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Comparison of Commercial DNA Extraction Kits with That of Organic Extraction Procedures Using Simulated Forensic Evidence Samples

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COMPARISON OF COMMERCIAL DNA EXTRACTION KITS WITH THAT OF ORGANIC EXTRACTION PROCEDURES USING SIMULATED FORENSIC EVIDENCE SAMPLES

by

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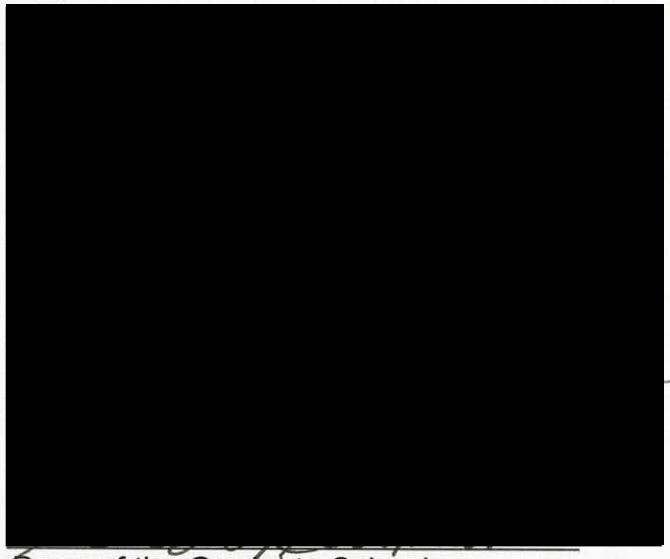

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ABSTRACT

COMPARISON OF COMMERCIAL DNA EXTRACTION KITS WITH THAT OF ORGANIC EXTRACTION PROCEDURES USING SIMULATED FORENSIC SAMPLES

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DNA analysis has become essential to the world of forensics in recent years. The success of such analysis requires effective methods for the extraction of DNA. Two straight extraction methods: the organic phenol-chloroform extraction method and the ReliaPrep™ Blood gDNA Miniprep System (Promega Corporation, Madison WI), and two differential extractions: the differential organic phenol-chloroform extraction method and a Differex extraction method (Promega Corp.), were compared and assessed to determine their effectiveness in extracting DNA from blood and semen in simulated forensic samples. Real-time PCR quantitation was used to quantify the concentration of DNA recovered from each extraction. Once the samples were quantitated, few selected samples were PCR amplified using the Identifiler human identification kit (Applied Biosystems, Foster City, CA) and analyzed using the ABI 310 genetic analyzer to assess the quality and purity of the recovered DNA from the samples. For the straight extraction both methods recovered DNA, but the commercial kit recovered more DNA and produced clean profiles with no alleleic drop outs. For the differential extractions, both

methods recovered DNA; however, the commercial kits recovered significantly more DNA than its organic counterpart in addition to producing cleaner profiles.

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CHAPTER I

INTRODUCTION

Deoxyribonucleic acid, DNA, is the genetic material located in the nucleus of cells that contains the genetic instructions used in the development and functioning of all known living organisms. DNA also contains the informational code for replicating cells and constructing needed enzymes, and therefore is known as the master copy of an organism's information code (Biggs, 2000). With this being said, it is understood that DNA forms the genetic code that determines how an organism looks and functions. The two primary purposes of DNA are to replicate for cell growth and to carry instructions for protein production, and for these reasons alone DNA can be used generally in science. DNA is replicated each time a cell divides and is passed on from generation to generation with one half of information coming from the mother and the other half coming from the father. The particulate nature of the DNA molecule is what allows for DNA fingerprinting and DNA analysis (Butler, 2005).

DNA analysis has been extremely beneficial in the field of human identification in forensic sciences. The fact that DNA is extremely stable and that it is only required in minute amounts for analysis, has essentially led to the evolution of DNA typing. DNA typing, also known as DNA fingerprinting, is used to identify individuals. This methodology was developed by Sir Alec Jeffreys in 1985, and was first used in forensic science in 1986 in a murder case (Butler, 2005). This methodology can also be used to establish paternity or other family

relationships, match organ donors with recipients, identify catastrophe victims, and help in the development of cloning applications.

