS393	Y_DYS391	Y_DYS439	Y_DYS635	Y_DYS392	R_Y_GATA H4	R-DYS437	R-DYS438	R_DYS448
2A26	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A27	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A28	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A29	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A30	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A31	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A32	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A33	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A34	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A35	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A36	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A37	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A38	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A39	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A40	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A41	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A42	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

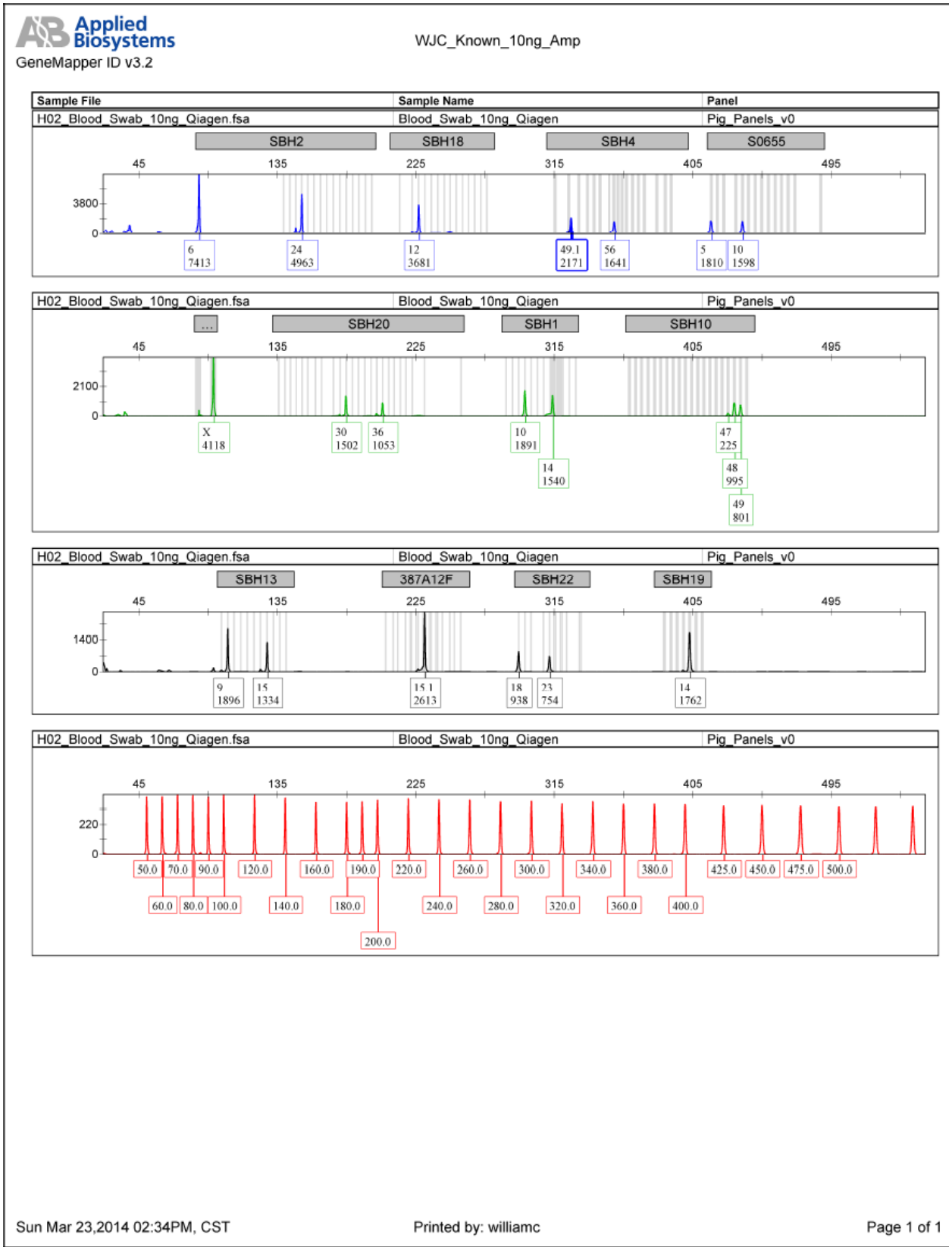
Genetic Loci / Item Number Name	B_DYS456	B_DYS389I	B_DYS390	B_DYS389II	G_DYS458	G_DYS19	G_DYS385	Y_DYS393	Y_DYS391	Y_DYS439	Y_DYS635	Y_DYS392	R_Y_GATA H4	R-DYS437	R-DYS438	R_DYS448
2A43	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A44	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A45	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A46	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A47	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A48	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A49	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2A50	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Known Semen Swab	13	12	22	28	15	14	13-14	14	10	12	22	11	11	16	10	20

Appendix N: Electropherograms

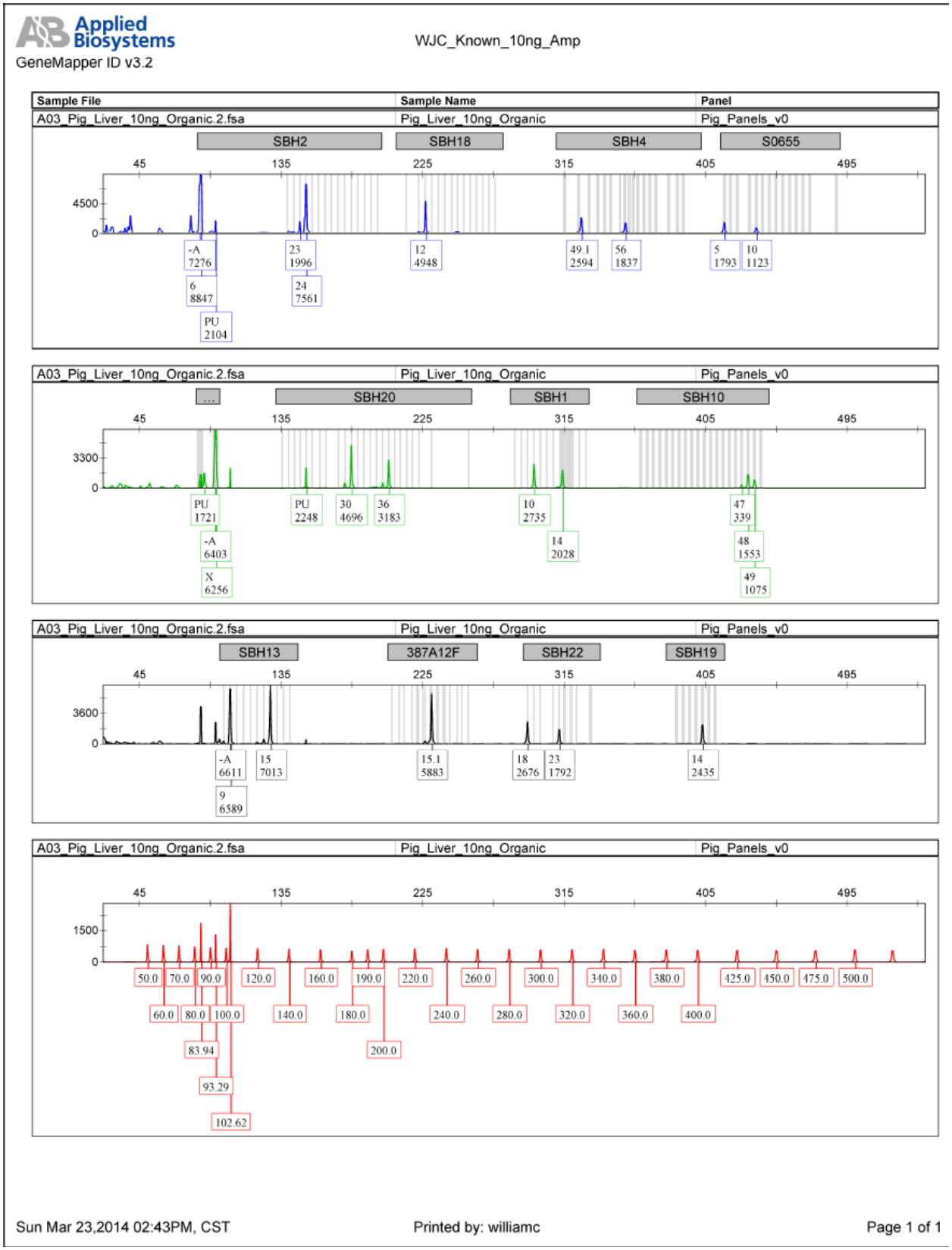
Pilot Study: Known Liver Sample 10 ng Qiagen Extraction



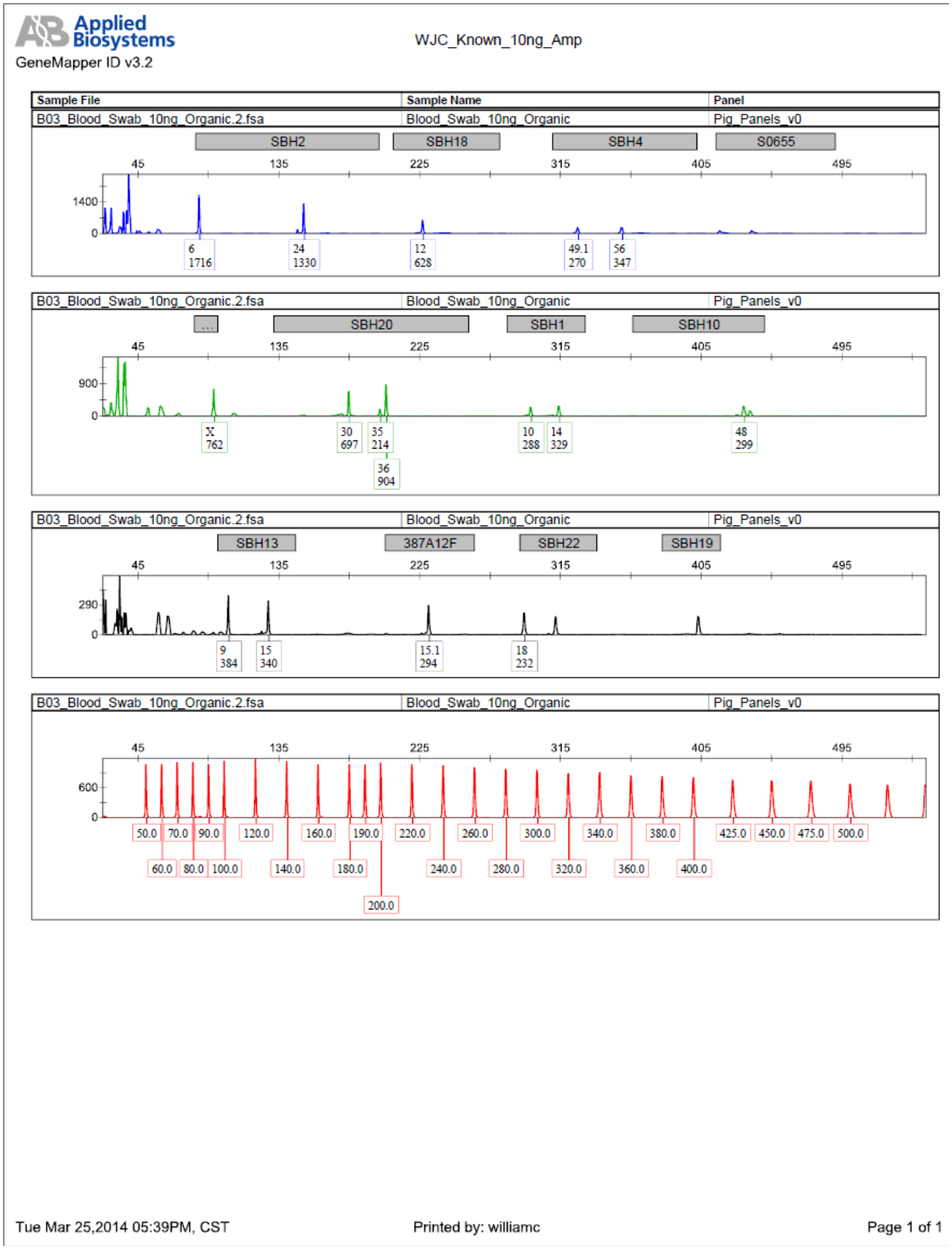
Pilot Study: Pig Blood Swab Qiagen Extraction



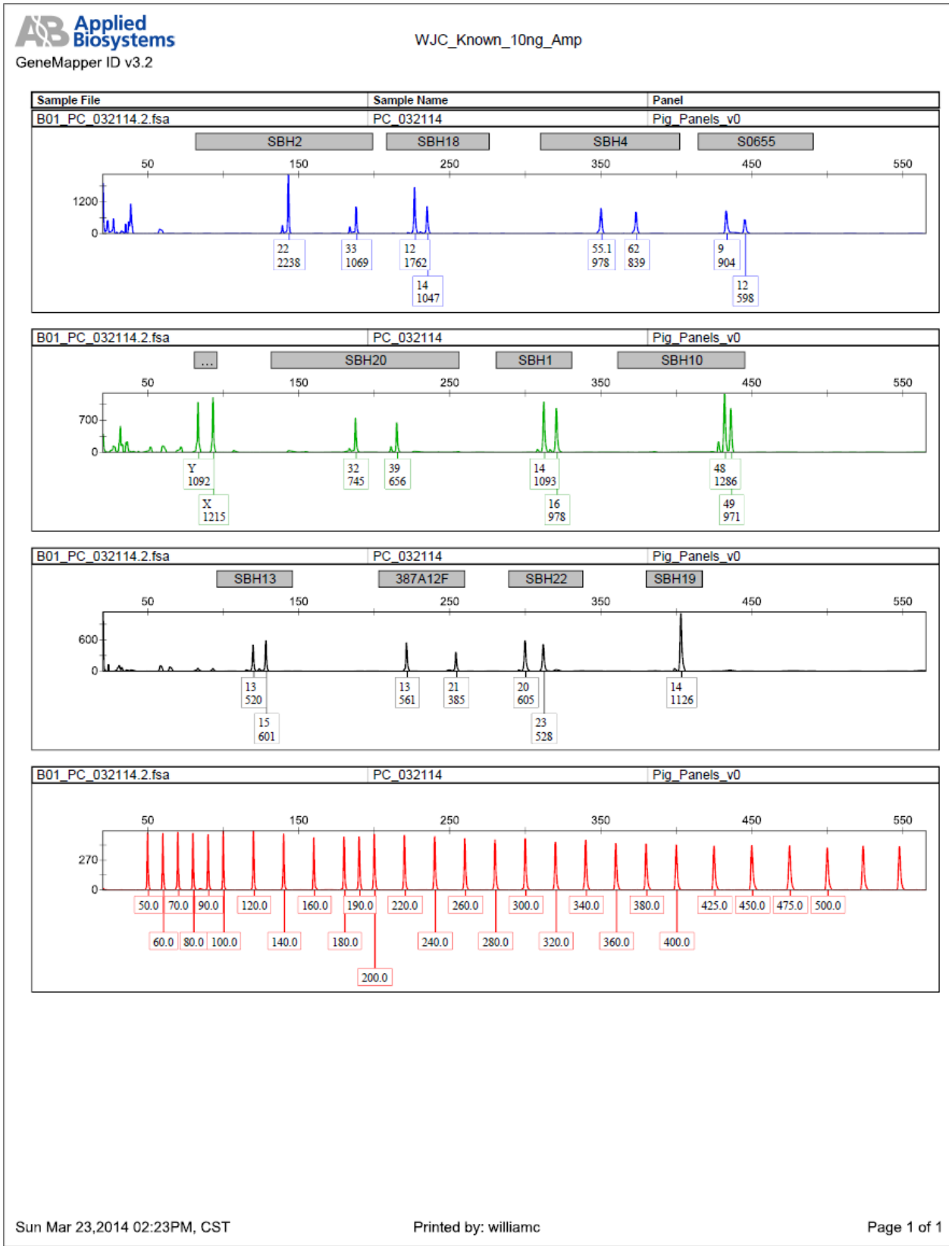
Pilot Study: Pig Liver Organic Extraction (10 seconds)