Forensic DNA typing relies on polymorphisms in the genome, and in humans only about 0.1% of the genome is different from one person to another (Lewis, 2008). In order for DNA analysis to be effective, several techniques have been developed for practical application in laboratories. The protocols used today in DNA typing and analysis have been optimized so that they do not take an extensive amount of time to complete, while maximizing reliability and detection sensitivity; however, there is always room for improvement. DNA typing can be accomplished by using loci that contain tandem repetitive DNA sequences. Oftentimes this is accomplished with microsatellites or short tandem repeats. Here comparisons are made between several polymorphic markers in two people. The vast majority of DNA molecules are the same between people. Only about four million nucleotides differ between people, and this is what makes each individual unique (Butler, 2005). However, DNA variation is exhibited in the form of different alleles. There are two known types of variation and these are known as sequence polymorphism and length polymorphism.

In DNA typing, multiple markers or loci are examined. With more loci being compared, the probability of two nonrelated individuals having different genotypes increases tremendously. This also means that the probability of connecting two matching DNA profiles is increased.

DNA profiles will be constant regardless of where the sample originated. Be it saliva, semen, blood, hair, etc. Since each individual has a unique profile

with the exception of identical twins, DNA analysis has become essential in solving crimes. Profile frequencies can be accomplished by multiplying each individual locus genotype frequency together using what is known as the product rule. These calculations are made to decrease the odds of random matches in unrelated individuals.

Biological samples obtained from crime scenes as well as the blood samples used in paternity tests contain a number of substances in addition to DNA; therefore, the DNA molecules must be separated from the other extracellular materials. This separation is known as extraction, and there are several techniques used for DNA extraction in Forensic laboratories. These techniques include: the organic extraction, Chelex extraction, extraction from FTA paper, robotic extraction and extractions using commercial kits.

The extraction process has undergone significant changes throughout history. The very first DNA isolation extraction was accomplished by Friedrich Miescher in 1869, while attempting to determine the chemical composition of cells. Miescher used leucocytes to separate the nuclei of the cells from the cytoplasm where he then isolated the DNA. From here technological advances led to extractions based on density gradients and centrifugation strategies. These advances led to Meselson and Stahl demonstrating the semiconservative replication nature of DNA in 1958. In 1985, Alec Jeffreys discovered the multilocus Variable Number Tandem Repeats (VNTR) probes, and by 1988 the FBI began DNA case work.

The original process for extraction and purification of DNA in the forensic laboratory was extremely complicated, time consuming, labor intensive and had limited overall throughput (Tan & Yiap, 2009). However technological advances as well as an increase in sexually related crimes, has led to a demand for quick and efficient extraction methods. According to RAINN, in 2007, there were approximately 248,300 victims of rape, attempted rape or sexual assault. This has lead to the increase of what is known as backlogs in forensic laboratories. According to the National Institute of Justice (NIJ), backlogs can be defined as evidence that has not been processed for 30 days after it was submitted to the laboratory. Backlogs are becoming major issues in the forensic laboratory due to the needed testing at rates faster than labs can process them. Although a large volume of backlogged evidence does exist, several methods and technologies like Polymerase Chain Reaction (PCR) have been incorporated in an effort to reduce the backlog.

PCR is the scientific technique used to amplify a single or a few copies of a piece of DNA in order to generate thousands of copies of that particular DNA sequence. PCR has been a hot topic in recent years, and there has been a great deal of information published in regards to PCR applications and protocols. However, this is not the case for information available regarding processing of specimen for optimal DNA recovery before amplification. This issue is crucial for improving time management in processing, reducing backlog of cases in forensic laboratories, and obtaining optimal quantities of DNA for profiling. Real-time PCR methods are used as a method of quantitation to determine the

quantity of DNA recovered from samples and evidence. Quantitation is extremely important due to the fact that most multiplex PCR kits require between 0.5 to 2.0ng of sample to produce optimal results. Ideally, there would be an extraction procedure that is efficient and has the ability to be used with a broad range of specimen types in a small window of time for DNA analysis.

Aims and Objectives

The primary objective of this research is to compare different commercial DNA extraction kits with that of traditional organic extraction procedures involving simulated forensic evidence samples. Genomic DNA were assessed and compared to test the efficiency of the ReliaPrep™ Blood gDNA Miniprep system, provided by the Promega Corporation, with that of the phenol chloroform organic extraction, in addition to differential mixtures being assessed and compared to test the efficiency of the Differex system, also provided by the Promega Corporation, with that of the differential phenol chloroform organic extraction. These comparisons were made in an effort to determine which method provides more extracted DNA in a laboratory setting. In order to assess the sensitivity and efficiency of these methods, blood samples as well as mixed samples containing male and female cells of different concentrations were extracted using both the organic method as well as commercial kits. The commercial kits use magnetic particles to prepare clean samples for Short Tandem Repeat (STR) analysis, which is essential to the efficiency of analysis. The methods that provide the more robust results were examined in this study.