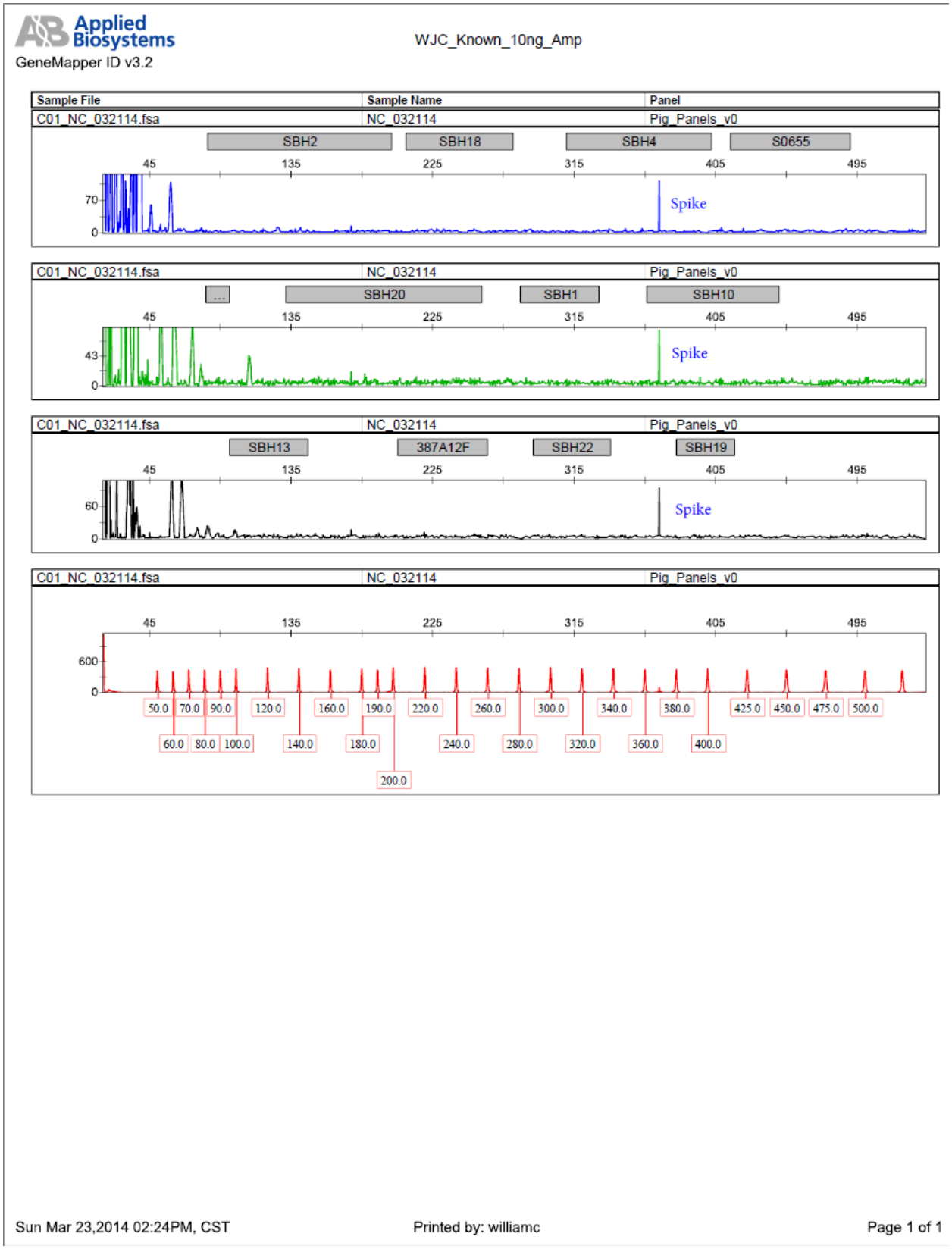
Pilot Study: Pig Blood Swab Organic Extraction (10 seconds)



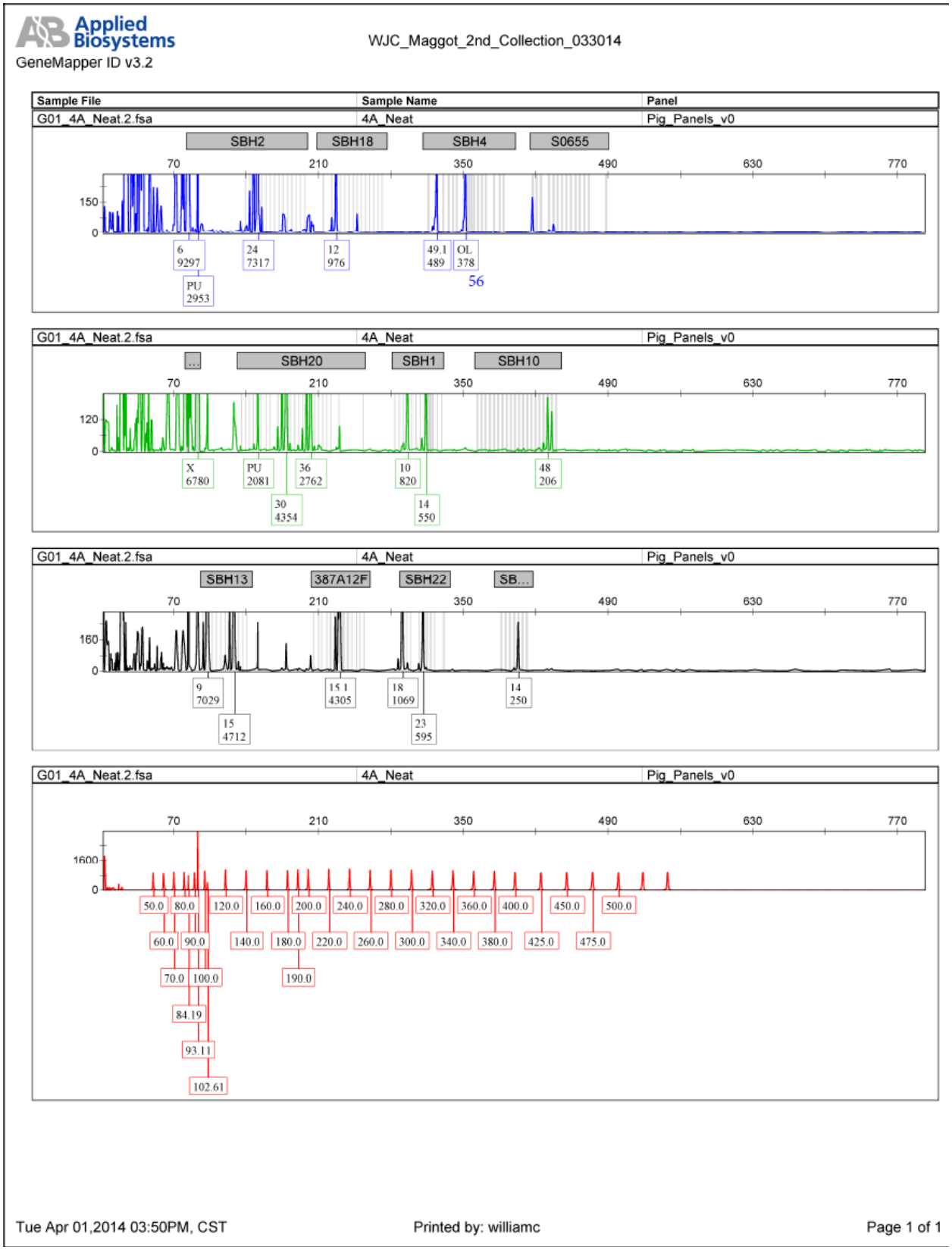
Pilot Study: Positive Control 032114 for Known Samples



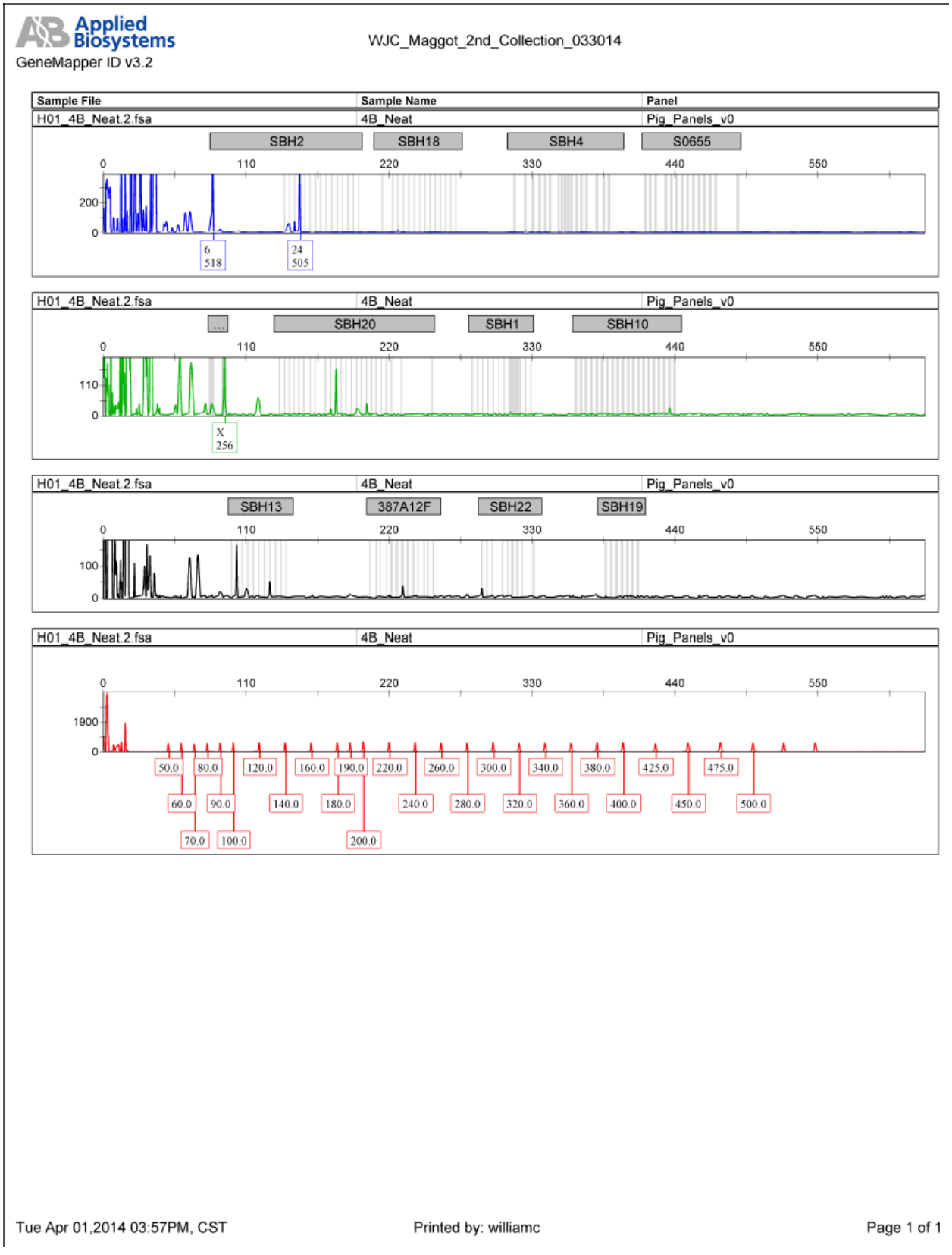
Pilot Study: Negative Control 032114 for Known Samples



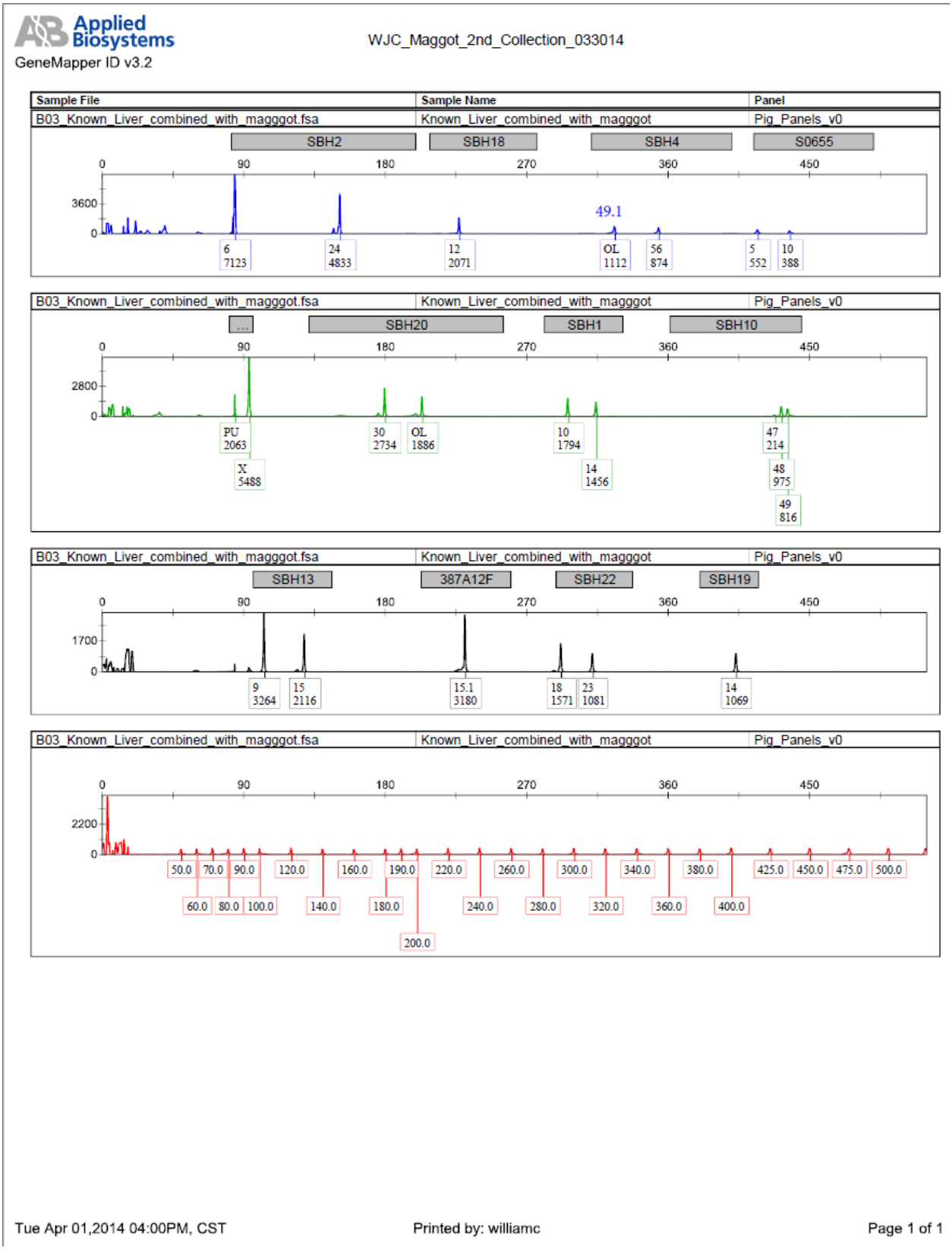
Pilot Study: Sample 4A Neat Preserved in Ethanol (10 seconds)



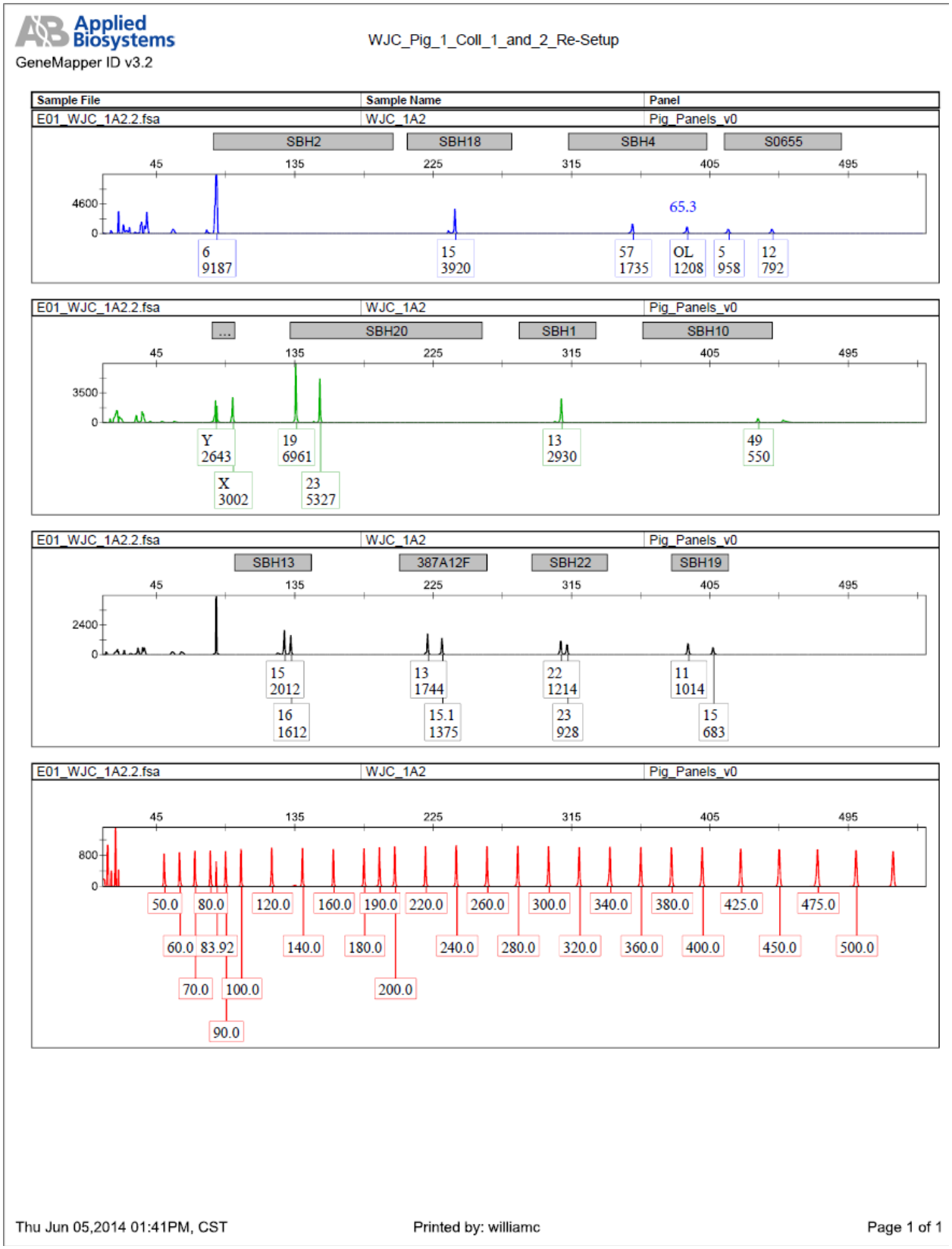
Pilot Study: Sample 4B Neat Preserved in Ethanol (10 seconds)



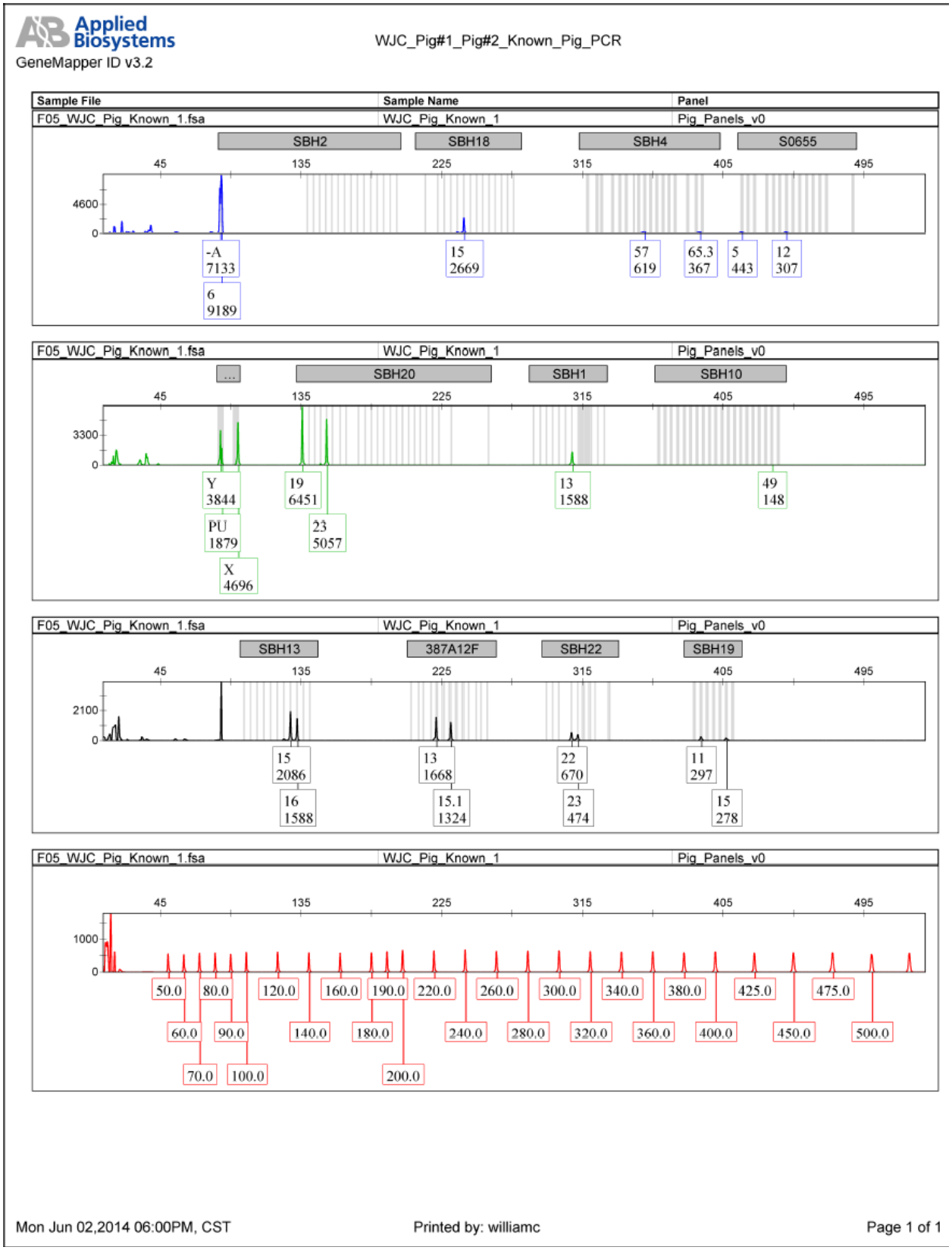
Pilot Study: Known Liver Sample with Maggot Extract (Rule out Inhibition from Larvae Extract) (5 seconds)



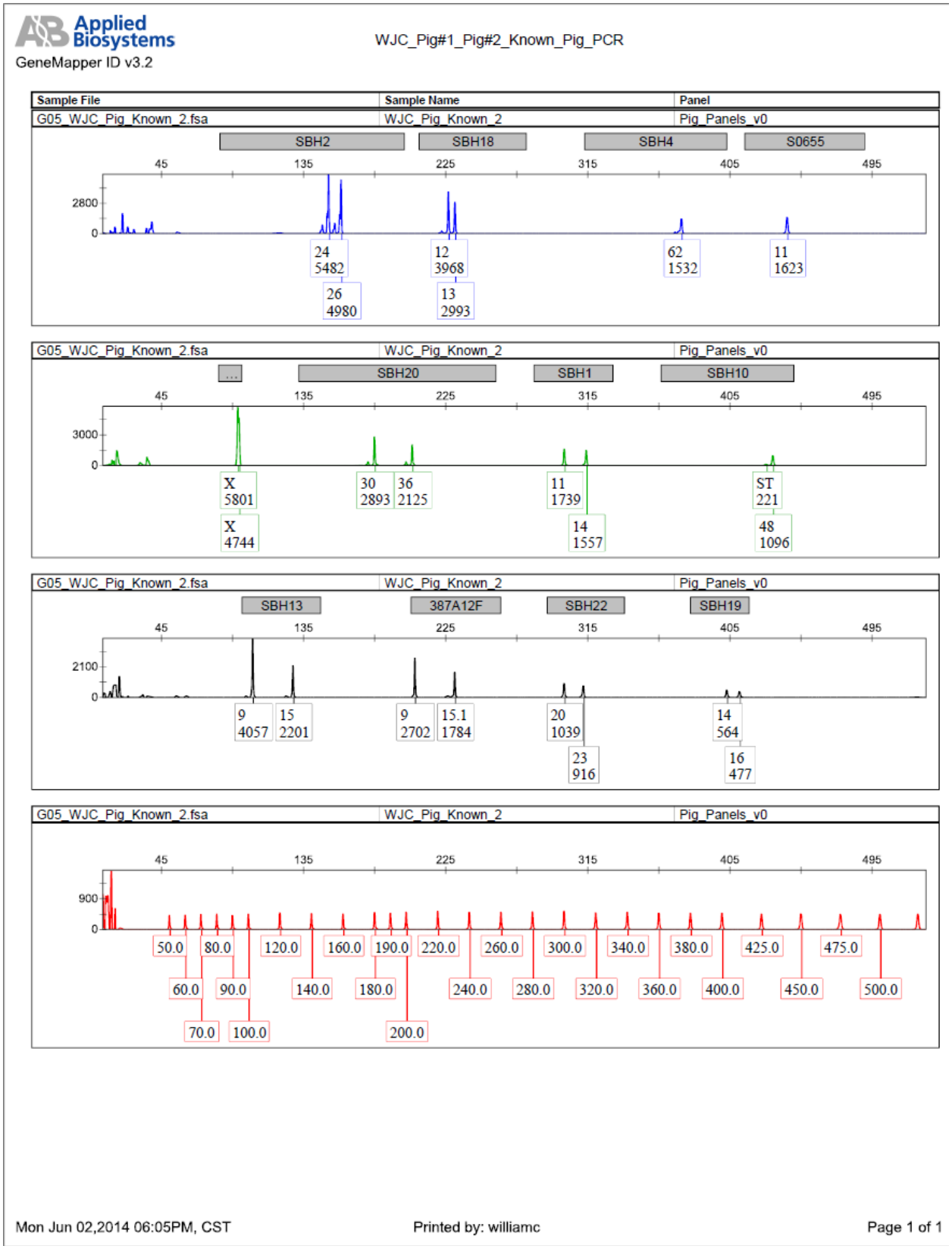
Pig #1 Sample 1A2 Collection 1 Preserved in Ethanol (10 seconds)