CHAPTER II

REVIEW OF LITERATURE

Organic DNA extraction method, that involves phenol, chloroform, and Isoamyl alcohol (25:24:1), has been used for the longest period of time. This method of extraction can be used for both RFLP and PCR typing technologies. Organic extraction usually involves epithelial cells, being extracted from other cellular material. Organic extractions can be separated into two categories: straight extraction and differential extraction methods.

Straight extraction is the extraction of DNA involving non- sperm cells from other cellular materials. Salt can be described as one of the most common impurities that can be found in DNA, therefore, in order to successfully separate DNA from the extracellular material several steps must take place (Tan & Yiap, 2009). The first step in the process is cell lysis, and this disrupts the cellular structure. Next is the inactivation of proteins, and lastly is separation of DNA from the other cellular material.

Extra cellular materials can be removed from DNA, since DNA is more soluble in the aqueous portion of the organic-aqueous mixture. Centrifugation of the aqueous-organic mixture leads to the unwanted proteins and cellular debris being separated from the aqueous phase. Once this is done, the double-stranded DNA molecules can be concentrated and quantitated.

Some of the advantages of using the straight extraction method include that it produces double-stranded DNA, and has high molecular weight recovery. Some of the disadvantages for this type of method includes that it is time

consuming, it uses hazardous chemicals, and the transfer between tubes increases the risk for error and contamination.

Differential extraction is the other category of organic extraction. Differential extraction refers to the process by which the DNA from two different types of cells can be separated without mixing their contents (Butler 2005). The most common application of this method is the extraction of DNA from vaginal epithelial cells and sperm cells from sexual assault cases in order to determine the DNA profiles of the victim and the perpetrator. Its success is based on knowing that sperm cells have protein disulfide bonds in their outer membrane which makes them more resistant to breaking. The primary advantage of using this method is the separation of epithelial cells from sperm cells. One disadvantage involved with using this method includes the failure to separate male and female portions will result in mixed DNA profiles. Another disadvantage is that if a person is azoospermic, or has had a vasectomy, then the absence of spermatozoa in these individuals leads to no recovery of sperm cell DNA in these individuals.

Although organic extraction techniques are extremely efficient, this method is time consuming, uses toxic chemicals, and does not always remove PCR inhibitors. Since organic extraction is not the most ideal in a laboratory setting, several commercial kits for extraction have become available in recent years (Tan & Yiap, 2009). Many of the commercial kits for extraction have been formulated in order to reduce the amount of time it takes to extract DNA while not using toxic chemicals. Some of the kits that have been created for use in the

extraction of DNA are: DNA IQ system, provided by the Promega Corporation, PrepFiler, provided by Applied Biosystems, Chelex, and FTA Paper.

The Chelex® Extraction method is one of the extraction methods that can be completed in less time than that of the organic extraction. Chelex® is composed of styrene divinylbenzene copolymers containing paired iminodiacetate ions that act as chelating groups in binding polyvalent metal ions like that of Magnesium. Some of the advantages of using the Chelex® procedure is that it is less time consuming, involves fewer steps than that of an organic extraction, the use of one tube leads to fewer opportunities for sample to sample contamination, and it removes most inhibitors of the PCR which is important when using PCR. Some of the disadvantages include that Chelex® produces single-stranded DNA that is not suitable for RFLP typing, and the addition of too much whole blood or bloodstained sample can result in PCR inhibition (Butler, 2005).

FTA Paper is yet another approach to DNA extraction. It was developed by Lee Burgoyne at Flinders University, as a method for storage of DNA. The paper is an absorbent cellulose based paper that contains four chemical substances formulated to protect DNA molecules from nuclease degradation and preserve the paper from bacterial growth (Butler, 2005).

Differex™ is a commercial extraction kit that can be used as an efficient method to separate sperm cells from epithelial cells. This kit was designed to extract sexual assault samples quickly and efficiently, resulting in a fast method for separating male and female fractions for analysis.

In addition to the many commercial kits dedicated to the extraction processes, other methods for extraction have also been developed in recent years. One of the more recent techniques for differential extraction involves research focused on the development of microfluidic devices. This method has been designed to reduce time and cost of the forensic analysis procedures, which will eventually diminish the potential for contamination or loss of samples. Through the use of microdevices, acoustic forces offer an efficient, alternative method for retaining and manipulating particles in the microfluidic system (Norris et al., 2009). Acoustic Differential Extraction, ADE, is the method involving acoustic particle trapping. The acoustic cell and particle manipulation techniques are based on forces acting on an object entering an acoustic standing wave. The forces will direct the object either to a pressure node or a pressure antinode of the wave depending on the material parameters of the object and the surrounding fluid. The ADE method utilizes noncontact acoustic forces in a valveless, microfluidic device to retain sperm cells form biological evidence found in sexual assault evidence. ADE has the potential to accommodate a large range of sample volumes, and this makes it ideal for forensic applications.

Although this method has many advantages over conventional extraction, it is not without limitations. Microdevices have high specificity, but the techniques require a complicated multilayer microchip structure that requires a number of processing steps to fabricate or narrow shallow channels at the detector (Norris

et al., 2009). This increases the risk for clogging when biological materials, like those involved in forensic analysis are used.

Applied Biosystems and Promega Corporation are two commercial companies among others that produce commercial DNA extraction kits for the extraction of genomic DNA from biological samples. The Prepfiler™ Extraction Kit (Applied Biosystems) and the DNA IQ™ Extraction Kit (Promega Corp.) are commonly used commercial kits used in forensic laboratories, and these kits were tested for their ability to recover DNA from various concentrations of whole blood.

The Prepfiler™ Automated Forensic DNA Extraction Kit is the extraction kit provided by Applied Biosystems, and it is used for the extraction of DNA from forensic samples. This kit was developed for the isolation of high quality genomic DNA from a variety of samples, and it contains the reagents that are necessary for lysis of cells, removal of PCR inhibitors, and the elution of bound DNA. It was designed to improve the overall yield, concentration, and purity of DNA isolated from routine and challenging samples. In previous tests, this kit was compared to the phenol chloroform technique as well as some other commercial kits, and the DNA yields for all sample types were either equal to or better than the other commercial kits used in the tests (Prepfiler, 2008). Research has shown that in addition to not containing harmful chemicals, this kit can deliver higher concentrations and higher total DNA yields. This kit also has shown consistent levels of performance between test sites, which demonstrates robustness of performance in different environments.

The DNA IQ™ System is one of the extraction kits supplied by Promega Corporation, and is designed for the isolation of biological samples in the forensic realm as well. It uses a silica-based paramagnetic resin to isolate all DNA from liquid samples and samples on solid supports. It is also used to extract DNA from liquid samples or stains. This kit contains a unique resin that is designed to eliminate PCR inhibitors and purify the contaminant that is frequently encountered in the forensic samples. According to Promega Corporation, sample size is not a problem because as sample size decreases this system becomes more efficient.

MagneSil ® Blood Genomic, Max Yield System is another kit produced by the Promega Corporation, and it is designed for the purification of DNA from 200µl of whole blood and is normally used in conjunction with automated purification protocols on robotic workstations. This system provides purified DNA that can be used in STR and PCR applications as well as multiplexed PCR.

ReliaPrep™ Blood gDNA Miniprep System is also produced by Promega Corporation and it provides a simplified technique for the preparation of purified and intact DNA from mammalian blood. The samples are processed using a binding column in a microcentrifuge tube. Up to 200µl of blood can be processed using this type of extraction per purification. The genomic DNA isolated is considered high-quality, and can be used in common applications such as agarose gel analysis, restriction enzyme digestion, and PCR analysis. The ReliaPrep system consists of four simple steps. 1. To effectively disrupt or

homogenize the starting material to release the DNA; 2. Binding the DNA to the ReliaPrep™ Binding Column; 3. Removing impurities with wash solution, and 4. Eluting the purified DNA. There is no ethanol used in the purification protocol which eliminates the downstream problems caused by ethanol carryover.

Since extractions are necessary in forensic analysis, the commercial kits used in laboratories need to be practical as well as efficient. When choosing an extraction kit to use, there are several factors that should be addressed. Some of these factors include: suitability for sample types, if the samples to be used are multiple or not, is the kit effective in the removal of PCR inhibitors, and if there will be good DNA recovery for smaller sample size.

CHAPTER III

MATERIALS AND METHODS

The Institutional Review Board of Human Subjects Protection Review Committee approval was obtained for using the human blood samples in this research. Anonymous blood samples were provided from Forrest General Hospital Clinical Laboratory, and the semen samples used in this research were provided from within the laboratory.