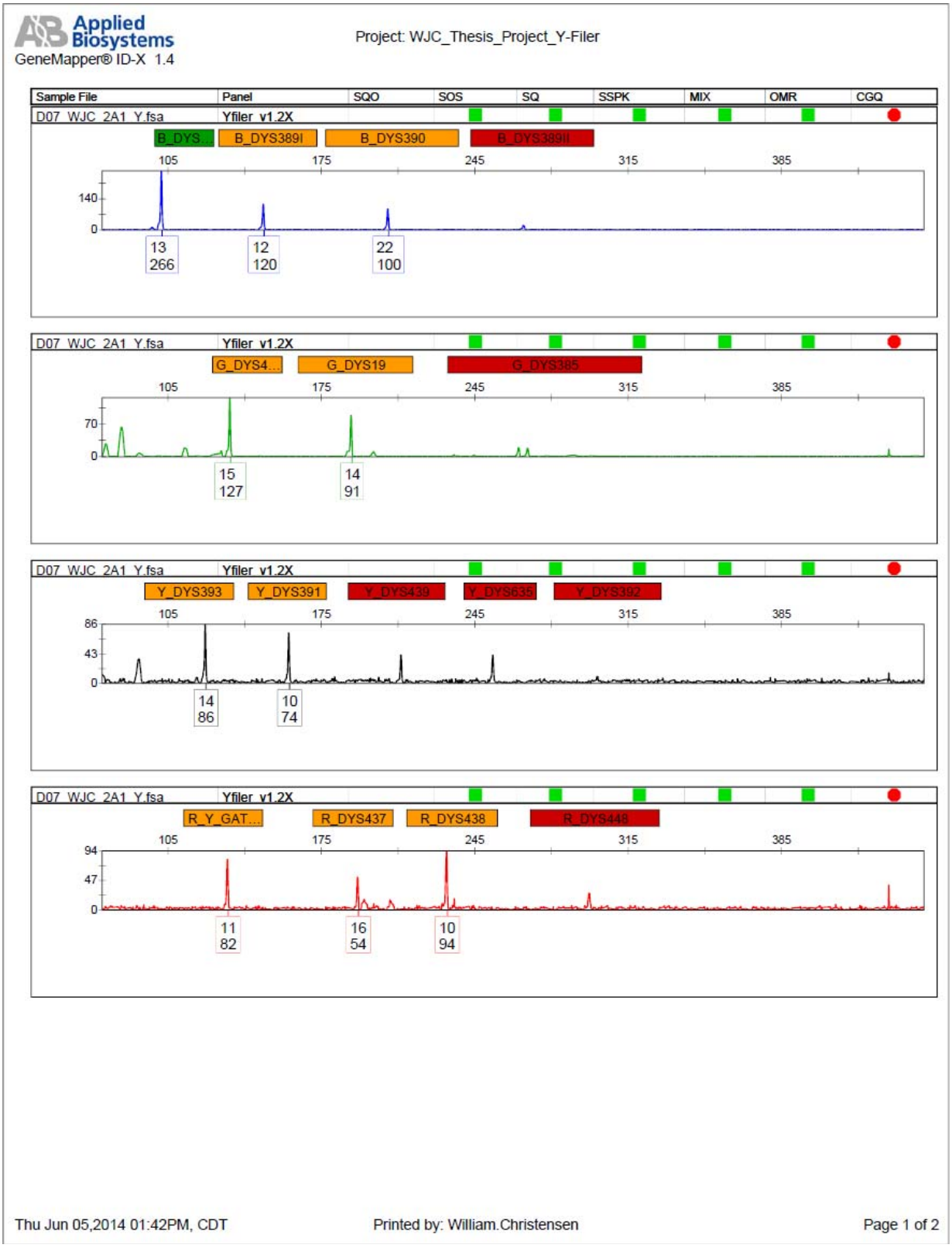
Pig #1 Known Sample



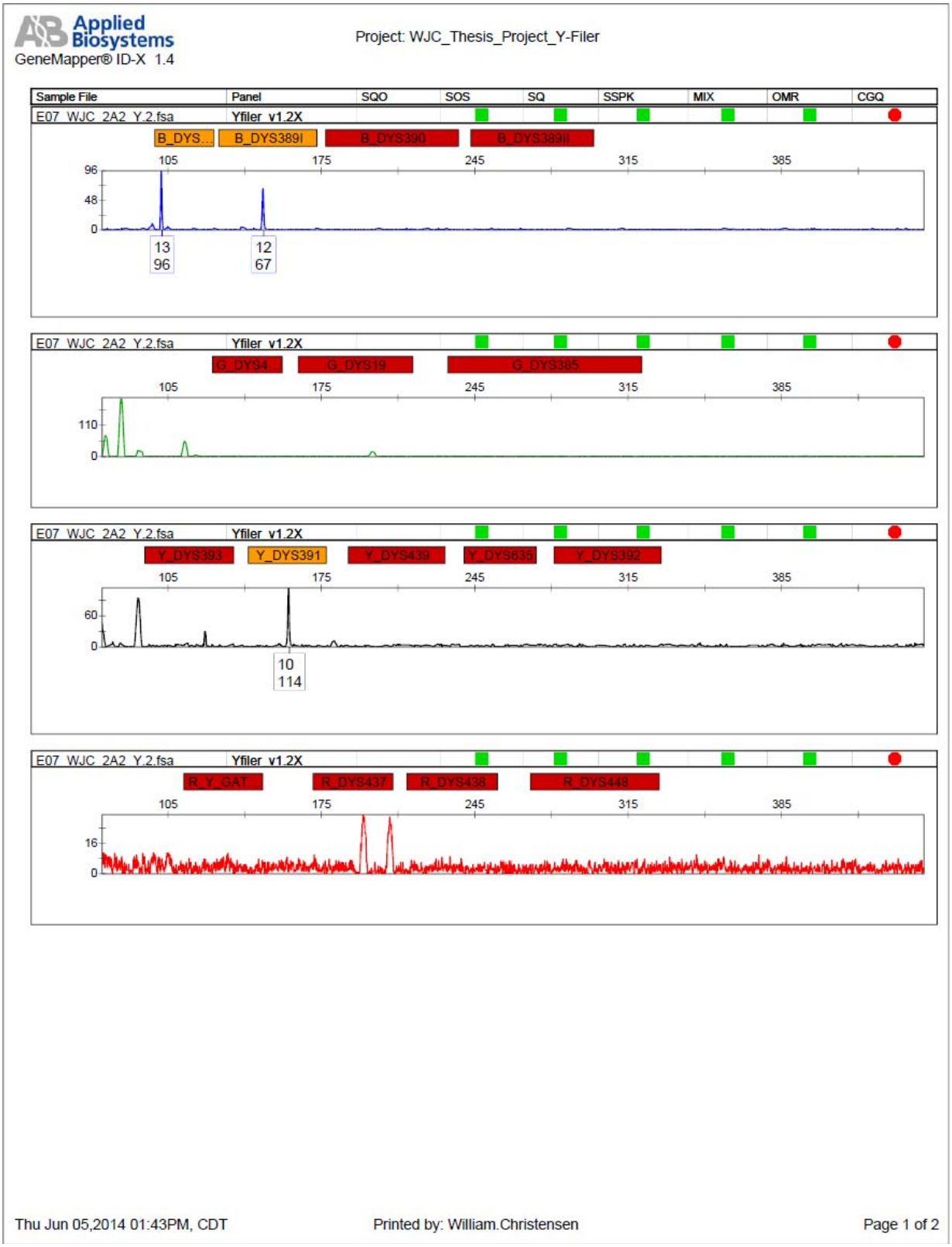
Pig #2 Known Sample



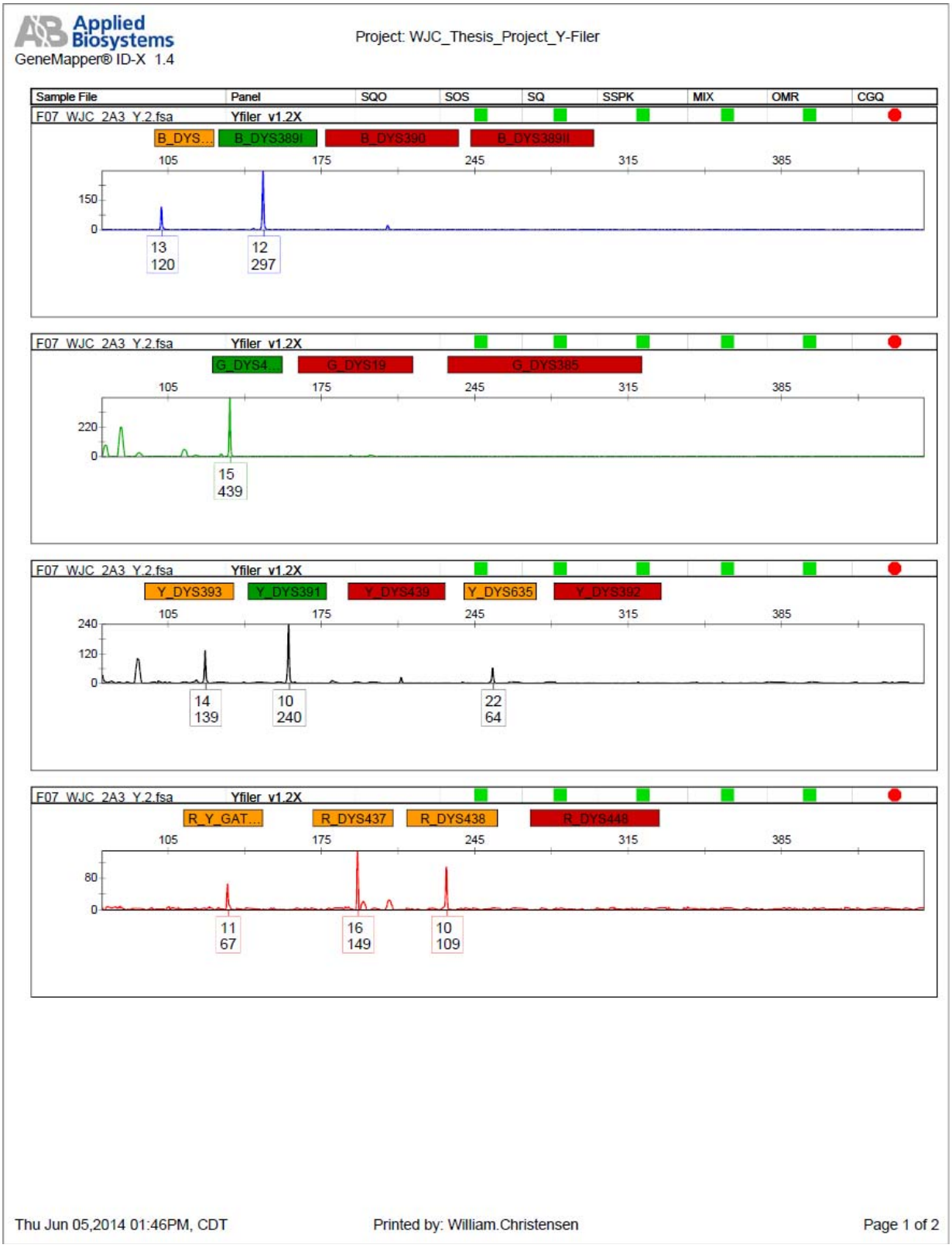
Pig #2 Sample 2A1 (Y-Filer) (5 seconds)



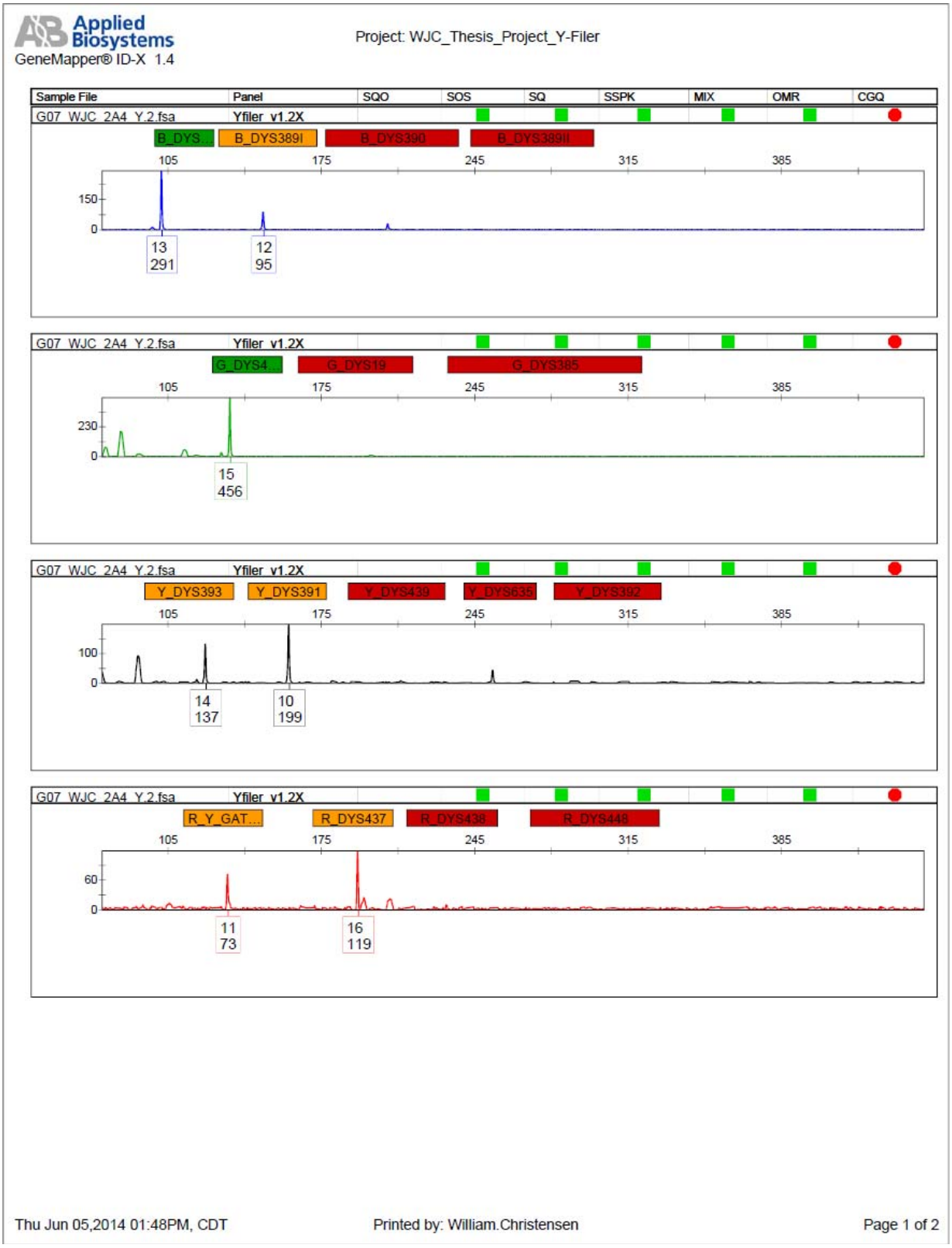
Pig #2 Sample 2A2 (Y-Filer) (10 seconds)



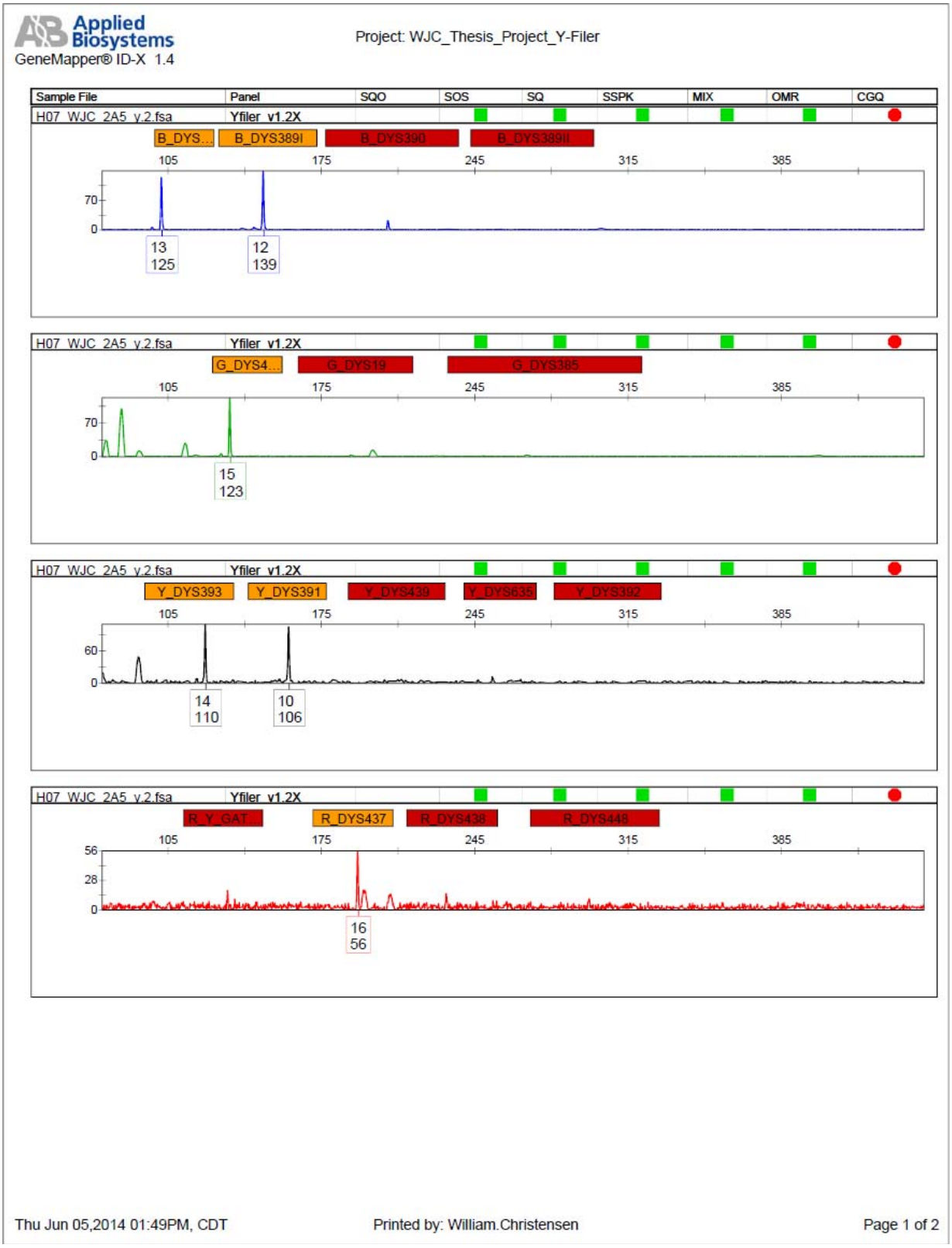
Pig #2 Sample 2A3 (Y-Filer) (10 seconds)



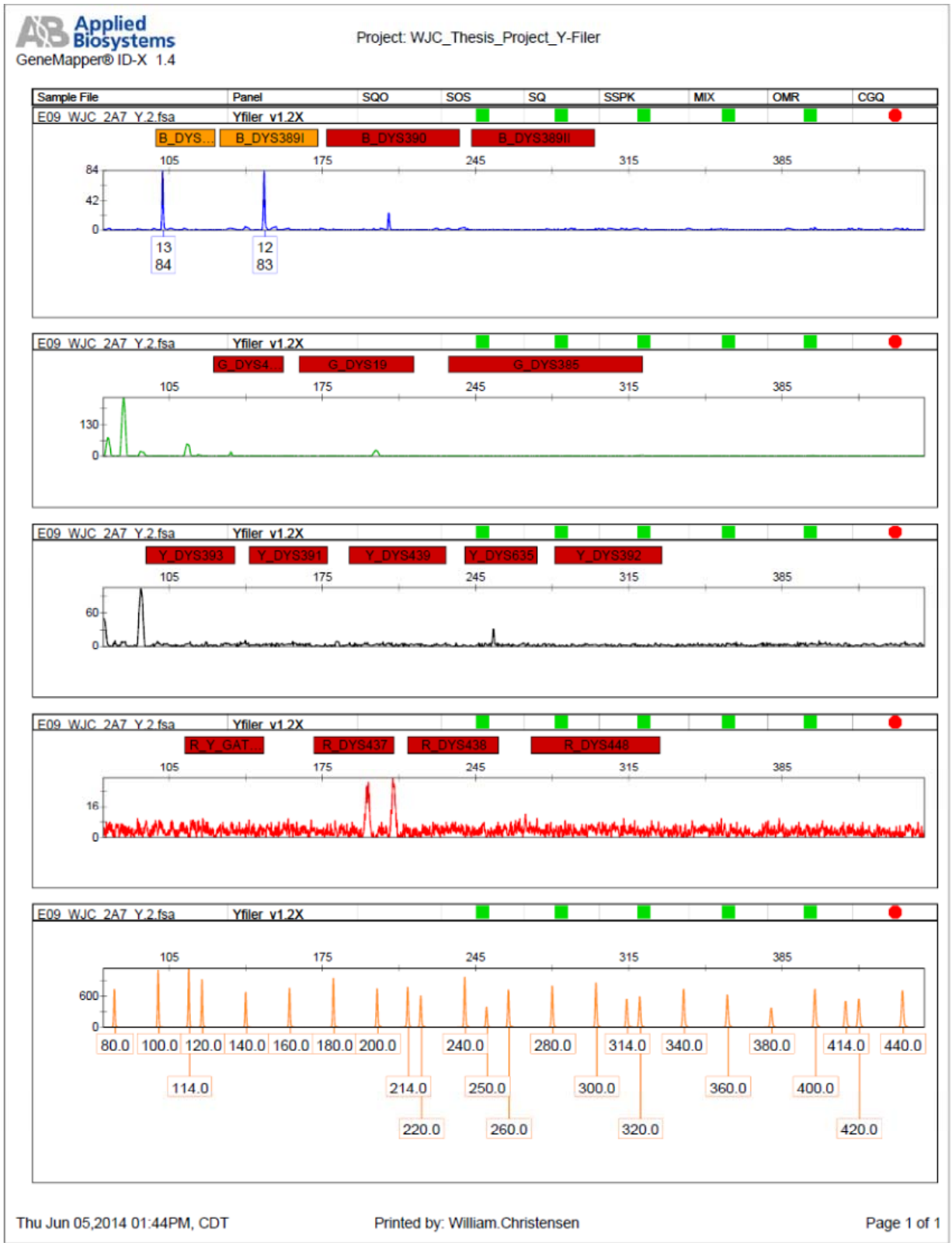
Pig #2 Sample 2A4 (Y-Filer) (10 seconds)



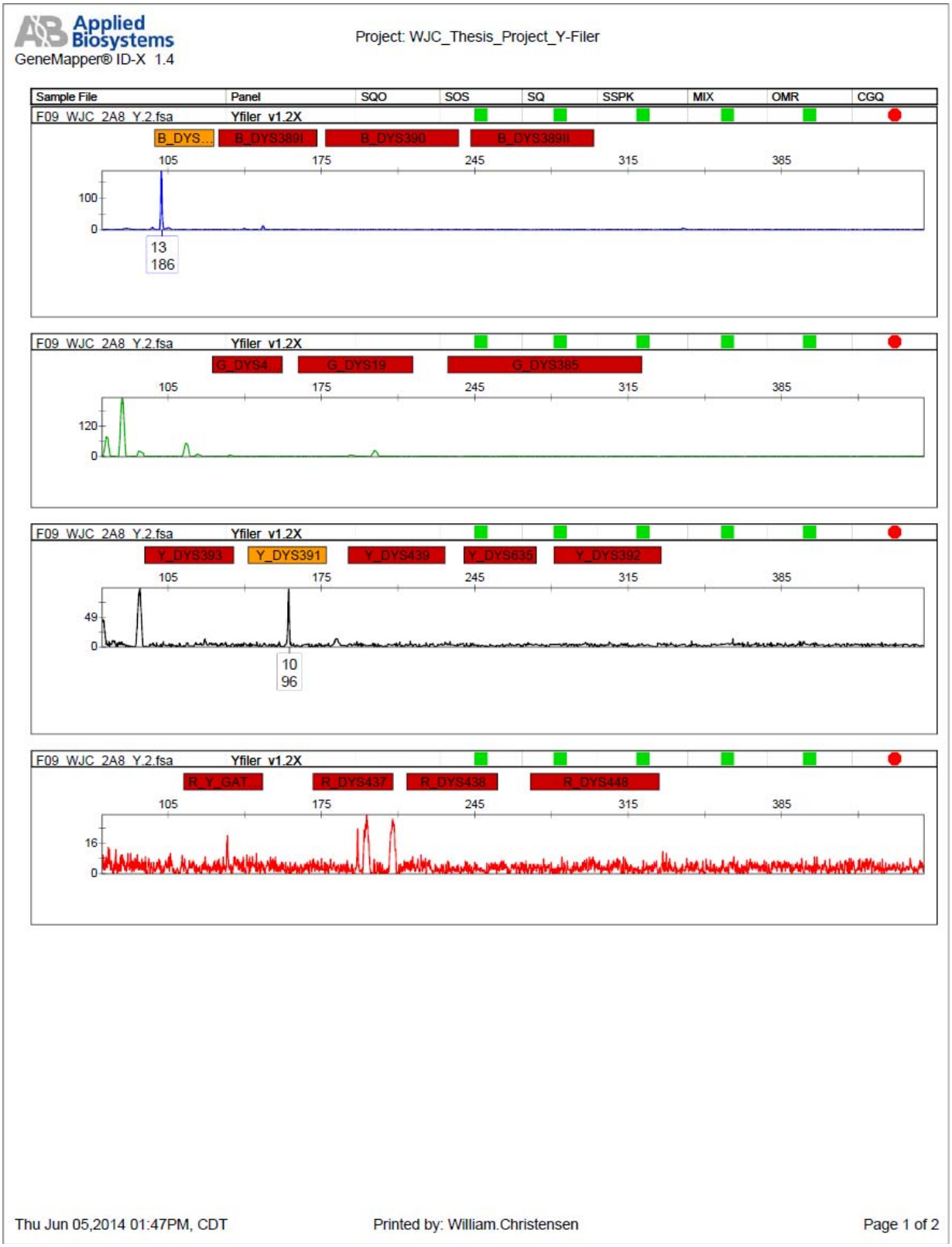
Pig #2 Sample 2A5 (Y-Filer) (10 seconds)



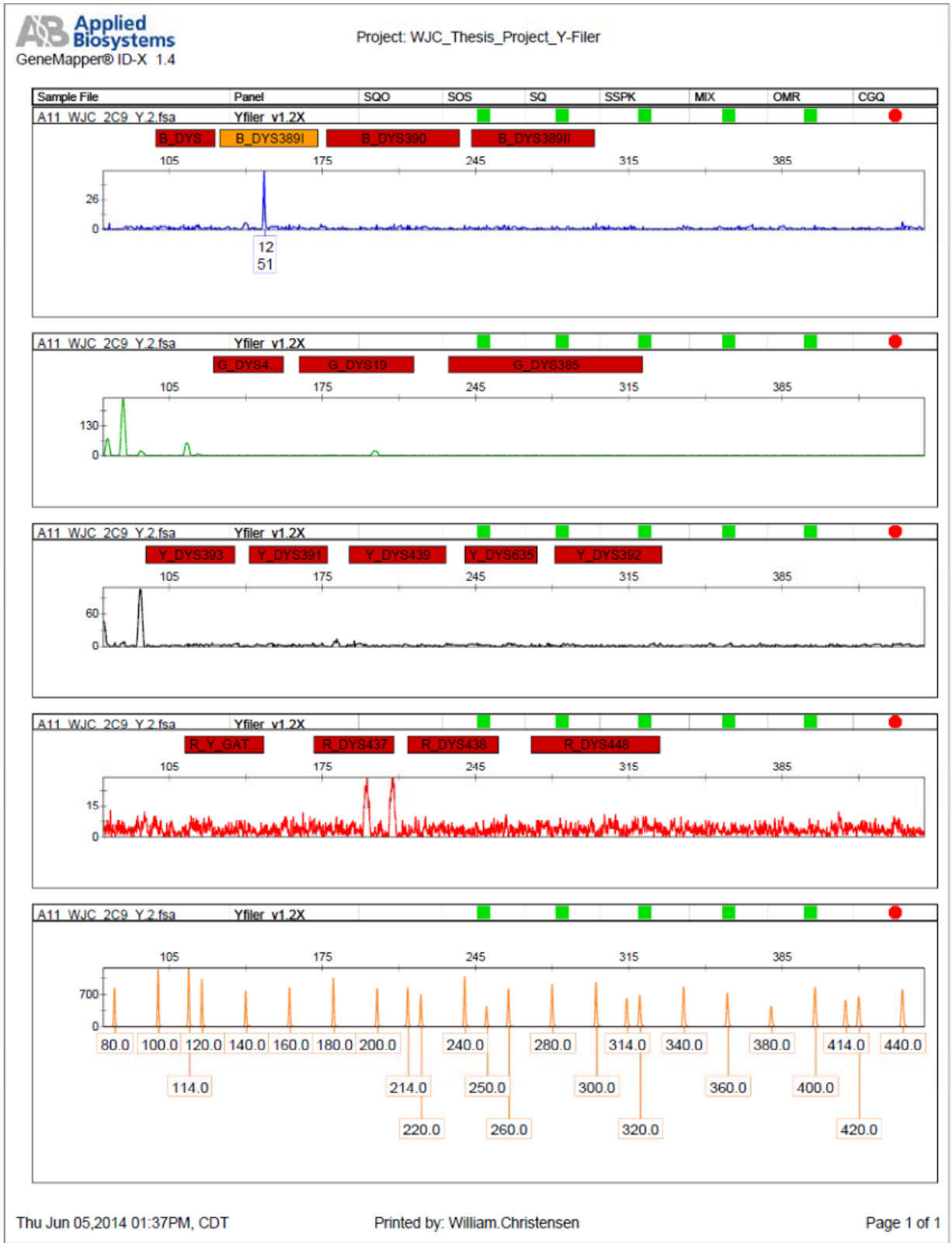
Pig #2 Sample 2A7 (Y-Filer) (10 seconds)



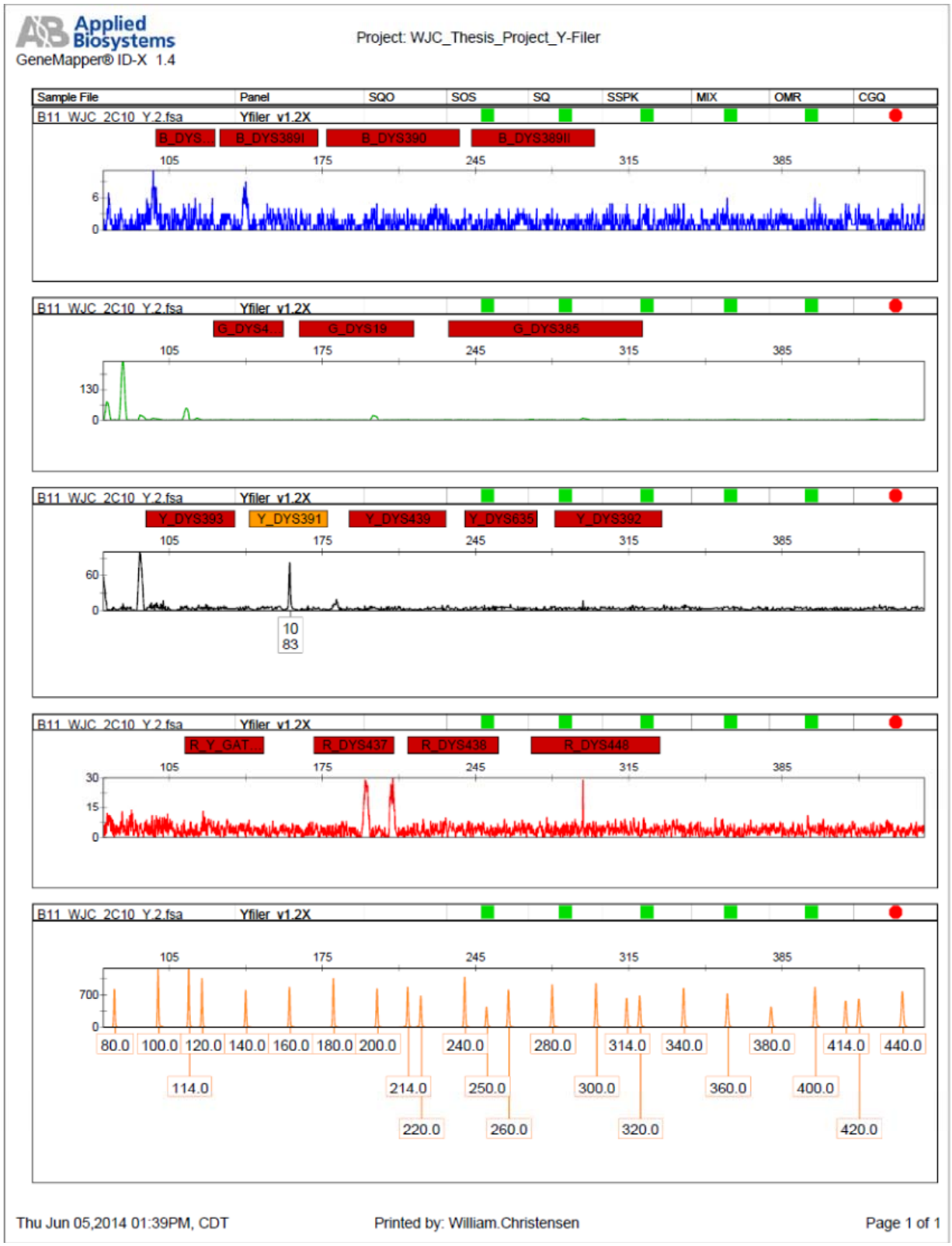
Pig #2 Sample 2A8 (Y-Filer) (10 seconds)



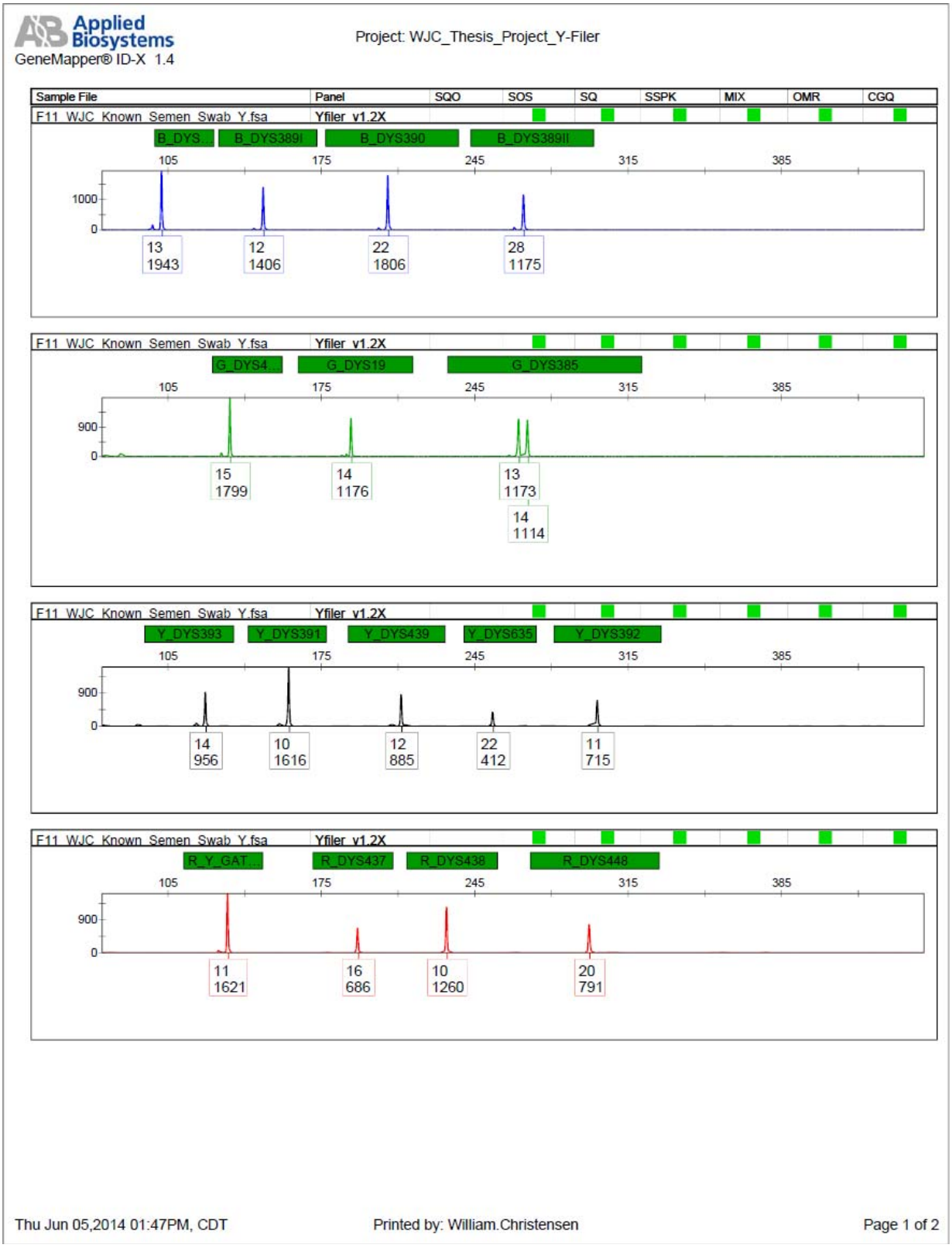
Pig #2 Sample 2C9 (Y-Filer) (10 seconds)



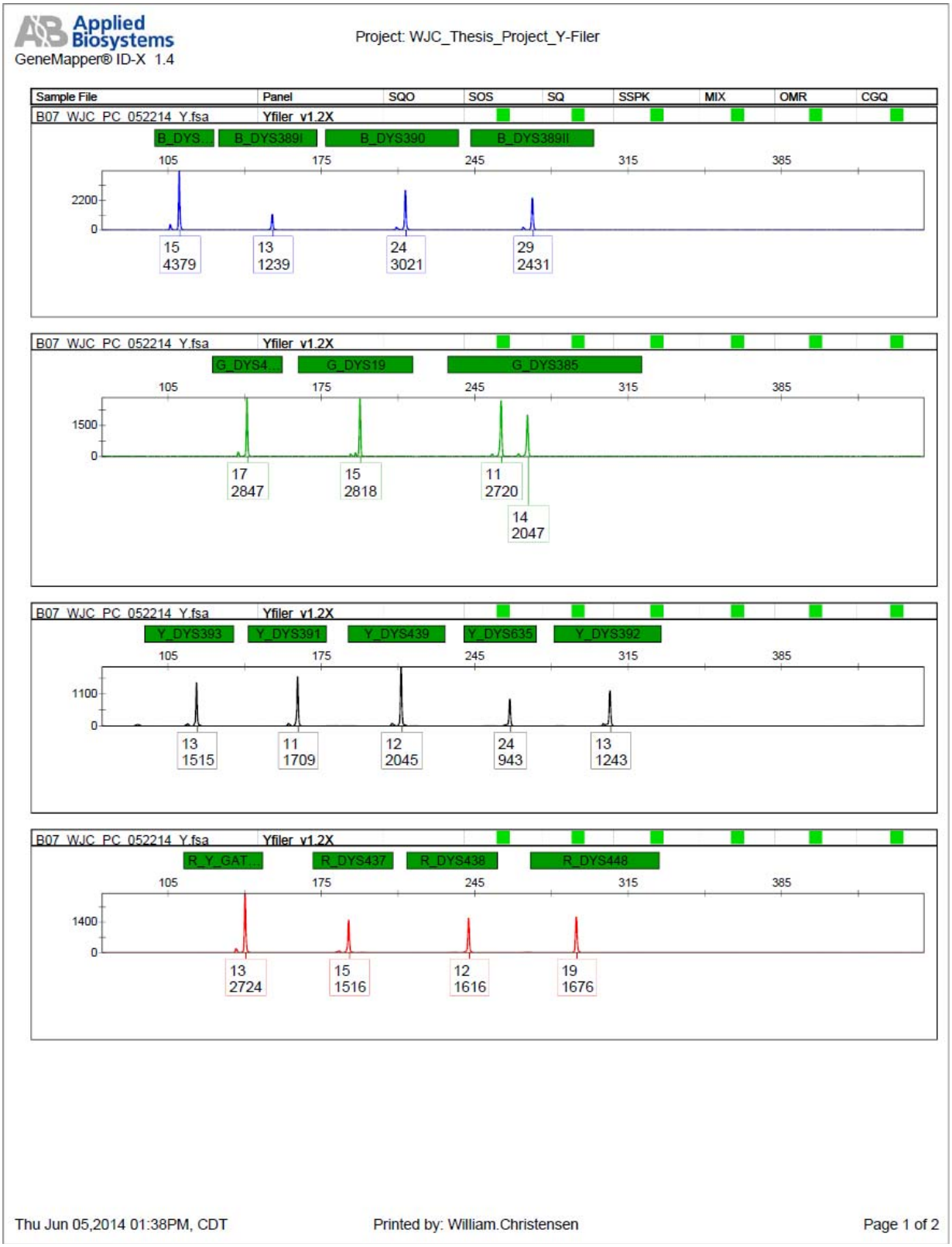
Pig #2 Sample 2C10 (Y-Filer) (10 seconds)