Sample Preparation for Straight Extractions

Liquid blood samples at various quantities ranging in the following amounts: 0.1µl, 0.2µl, 0.3µl, 0.5µl, 1.0µl, 2.0µl, 3.0µl, 5.0µl. 7.0µl and 10.0µl were pipeted onto filter paper and allowed to dry. The samples were dried at room temperature and stored at 4°C until extraction. Each of these stains was extracted separately using the straight organic extraction method. For the ReliaPrep™ Blood gDNA Miniprep system a 1:10 dilution was made for sample volumes less than 2µl. After the dilution was made, 1µl of the dilution was added to 199µl diH_2O for the 0.1µl sample volume, 2µl of the dilution was added to 198µl diH_2O for the 0.2µl sample volume, 3µl of the dilution was added to 197µl diH_2O for the 0.3µl sample volume, 5µl of the dilution was added to 195µl diH_2O for the 0.5µl sample volume, and 10µl of the dilution was added to 190µl diH_2O for the 1.0µl sample volume. For quantities ranging from 2 to 10µl, appropriate quantities of neat blood were added to diH_2O for a total volume of 200µl.

Sample Preparation for Differential Extractions

1:10 dilution of a semen sample was made and 1, 2, 3, 5 and 10 µl were deposited onto cotton swatches that already contained 5 µl of a female blood sample. These semen quantities represent 0.1, 0.2, 0.3, 0.5, and 1µl of neat semen sample respectively. Neat semen samples of 2, 3, 5, 7, and 10µl were added to another set of cotton swatches that already contained 5µl of female whole blood. Once the samples were prepared, they were allowed to dry for 30 – 60 minutes and then stored at 4°C until needed for extraction.

Part I: Sample Preparation for straight extraction techniques:

1. Whole blood in the following amounts were pipeted onto filter paper.

2. Pipette 0.1µl, 0.2µl, 0.3µl, 0.5µl, 1.0µl, 2.0µl, 3.0µl, 5.0µl and 10.0µl of whole blood in two sets of filter paper and allow blood to dry.

3. All samples were stored at 4°C until analysis.

Organic extraction: (Budowle, 2000)

1. Place stained material into micro centrifuge tube.

2. To the sample, add 400µl of extraction buffer and 10µl ProteinaseK, and vortex on low speed for 1sec. and centrifuge in the microcentrifuge for 2sec.

3. Incubate the tube at 56° C overnight.

4. Centrifuge in a microcentrifuge for 2 sec. to force the liquid to the bottom of the tube.

5. Using a wooden applicator stick, transfer the stained material into a Spin-X basket insert. Place the basket insert into the tube containing the stain extract. Spin in a microcentrifuge for 5 min at maximum speed.

6. Remove and discard basket insert, and add 500µl Phenol/Chloroform/ Isoamyl alcohol to the sample. Vortex (low speed) the mixture briefly to attain a milky emulsion. Spin the tube in a microcentrifuge for 5 min.

7. Assemble and label a Microcon 50 unit for each sample.

8. Transfer the aqueous phase from the tube in step 6 to the concentrator. Avoid pipeting organic solvent from the tube into the concentrator.

9. Place a cap on the concentrator and centrifuge in a microcentrifuge at 14,000 Xg (or 10,000 rpm) for 10 minutes.

10. Carefully remove the concentrator unit from the assembly and discard the fluid from the filtrate cap. Return the concentrator to the top of the unit assembly.

11. Add 500µl TE buffer to the concentrator. Replace the cap and centrifuge the assembly in a microcentrifuge at 14000Xg for 10 min. Repeat washings 3 times.

12. Remove the concentrator from the unit assembly and carefully invert the concentrator cup into a labeled sample collection tube. Discard the filtrate cup.

13. Centrifuge the assembly in a microcentrifuge at 5000 rpm for 3 min.

14. Discard the concentrator. Cap the collection tube.

15. Estimate the quantity of DNA in the sample by Agarose gel and by real time PCR system.

16. Store samples at 4°C or frozen.

ReliaPrep™ Blood gDNA Miniprep System Protocol

1. Thoroughly mix the blood sample for at least 10 minutes in a rotisserie shaker at room temperature. If blood is frozen, thaw completely before mixing for 10 minutes.

2. Dispense 20µl of Proteinase K Solution into a 1.5ml microcentrifuge tube.

3. Add 200µl of blood (or dilutions made) to the tube containing the Proteinase K solution, and briefly mix.

4. Add 200µl of Cell Lysis Buffer to the tube. Cap and mix by vortexing for at least 10 seconds.

5. Incubate at 56°C for 10 minutes.

6. While the blood samples are incubating, place a ReliaPrep™ Binding Column into an empty Collection Tube.

7. Remove the tube from the heating block. Add 250µl of Binding Buffer, cap the tube, and mix by vortexing for 10 seconds with a vortex mixer.

8. Add the contents of the tube to the ReliaPrep™ Binding Column, cap it, and place in a microcentrifuge.

9. Centrifuge for 1 minute at maximum speed. Check the binding column to make sure the lysate has completely passed through the membrane. If lysate is still visible on top of the membrane, centrifuge the column for another minute.