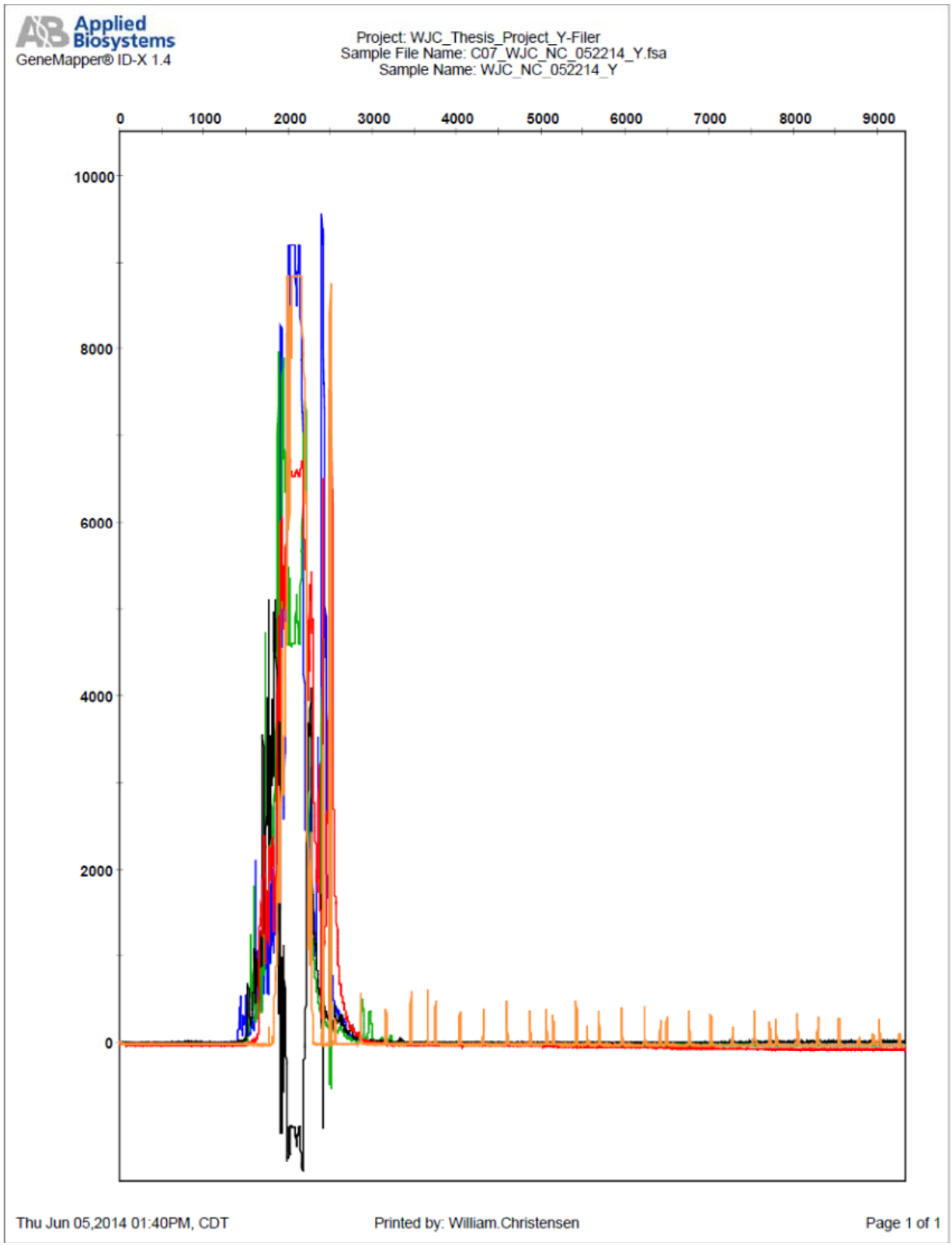
Known Semen Swab (Y-Filer)



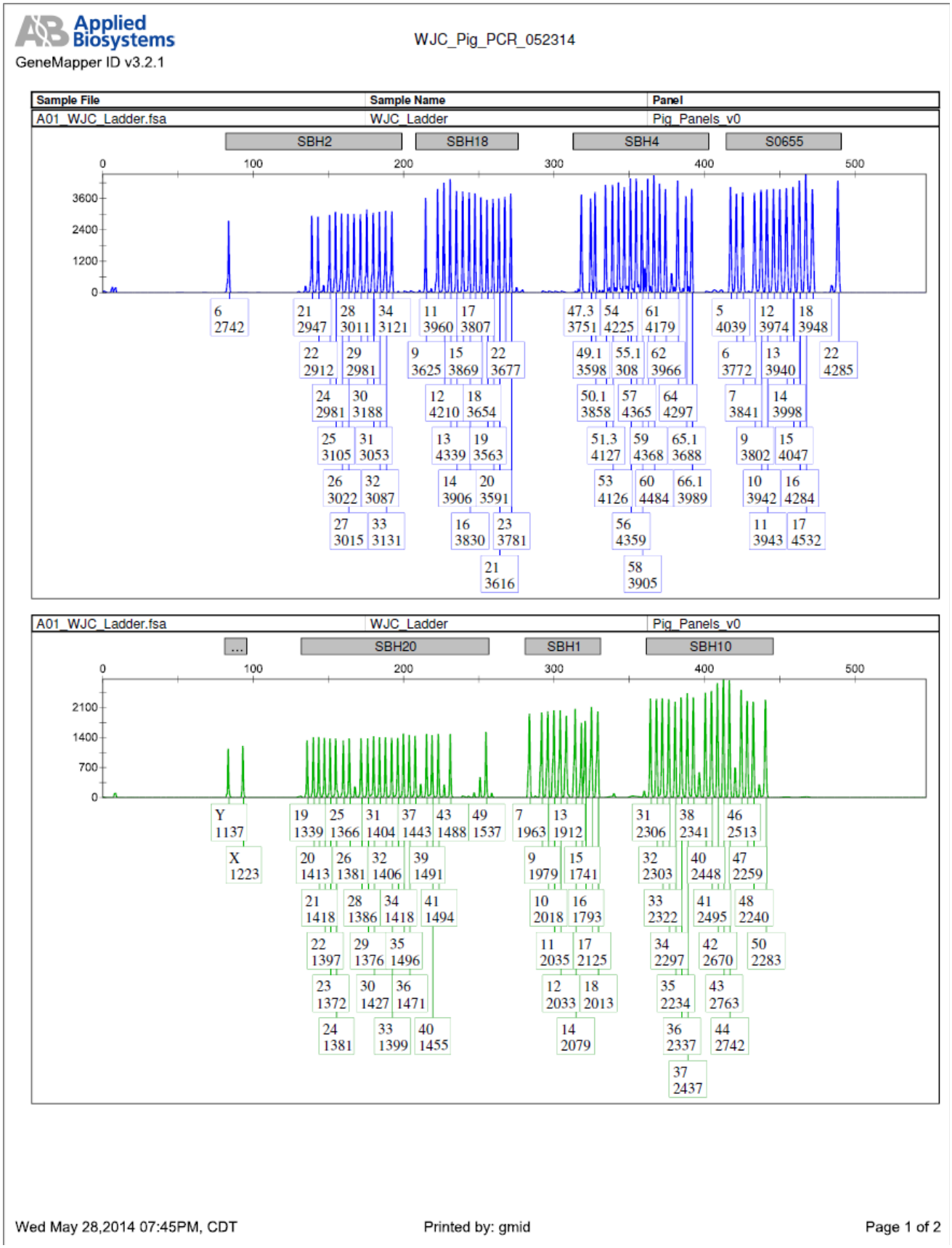
Positive Control 052214 Y-Filer



Negative Control 052214 (Y-Filer)



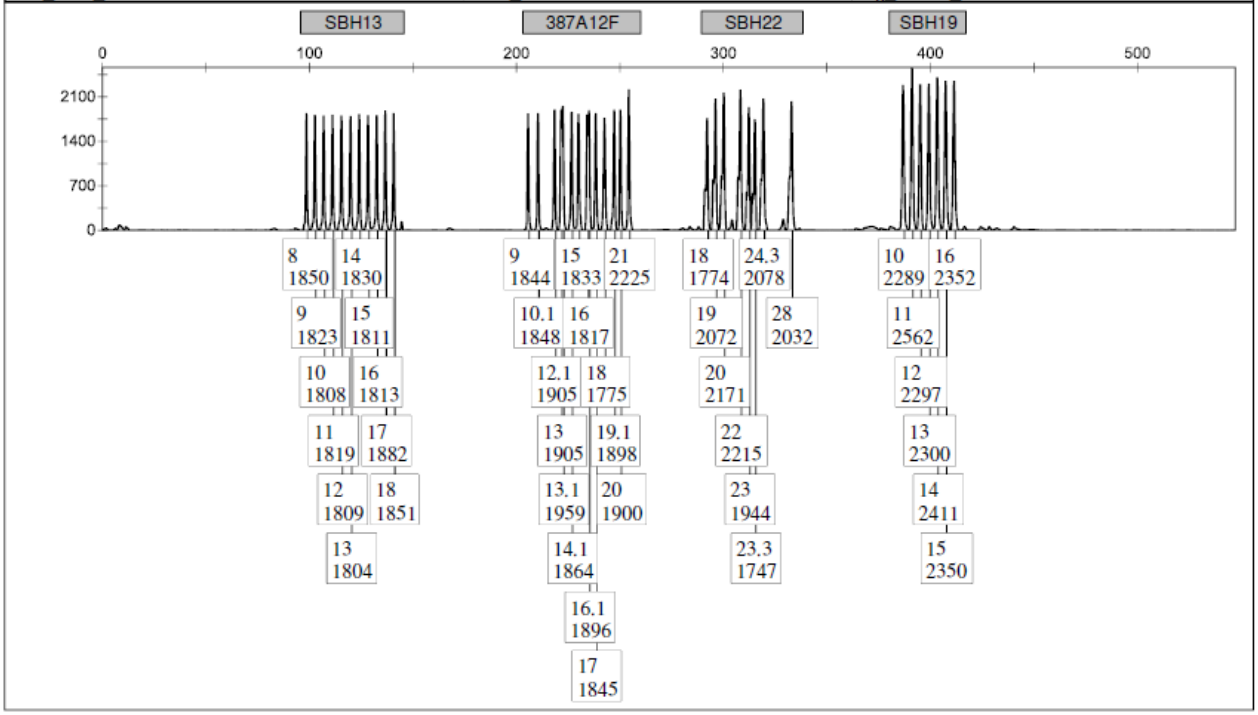
Animaltype Pig PCR Kit Ladder Obtained from Analysis



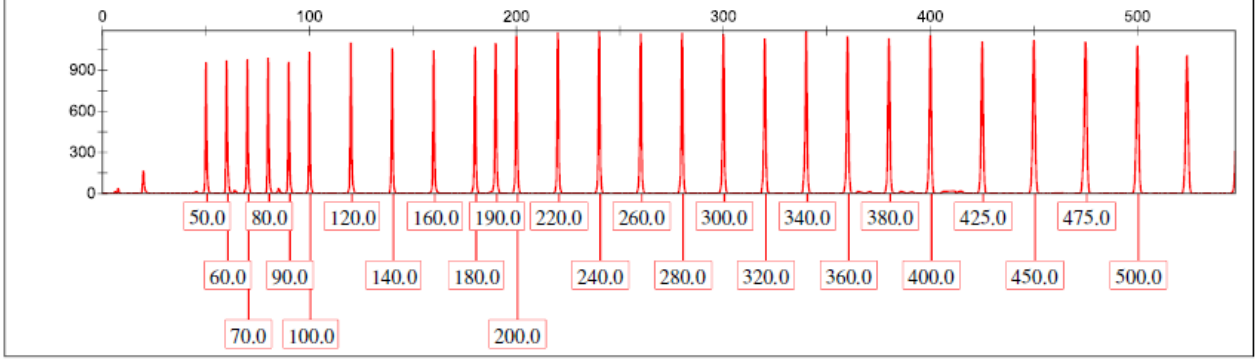
Applied Biosystems
GeneMapper ID v3.2.1

WJC_Pig_PCR_052314

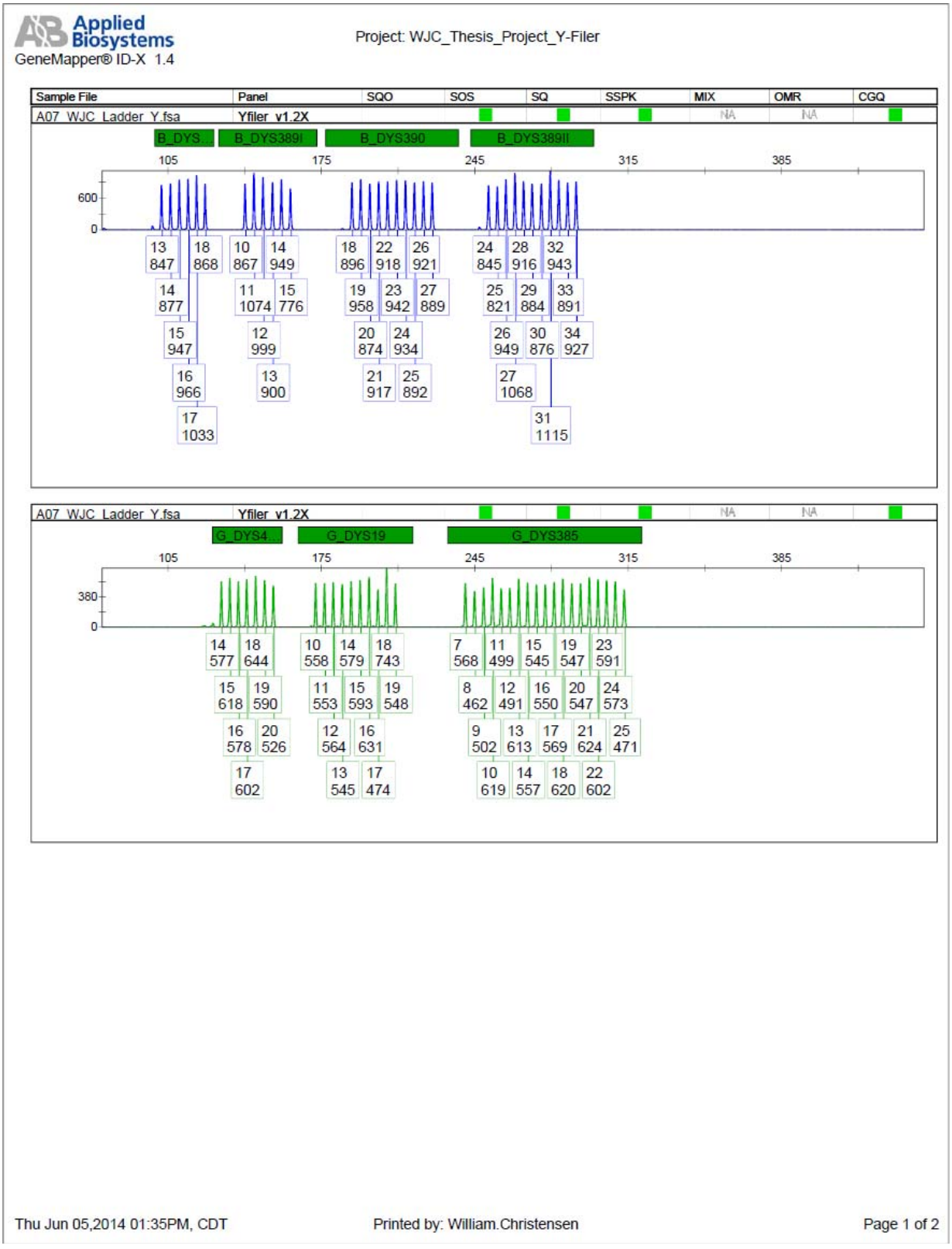
Sample File	Sample Name	Panel
A01_WJC_Ladder.fsa	WJC_Ladder	Pig_Panels_v0

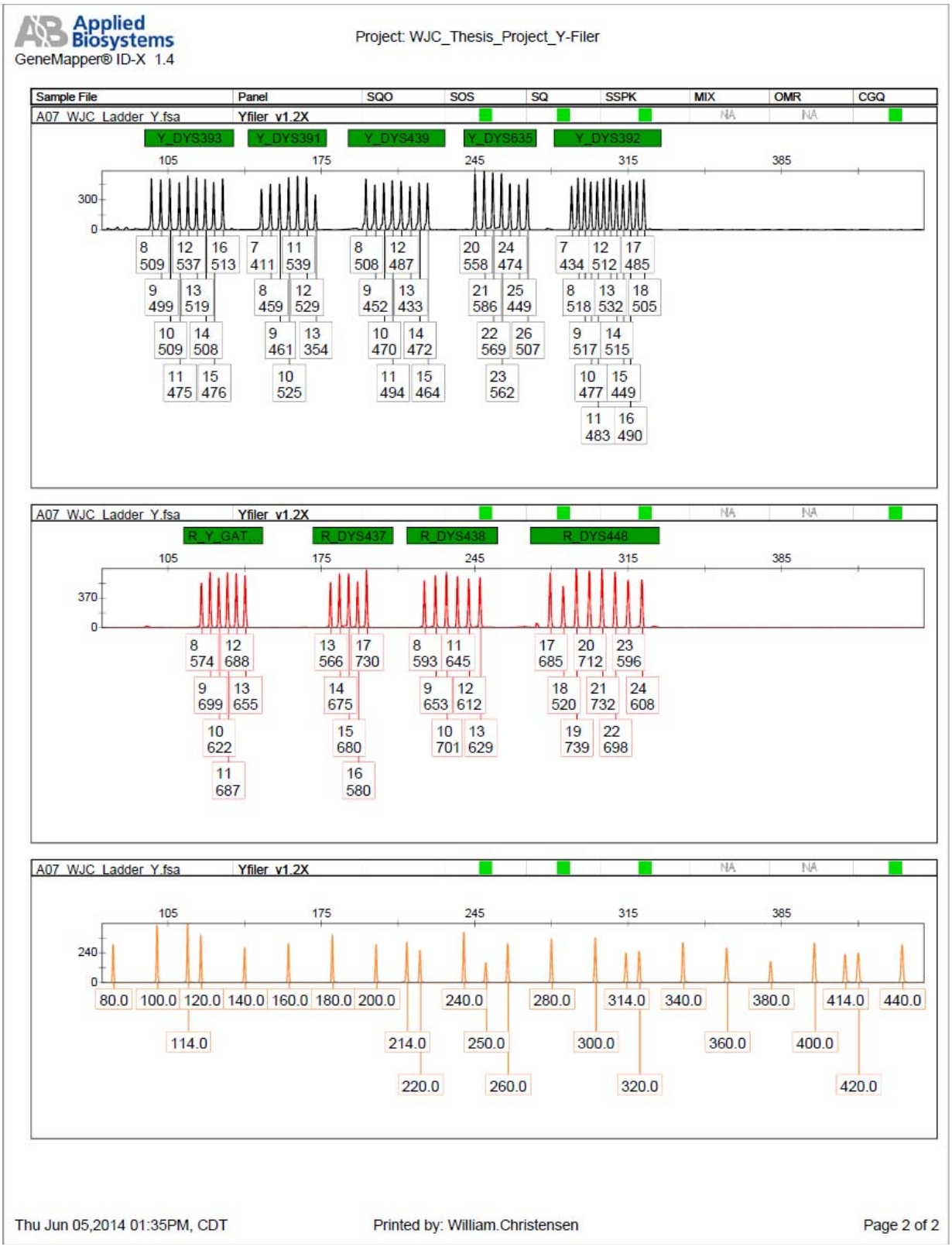


A01_WJC_Ladder.fsa	WJC_Ladder	Pig_Panels_v0



Y-Filer Ladder obtained from Analysis




Applied Biosystems
GeneMapper® ID-X 1.4
Project: WJC_Thesis_Project_Y-Filer
Sample File
Panel
SQO
SOS
SQ
SSPK
MIX
OMR
CGQ
A07_WJC_Ladder_Y.fsa
Yfiler_v1.2X
Y_DYS393
Y_DYS391
Y_DYS439
Y_DYS635
Y_DYS392
R_Y_GAT...
R_DYS437
R_DYS438
R_DYS448
105
175
245
315
385
Thu Jun 05,2014 01:35PM, CDT
Printed by: William.Christensen
Page 2 of 2

Positive Control (Animaltype Pig PCR Amplification Kit, December 2007)

Figure 7

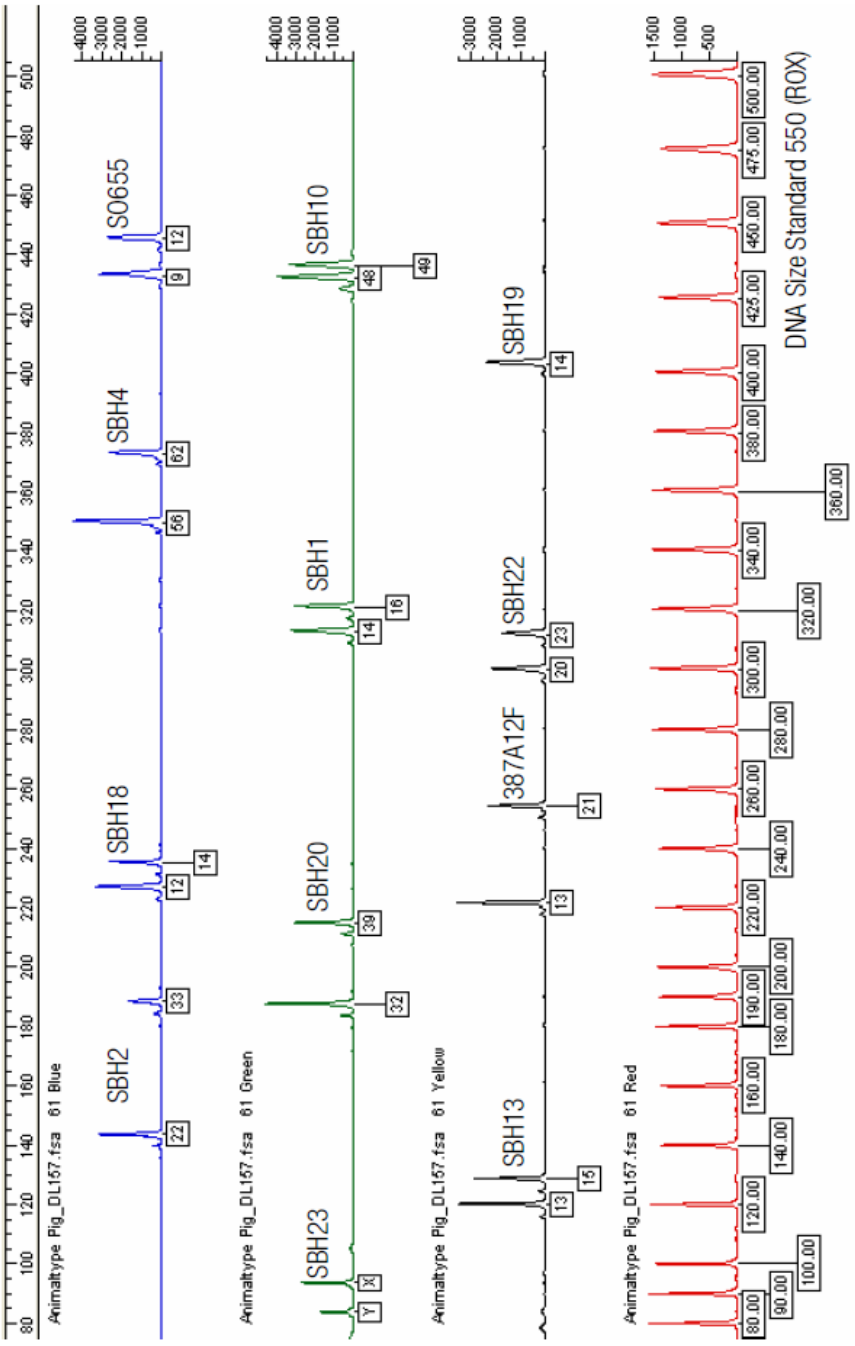


Fig. 7 Electropherogram of the Animaltype **Pig** using 2 ng Control DNA DL157. Analysis was done on an ABI PRISM® 310 Genetic Analyzer with the DNA Size Standard 550 (ROX). Allele assignment was performed using the Genotyper® Software and the Animaltype **Pig** Template File.

Pig Allelic Ladder (Animaltype Pig PCR Amplification Kit, December 2007)

26

Figure 8

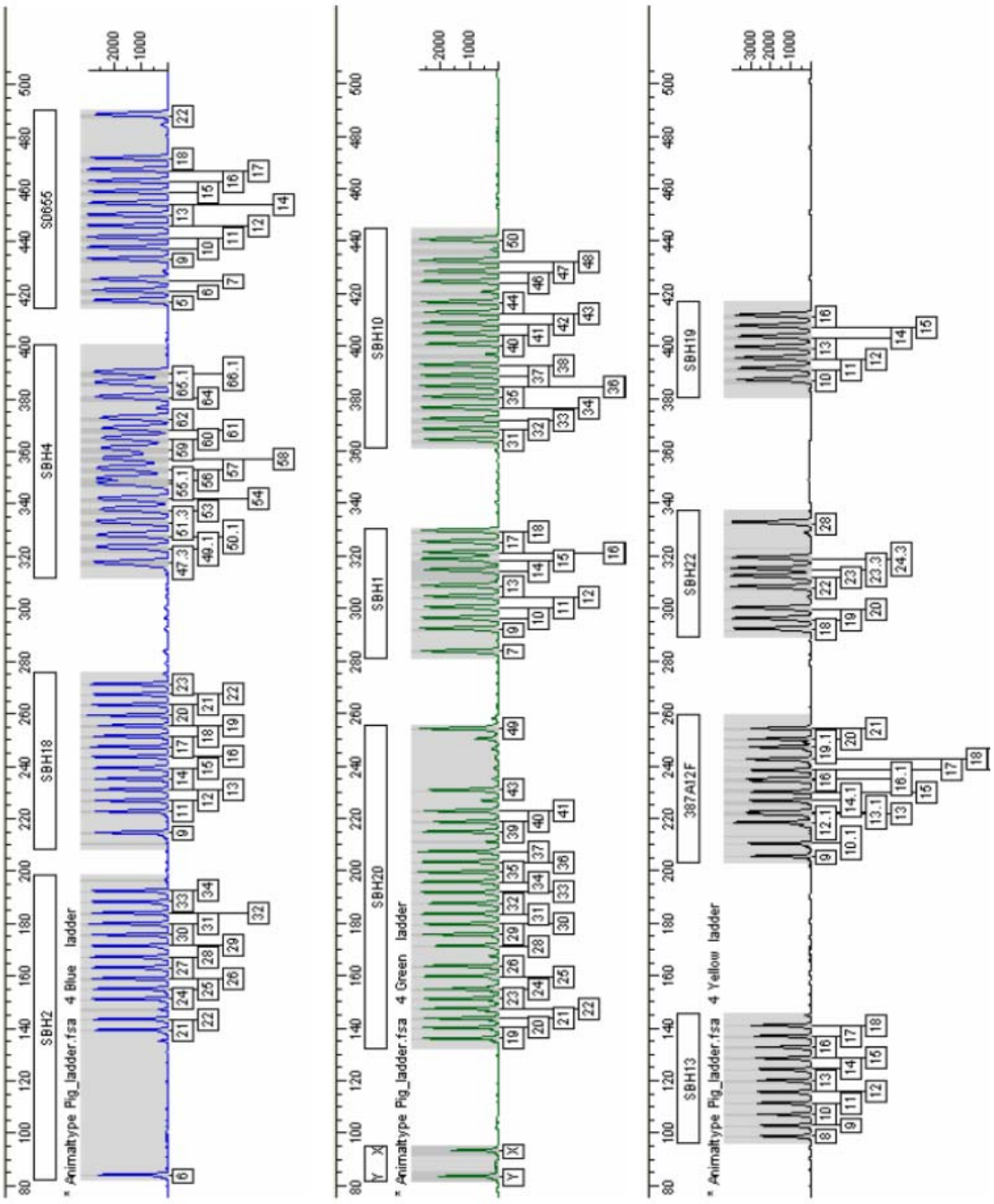


Fig. 8 Electropherogram of the Animaltype **Pig** Allelic Ladder analysed on an ABI PRISM® 310 Genetic Analyzer. Allele assignment was performed using the Genotyper® Software and the Animaltype **Pig** Template File.

Appendix O: Certificate of Analysis for Commercial Semen from LEE Biosolutions

LEE BIOSOLUTIONS
2924 MARY AVENUE, ST. LOUIS, MISSOURI, USA 63144 T: 314.968.1091 F: 314.968.9851 WWW.LEEBIO.COM

CERTIFICATE OF ANALYSIS

PRODUCT:	Semen		
SOURCE:	Single Human Donor		
CAT NO:	991-04-S	**DONOR NO:**	T4088
APPEARANCE:	Yellowish to white milky viscous liquid.		
FORM:	Raw, frozen semen.		
NOTE:	Material contains no preservatives and is not sterile.		
STORAGE:	Below -20°C		
CONSENT:	Donor Consents on File.		

Non-Infectious Disease Statement: Non-reactive for HIV-1/HCV/ HBV by NAT, HBsAg, HCV Ab, HIV 1&2 Ab, and RPR by currently approved FDA methods. However, because no test method can offer complete assurance that infectious agents are absent, this material should be handled at the Bio-safety Level 2 (BSL 2) as recommended for any potentially infectious human serum or blood specimen in the CDC/NIH manual "Bio-safety in microbiological and Biomedical Laboratories", 1999. This material is sold for in-vitro diagnostic use only in manufacturing and research. This material is not suitable for human use. We certify this to be true to the best of our knowledge. The statements herein are offered for informational purposes only and are intended to be used solely for your consideration, investigation and verification only. It is up to the user to undertake sufficient verification and testing to determine the suitability for their own particular purpose. Use of product determined by end user.

CERTIFIED BY: Richard Lagomarsino **DATE:** 9-30-2013

Appendix P: Table of Adult Flies Collected for Identification

Accession Number	Liver Type or Pig#	Location	Date Collected	Family	Genus	Species
Pilot Study						
0001	Untreated	UCO	11/5/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0002	Treated	UCO	11/5/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0003	Treated	UCO	11/7/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0004	Treated	UCO	11/7/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0005	Treated	UCO	11/7/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0006	Untreated	UCO	11/7/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0007	Untreated	UCO	11/7/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0008	Untreated	UCO	11/7/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0009	Untreated	UCO	11/7/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0010	Untreated	UCO	11/7/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0011	Untreated	UCO	11/11/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0012	Untreated	UCO	11/11/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0013	Untreated	UCO	11/11/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0014	Untreated	UCO	11/11/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0015	Untreated	UCO	11/11/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0016	Treated	UCO	11/11/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0017	Treated	UCO	11/11/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0018	Treated	UCO	11/11/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0019	Treated	UCO	11/11/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0020	Treated	UCO	11/11/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0021	Treated	UCO	11/13/13	Abdomen missing		
0022	Treated	UCO	11/13/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0023	Treated	UCO	11/13/13	Head missing		

Accession Number	Liver Type or Pig#	Location	Date Collected	Family	Genus	Species
0024	Untreated	UCO	11/13/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0025	Untreated	UCO	11/13/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0026	Untreated	UCO	11/13/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0027	Untreated	UCO	11/13/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0028	Untreated	UCO	11/13/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0029	Untreated	UCO	11/13/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0030	Untreated	UCO	11/13/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0031	Treated	UCO	11/17/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0032	Treated	UCO	11/17/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0033	Treated	UCO	11/17/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0034	Treated	UCO	11/17/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0035	Treated	UCO	11/17/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0036	Untreated	UCO	11/17/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0037	Untreated	UCO	11/17/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0038	Untreated	UCO	11/17/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0039	Untreated	UCO	11/17/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0040	Untreated	UCO	11/17/13	Head Missing		
0041	Untreated	UCO	11/20/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0042	Untreated	UCO	11/20/13	Calliphoridae	*Calliphora*	*spp.*
0043	Untreated	UCO	11/20/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0044	Untreated	UCO	11/20/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0045	Untreated	UCO	11/20/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0046	Untreated	UCO	11/20/13	Calliphoridae	*Cynompyopsis*	*cadaverina*
0047	Treated	UCO	11/20/13	Sarcophagidae	*Sarcophaga*	Spp.
0048	Treated	UCO	11/20/13	Sarcophagidae	*Sarcophaga*	Spp.

Accession Number	Liver Type or Pig#	Location	Date Collected	Family	Genus	Species
0049	Treated	UCO	11/20/13	Calliphoridae	*Calliphora*	*spp.*
0050	Untreated	UCO	11/22/13	Calliphoridae	*Calliphora*	*spp.*
0051	Untreated	UCO	11/22/13	Calliphoridae	*Calliphora*	*spp.*
0052	Untreated	UCO	11/22/13	Calliphoridae	*Calliphora*	*spp.*
0053	Treated	UCO	11/22/13	Sarcophagidae	*Sarcophaga*	spp.
0054	Treated	UCO	11/22/13	Calliphoridae	*Calliphora*	*spp.*
Pig Collections						
0055	Pig #1	Arcadia Lake	4/28/14	Calliphoridae	*Phormia*	*regina*
0056	Pig #1	Arcadia Lake	4/28/14	Calliphoridae	*Phormia*	*regina*
0057	Pig #1	Arcadia Lake	4/28/14	Calliphoridae	*Phormia*	*regina*
0058	Pig #1	Arcadia Lake	4/28/14	Calliphoridae	*Phormia*	*regina*
0059	Pig #1	Arcadia Lake	4/28/14	Calliphoridae	*Phormia*	*regina*
0060	Pig #1	Arcadia Lake	4/28/14	Calliphoridae	*Phormia*	*regina*
0061	Pig #1	Arcadia Lake	4/28/14	Calliphoridae	*Phormia*	*regina*
0062	Pig #1	Arcadia Lake	4/28/14	Calliphoridae	*Phormia*	*regina*
0063	Pig #2	Arcadia Lake	4/28/14	Sarcophagidae	*Sarcophaga*	spp.
0064	Pig #2	Arcadia Lake	4/28/14	Sarcophagidae	*Sarcophaga*	spp.
0065	Pig #2	Arcadia Lake	4/28/14	Sarcophagidae	*Sarcophaga*	spp.
0066	Pig #2	Arcadia Lake	4/28/14	Sarcophagidae	*Sarcophaga*	spp.
0067	Pig #2	Arcadia Lake	4/28/14	Sarcophagidae	*Sarcophaga*	spp.