10. Remove the collection tube containing flowthrough, and discard the liquid as hazardous waste.

11. Place the binding column into a fresh collection tube. Add 500µl of Column Wash Solution to the column, and centrifuge for 3 minutes at maximum speed. Discard the flowthrough.

12. Repeat step 11 twice for a total of three washes.

13. Place the column in a clean 1.5ml microcentrifuge tube.

14. Add 50-100µl of Nuclease-Free Water to the column. Centrifuge for 1 minute at maximum speed.

15. Discard the ReliaPrep™ Binding Column, and save the eluate. Do not reuse binding columns or collection tubes.

Preparation of Differential Stained Samples

1. 1:10 dilution of semen stains were used for the 0.1, 0.2., 0.3, 0.5, and 1.0µl sample volumes. Neat semen were pipeted to filter paper for the 2, 3, 5, 7, and 10µl sample volumes.

2. Pipette 5µl of whole blood onto the filter paper and dry for approximately 15 minutes.

3. Pipette either the dilute or neat semen sample as in step 1 onto the filter paper that contain dried blood and allow to air dry for approximately 15 minutes

4. Package samples and store at 4°C.

Organic Extraction of DNA from Differential Stains

1. Using a clean surface for each swab, dissect the stained material from the applicator stick and place it into a 2.0 mL microcentrifuge tube.

2. To the sample, add 400µl TNE, 25µl 20% sarkosyl, 75µl H_2O and 5µl proteinase K. Vortex and quick spin mix for 1second to force the material into the extraction solution.

3. Incubate at 37°C for 2hrs.

4. Using a wooden applicator stick, transfer the swab material into a Spin-basket insert, and place the basket insert into the tube containing the stain extract. Centrifuge in a microcentrifuge tube at maximum speed for 5 minutes.

5. Remove basket insert from the extract tube. Remove the material from the basket and place in a clean microcentrifuge tube. (Freeze if storage is required or discard the swab material.)

6. While being careful not to disturb any pellet, remove the supernatant fluid from the extract and place in a new labeled tube. This supernatant in the female (E. cell) fraction. Analysis of the female fraction resumes at step 11.

7. Wash the cell pellet by resuspending it in 1.5ml TNE, vortex-mixing the suspension briefly, and centrifuging the tube in a microcentrifuge at maximum speed for 5 minutes. Remove and discard the supernatant, being careful not to disturb the pellet.

8. Repeat the wash step two additional times for a total of 3 washes of the cell pellet.

9. To the tube containing the washed pellet, add 150µl TNE, 50µl 20% sarkosyl, 40 µl 0.39M DTT, 150µl water, and 10µl proteinase K. Close the tube caps and vortex-mix for 1sec and centrifuge in a microcentrifuge for 2sec. to force all of the fluid and material to the bottom of the tubes.

10. Incubate at 37°C for 2 hours.

11. To the tube containing the pellet and the tube containing the female fraction, add 400µl Phenol/Chloroform/Isoamylalcohol (PCIA). Vortex-mix at low speed briefly to attain a milky emulsion. Centrifuge the tube in a microcentrifuge for 5 minutes.

12. Assemble a Microcon 50 unit. Transfer the aqueous phase from the tube in step 11 to the top of the concentrator. Avoid pipetting organic solvent form the tube into the concentrator.

13. Place a cap on the concentrator and centrifuge in a microcentrifuge at 14000xg for 10 minutes.

14. Carefully remove the concentrator unit form the assembly and discard the filtrate fluid from the filtrate cap. Return the concentrator to the top of the filtrate cup.

15. Add 400µl TE buffer to the concentrator. Replace the cap and centrifuge the assembly in a microcentrifuge at 14000xg for 10 min. Discard waste.

16. Repeat washing with TE buffer 2 more times. Remove the concentrator form the filtrate cup and carefully invert the concentrator onto a labeled retentate cup. Discard filtrate cup.

17. Centrifuge the assembly in a microcentrifuge at 5000 rpm for 5 min.

18. Discard the concentrator. Cap the retentate cup that contain the DNA.

19. The samples can then be quantitated using the Quantifiler Human DNA Quantification Kit.

20. After the samples were quantitated, the samples were amplified using the AmpFISTR Identifiler PCR Amplification Kit to determine the quality and purity of the DNA.

The Stepwise Protocol for the Differex Extraction Kit

Preparation of Digestion Solution

1. Add proteinase K to the digestion Buffer to a final concentration of 270µg/ml to prepare the digestion solution. Each sample requires 0.4ml of digestion solution. This solution is not to be reused once the proteinase K has been added.

2. Mix and use immediately.

Differential Extraction Protocol

1. Place the solid support containing sperm in a microcentrifuge tube.

2. Add 0.4ml of yellow digestion solution to the sample.

3. Close the cap and vortex at high speed for 30 seconds. Be sure to keep the tube vertical while vortexing. Place the tube at 56°C for 90 minutes.

4. For each sample processed in Step 3, place a DNA IQ spin basket into a new labeled 1.5ml microtube.

5. After the proteinase K digestion, remove the solid support from the digestion solution and place it in the spin basket prepared in step 4. Slowly pipet the remaining digestion solution into the spin basket. Some of the solution may flow through the spin basket.

6. Close the cap on the spin basket. Mark the tube where you expect the sperm pellet to form. Centrifuge for 10 minutes at 14,000rpm in a

microcentrifuge at room temperature. The tube may contain a small slightly yellow or white pellet of sperm and a yellow aqueous layer containing the epithelial DNA in digestion solution.

Substrate Removal

1. Remove and discard the spin basket. Remove any yellow digestion solution from the tube cap. To avoid carryover of epithelial DNA into the sperm fraction, be sure that no liquid remains in the cap after centrifugation.

2. Vortex the stock DNA IQ Resin bottle for 10 seconds at high speed. Add 3.5 µl of DNA IQ resin near the bottom of the tube on the side opposite the sperm pellet. Place the tube into the Differex Magnet, being careful to align the pellet with the magnet to allow the resin to cover the pellet. Leave the tube in position for steps 3 and 4

3. Remove and reserve as much of the yellow aqueous layer as possible for epithelial DNA purification

4. Wash the resin-capped pellet by slowly adding 500µl Nuclease-Free Water, being careful to rinse the tube walls of any residual yellow digestion solution. Remove and discard the water. Repeat this wash step with another 500µl of Nuclease-Free Water.

5. Add a third wash volume of 500µl Nuclease-Free Water to the sample tube, and vortex sample briefly to resuspend resin particles.

6. Replace tubes into microcentrifuge, and centrifuge for 10 minutes at maximum speed at room temperature.

7. Vortex the stock DNA IQ Resin bottle for 10 seconds at high speed or until resin is thoroughly mixed. Following centrifugation, immediately add 7µl of DNA Resin near the bottom of the tube opposite the new pellet. This pellet will be in a different position than the pellet previously formed, and will also contain the DNA IQ resin. Place spin tubes into DIfferex Magnet (position 2), be careful to align sperm/resin pellet with magnet, and allow the additional resin to cover the pellet with magnet, Leave the tube in position 2 of the Differex Magnet for steps 8 and 9.

8. Remove wash solution, and add a fourth 500µl wash solution.

9. Slowly add 100µl of separation solution to the side of the tube, and let it settle beneath the aqueous layer so that it covers the resin pellet. Be careful not to disturb the pellet.

10. Remove wash solution and as much separation solution as possible without disrupting the pellet. Reserve contents of tube as the sperm fraction.

11. Add 100µl TNE to the sample vortex for 10 seconds, and replace tube back in magnet position 2 to separate the magnetic particles from the sperm cells.

12. To extract DNA from the sperm fraction, add 250µl of DNA IQ lysis buffer containing DTT. The separation solution will completely dissolve in the lysis buffer, and the sperm will lyse.

13. Dispense 20µl of Proteinase K Solution into the 1.5ml microcentrifuge tube containing the sperm fraction, and vortex for 10 seconds.

14. Incubate at 56°C for 10 minutes.

15. While the samples are incubating, place a ReliaPrep™ Binding column into an empty collection tube.

16. Remove the tube from the heating block. Add 250µl of Binding Buffer, cap the tube, and mix by vortexing for 10 seconds with a vortex mixer.

17. Add the contents of the tube to the ReliaPrep™ binding column, cap it, and place in a microcentrifuge.

18. Centrifuge for 1 minute at maximum speed. Check the binding column to make sure the lysate has completely passed through the membrane. If lysate is still visible on top of the membrane, centrifuge the column for another minute.

19. Remove the collection tube containing flowthrough, and discard the liquid as hazardous waste.

20. Place the binding column into a fresh collection tube. Add 500µl of Column wash solution to the column, and centrifuge for 3 minutes at maximum speed. Discard the flowthrough.