Accession Number	Liver Type or Pig#	Location	Date Collected	Family	Genus	Species
0068	Pig #2	Arcadia Lake	4/28/14	Sarcophagidae	*Sarcophaga*	spp.
0069	Pig #2	Arcadia Lake	4/28/14	Sarcophagidae	*Sarcophaga*	spp.
0070	Pig #2	Arcadia Lake	4/28/14	Sarcophagidae	*Sarcophaga*	spp.
0071	Pig #2	Arcadia Lake	4/28/14	Sarcophagidae	*Sarcophaga*	spp.
0072	Pig #2	Arcadia Lake	4/28/14	Sarcophagidae	*Sarcophaga*	spp.
0073	Pig #2	Arcadia Lake	4/28/14	Sarcophagidae	*Sarcophaga*	spp.
0074	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0075	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0076	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0077	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0078	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0079	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0080	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0081	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0082	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0083	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0084	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0085	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*

Accession Number	Liver Type or Pig#	Location	Date Collected	Family	Genus	Species
0086	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0087	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0088	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0089	Pig #1	Arcadia Lake	5/1/14	Unable to determine		
0090	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0091	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0092	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0093	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0094	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0095	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0096	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0097	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0098	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0099	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0100	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0101	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0102	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0103	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*

Accession Number	Liver Type or Pig#	Location	Date Collected	Family	Genus	Species
0104	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0105	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0106	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0107	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0108	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0109	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0110	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0111	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0112	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0113	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0114	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0115	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0116	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0117	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0118	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0119	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0120	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0121	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*

Accession Number	Liver Type or Pig#	Location	Date Collected	Family	Genus	Species
0122	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0123	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0124	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0125	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0126	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0127	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0128	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0129	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0130	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0131	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0132	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0133	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0134	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0135	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0136	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0137	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0138	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0139	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*

Accession Number	Liver Type or Pig#	Location	Date Collected	Family	Genus	Species
0140	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0141	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0142	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0143	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0144	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0145	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0146	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0147	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0148	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0149	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0150	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0151	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0152	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0153	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0154	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0155	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0156	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0157	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*

Accession Number	Liver Type or Pig#	Location	Date Collected	Family	Genus	Species
0158	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0159	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0160	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0161	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0162	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0163	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0164	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0165	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0166	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0167	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0168	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0169	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0170	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0171	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0172	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0173	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0174	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0175	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*

Accession Number	Liver Type or Pig#	Location	Date Collected	Family	Genus	Species
0176	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0177	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0178	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0179	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0180	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0181	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0182	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0183	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0184	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0185	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0186	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0187	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0188	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0189	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0190	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0191	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0192	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0193	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*

Accession Number	Liver Type or Pig#	Location	Date Collected	Family	Genus	Species
0194	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0195	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0196	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0197	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0198	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0199	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0200	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0201	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0202	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0203	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0204	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0205	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0206	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0207	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0208	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0209	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0210	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0211	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*

Accession Number	Liver Type or Pig#	Location	Date Collected	Family	Genus	Species
0212	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0213	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0214	Pig #1	Arcadia Lake	5/1/14	Unable to determine		
0215	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0216	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0217	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0218	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0219	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0220	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0221	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0222	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0223	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0224	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0225	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0226	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0227	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0228	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0229	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*

Accession Number	Liver Type or Pig#	Location	Date Collected	Family	Genus	Species
0230	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0231	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0232	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0233	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0234	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0235	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0236	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0237	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0238	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0239	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0240	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0241	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0242	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0243	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0244	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0245	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0246	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0247	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*

Accession Number	Liver Type or Pig#	Location	Date Collected	Family	Genus	Species
0248	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0249	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0250	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0251	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0252	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0253	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0254	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0255	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0256	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0257	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0258	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0259	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0260	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0261	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0262	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0263	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0264	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0265	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*

Accession Number	Liver Type or Pig#	Location	Date Collected	Family	Genus	Species
0266	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0267	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0268	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0269	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0270	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0271	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0272	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0273	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0274	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0275	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0276	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0277	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0278	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0279	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0280	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0281	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0282	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0283	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*

Accession Number	Liver Type or Pig#	Location	Date Collected	Family	Genus	Species
0284	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0285	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0286	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0287	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0288	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0289	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0290	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0291	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0292	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0293	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0294	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0295	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0296	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0297	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0298	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0299	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0300	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0301	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*

Accession Number	Liver Type or Pig#	Location	Date Collected	Family	Genus	Species
0302	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0303	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0304	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0305	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0306	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0307	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0308	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0309	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0310	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0311	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0312	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0313	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0314	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0315	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0316	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0317	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0318	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0319	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*

Accession Number	Liver Type or Pig#	Location	Date Collected	Family	Genus	Species
0320	Pig #1	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0321	Pig #2	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0322	Pig #2	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0323	Pig #2	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0324	Pig #2	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0325	Pig #2	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0326	Pig #2	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0327	Pig #2	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0328	Pig #2	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0329	Pig #2	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0330	Pig #2	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0331	Pig #2	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0332	Pig #2	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0333	Pig #2	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0334	Pig #2	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0335	Pig #2	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0336	Pig #2	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0337	Pig #2	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*

Accession Number	Liver Type or Pig#	Location	Date Collected	Family	Genus	Species
0338	Pig #2	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0339	Pig #2	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0340	Pig #2	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0341	Pig #2	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0342	Pig #2	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0343	Pig #2	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0344	Pig #2	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0345	Pig #2	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0346	Pig #2	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0347	Pig #2	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0348	Pig #2	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0349	Pig #2	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0350	Pig #2	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0351	Pig #2	Arcadia Lake	5/1/14	Calliphoridae	*Phormia*	*regina*
0352	Pig #2	Arcadia Lake	5/1/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0353	Pig #1	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0354	Pig #1	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0355	Pig #1	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*

Accession Number	Liver Type or Pig#	Location	Date Collected	Family	Genus	Species
0356	Pig #1	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0357	Pig #1	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0358	Pig #1	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0359	Pig #1	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0360	Pig #1	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0361	Pig #1	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0362	Pig #1	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0363	Pig #1	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0364	Pig #1	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0365	Pig #1	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0366	Pig #1	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0367	Pig #1	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0368	Pig #1	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0369	Pig #1	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0370	Pig #1	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0371	Pig #1	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0372	Pig #1	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0373	Pig #1	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*

Accession Number	Liver Type or Pig#	Location	Date Collected	Family	Genus	Species
0374	Pig #1	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0375	Pig #1	Arcadia Lake	5/5/14	Unable to determine		
0376	Pig #1	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0377	Pig #1	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0378	Pig #1	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0379	Pig #1	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0380	Pig #1	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0381	Pig #1	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0382	Pig #1	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0383	Pig #1	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0384	Pig #1	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0385	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0386	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0387	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0388	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0389	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0390	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0391	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*

Accession Number	Liver Type or Pig#	Location	Date Collected	Family	Genus	Species
0392	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0393	Pig #2	Arcadia Lake	5/5/14	Unable to determine		
0394	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0395	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0396	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0397	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0398	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0399	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0400	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0401	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0402	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0403	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0404	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0405	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0406	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0407	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0408	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0409	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*

Accession Number	Liver Type or Pig#	Location	Date Collected	Family	Genus	Species
0410	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0411	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0412	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0413	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0414	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0415	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0416	Pig #2	Arcadia Lake	5/5/14	Unable to determine		
0417	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0418	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0419	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0420	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0421	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0422	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Cochiliomyia*	*macellaria*
0423	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*
0424	Pig #2	Arcadia Lake	5/5/14	Calliphoridae	*Phormia*	*regina*

Appendix Q: Test Statistic Calculations for the Animaltype Pig PCR Amplification Kit

Maggots Stored in EtOH Results 1= Alleles Called at Locus, 0= No Allele calls at Locus

	Loci												
	SBH3	SBH18	SBH4	SO655	SBH23	SBH20	SBH1	SBH10	SBH13	387A12F	SBH22	SBH19	Total Loci
4A Neat (EtOH) 5 secs	1	1	1	0	1	1	1	0	1	1	1	0	
4A Neat (EtOH) 10 secs	1	1	1	0	1	1	1	1	1	1	1	1	11
4A (1:10) 5 secs	1	0	0	0	1	1	0	0	1	1	0	0	
4A (1:10) 10 secs	1	0	0	0	1	1	0	0	1	1	0	0	
4B Neat (EtOH) 10 secs	1	0	0	0	1	0	0	0	0	0	0	0	2
4C Neat (EtOH) 10 secs	0	0	0	0	0	0	0	0	0	0	0	0	0
1A1	0	0	0	0	0	0	0	0	0	0	0	0	0
1A2	1	1	1	1	1	1	1	1	1	1	1	1	12
1A3	0	0	0	0	0	0	0	0	0	0	0	0	0
1A4	0	0	0	0	0	0	0	0	0	0	0	0	0
1A5	0	0	0	0	0	0	0	0	0	0	0	0	0
1A6	0	0	0	0	0	0	0	0	0	0	0	0	0
1A7	0	0	0	0	0	0	0	0	0	0	0	0	0
1A8	0	0	0	0	0	0	0	0	0	0	0	0	0
1A9	0	0	0	0	0	0	0	0	0	0	0	0	0
1A10	0	0	0	0	0	0	0	0	0	0	0	0	0
1A11	0	0	0	0	0	0	0	0	0	0	0	0	0
1A12	0	0	0	0	0	0	0	0	0	0	0	0	0
1A13	0	0	0	0	0	0	0	0	0	0	0	0	0
1A14	0	0	0	0	0	0	0	0	0	0	0	0	0
1A15	0	0	0	0	0	0	0	0	0	0	0	0	0
1A16 (Bad Injection)	0	0	0	0	0	0	0	0	0	0	0	0	
1A17	1	0	0	0	0	0	0	0	0	0	0	0	1
1A18	0	0	0	0	0	0	0	0	0	0	0	0	0
1A19	0	0	0	0	0	0	0	0	0	0	0	0	0
1A20	0	0	0	0	0	0	0	0	0	0	0	0	0
1A21	0	0	0	0	0	0	0	0	0	0	0	0	0
1A22	0	0	0	0	0	0	0	0	0	0	0	0	0
1A23	0	0	0	0	0	0	0	0	0	0	0	0	0
1A24	0	0	0	0	0	0	0	0	0	0	0	0	0
1A25	0	0	0	0	0	0	0	0	0	0	0	0	0
1A26	0	0	0	0	0	0	0	0	0	0	0	0	0
1A27	0	0	0	0	0	0	0	0	0	0	0	0	0
1A28	0	0	0	0	0	0	0	0	0	0	0	0	0
1A29	0	0	0	0	0	0	0	0	0	0	0	0	0
1A30	0	0	0	0	0	0	0	0	0	0	0	0	0
1A31	0	0	0	0	0	0	0	0	0	0	0	0	0
1A32	0	0	0	0	0	0	0	0	0	0	0	0	0
1A33	0	0	0	0	0	0	0	0	0	0	0	0	0
1A34	0	0	0	0	0	0	0	0	0	0	0	0	0
1A35	0	0	0	0	0	0	0	0	0	0	0	0	0
1A36	0	0	0	0	0	0	0	0	0	0	0	0	0
1A37	0	0	0	0	0	0	0	0	0	0	0	0	0
1A38	0	0	0	0	0	0	0	0	0	0	0	0	0
1A39	0	0	0	0	0	0	0	0	0	0	0	0	0
1A40	0	0	0	0	0	0	0	0	0	0	0	0	0
1A41	0	0	0	0	0	0	0	0	0	0	0	0	0
1A42	0	0	0	0	0	0	0	0	0	0	0	0	0
1A43	0	0	0	0	0	0	0	0	0	0	0	0	0
1A44	0	0	0	0	0	0	0	0	0	0	0	0	0
1A45	0	0	0	0	0	0	0	0	0	0	0	0	0

Average X	0.553191
Std Dev	2.357306
Sample Size	47
D.F.	46

H_0: $\mu \leq 0$

H_1: $\mu > 0$

Count	47
Mean	0.553191489
Std Dev	2.357306904
Std Err	0.343848426
Hyp Mean	0
α	0.05
tails	1
df	46
t stat	1.60882368
p value	0.057248748
t crit	2.012895567
sig	no

Reject H_0 if t >2.01

Do not reject if H_0 t < 2.01

Showing Calculation Well positions

H_0: The average result of extracting entire larvae, which were collected between the time they were first observed to the point of total decomposition, using this extraction meth will provide genetic information that is concordant with what the larvae consumed at one locus at a minimum with this DNA amplification kit on a 3130 genetic analyzer.

H_1: The average result of extracting the entire larvae, which were collected between the time they were first observed to the point of total decomposition, using this extraction method will not provide any genetic information at any loci that is concordant with what the larvae consumed with this DNA amplification kit on a 3130 genetic analyzer.

Conclusion = Do not reject null hypothesis. Therefore, the average result of extracting the entire larvae, which were collected between the time they were first observed to the point of total decomposition, using this extraction method will not provide any genetic information at any loci that is concordant with what the larvae consumed using this DNA amplification kit on a 3130 genetic analyzer...

Appendix R: Test Statistic Calculations for the Applied Biosystems AmpFℓSTR® Y-Filer PCR amplification kit

Maggots Stored in EtOH Results 1= Alleles Called at Locus, 0= No Allele calls at Locus

	Loci																	
	B_DYS456	B_DYS389I	B_DYS390	B_DYS389II	G_DYS458	G_DYS19	G_DYS385	Y_DYS393	Y_DYS391	Y_DYS439	Y_DYS_635	Y_DYS392	R_Y_GATA H4	R-DYS437	R-DYS438	R_DYS448	Total Loci
2A1	1	1	1	0	1	1	0	1	1	0	0	0	1	1	1	0	10
2A2	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	3
2A3	1	1	0	0	1	0	0	1	1	0	1	0	1	1	1	0	9
2A4	1	1	0	0	1	0	0	1	1	0	0	0	1	1	0	0	7
2A5	1	1	0	0	1	0	1	1	1	0	0	0	0	1	0	0	6
2A6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A7	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
2A8	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2
2A9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A11 (Bad Injection)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2A12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A16 (Bad Injection)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2A17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A19 (Bad Injection)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2A20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2A50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Average X	0.8125
Std Dev	2.312374
Sample Size	48
D.F.	47
H_0: $\mu \leq 0$	
H_1: $\mu > 0$	
Count	48
Mean	0.8125
Std Dev	2.312374206
Std Err	0.333762468
Hyp Mean	0
α	0.05
tails	1
df	47
t stat	2.434365991
p value	0.009385267
t crit	2.01174048
sig	yes
Reject H_0 if t >2.01	
Do not reject if H_0 t < 2.01	

Showing Calculation Well positions

H_0- The average result of extracting entire larvae, which were collected between the time they were first observed to the point of total decomposition, using this extraction method will provide genetic information that is concordant with what the larvae consumed at one locus at a minimum with this DNA amplification kit on a 3130 genetic analyzer.

H_0+ The average result of extracting the entire larvae, which were collected between the time they were first observed to the point of total decomposition, using this extraction method will not provide any genetic information at any loci that is concordant with what the larvae consumed with this DNA amplification kit on a 3130 genetic analyzer..

Conclusion = Reject null hypothesis. Therefore, the average result of extracting entire larvae, which were collected between the time they were first observed to the point of total decomposition, using this extraction method will provide genetic information that is concordant with what the larvae consumed at one locus at a minimum with this DNA amplification kit on a 3130 genetic analyzer.

Appendix S: Lot Numbers used during Analysis

REAGENT LOT NUMBER WORKSHEET			
Reagent	**Lot #**	**Expiration**	**ADDITIONAL NOTES**
EXTRACTION/PURIFICATION			
Qiagen Kit	42794980 145034658	N/A	GS-600: Lot# 1105043 Y-Filer Ladder: 0802006 Exp: 4/12/2010
Organic Reagents	040413	10/4/2013	
PCI	105988	N/A	
TE^{-4}	040413	10/4/2013	
QUANTITATION			
Quantifiler Human			
Standards (Human)			
Quantifiler Human Male	1207081	7/16/13	
Standards (Human Male)	030714 051514	3/21/14 5/29/14	
AMPLIFICATION & GENETIC ANALYSIS SETUP			
Pig PCR Kit	CH1200231	04/2015	
YFiler Kit	1012063	12/8/2011	
GENETIC ANALYSIS			
HiDi Formamide	120213	6/2/14	
GS-550 (With Pig PCR Kit)	CH1200231	04/2015	
Buffer	1302419	N/A	
Sterile H_2O	126204	N/A	
3130 POP-4	1111092	7/12/13	