21. Repeat step 20 twice for a total of three washes.

22. Place the column in a clean 1.5ml microcentrifuge tube.

23. Add 50-100µl of Nuclease-free water to the column. Centrifuge for 1 minute at maximum speed.

24. Discard the ReliaPrep™ binding column, and save the eluate. Do not reuse binding columns or collection tubes.

DNA Quantification and Amplification

All the extracted DNA samples were quantitated using the Quantifiler® Human Quantitation Kit according to manufacturer's guidelines (Applied Biosystems,

Foster City, CA) to assess the total DNA quantity recovered from each extraction.

After the samples have been quantitated, a small aliquot of chosen samples were amplified using the AmpSTR® Identifiler® PCR Amplification Kit (Applied Biosystems) according to manufacturer's recommendations. Table 1 describes the thermal cycling conditions for amplifying the 15 autosomal Short Tandem Repeat (STR) markers. All the Amplicons were run on an automated ABI 310 genetic analyzer and analyzed using genemapper ID software. All experiments were performed in duplicates to ensure accuracy of results.

Table 1

Thermal Cycler Conditions for DNA Amplification Using the AmpSTR® Identifiler® PCR Amplification Kit

Initial Incubation Step	28 Cycles			Final Extension	Final Step
Hold	Denature	Anneal	Extend	Hold	Hold
95°C	94°C	59°C	72°C	60°C	4°C
11 min	1 min	1 min	1 min	60 min	∞

CHAPTER IV

ANALYSIS OF DATA

For each method evaluated, specific protocols for each sample was followed. In order to assess which method provides the most robust results, each of the samples and duplicates were extracted using the same basic methods to ensure the validity of the comparisons. Sample volumes used for digestion, volume of DNA recovered from the extraction, and total quantity of DNA recovered is listed in tables 2-5.

To assess the different DNA extraction methods, 2µl of the recovered DNA was run in a 1% agarose gel for comparison.

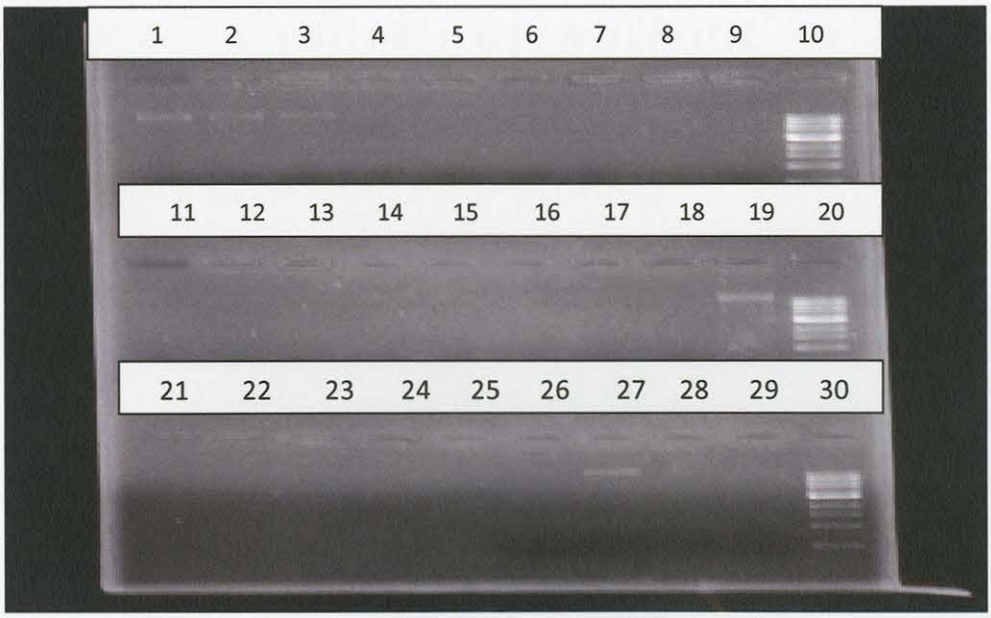

Figure 1. 1% agarose gel that contains 2µl of recovered DNA samples from the straight organic extraction. Samples are as follows: 1-R7, 2-R5, 3-R3, 4-R2, 5-R1, 6-R0.5, 7-R0.3, 8-R0.2, 9-R0.1, 10-1kb ladder, 11-S5, 12-S3, 13-S2, 14-S1, 15-S0.5, 16-S0.3, 17-S0.2, 18-S0.1, 19-R10, 20-1kbladder. 21-26-no samples; 27-S10, 28-S7, 29-S5, 30-1kbladder. (Key-R=reliaprep; S=straight organic, and the number following R or S is the actual volume of blood used for extraction)

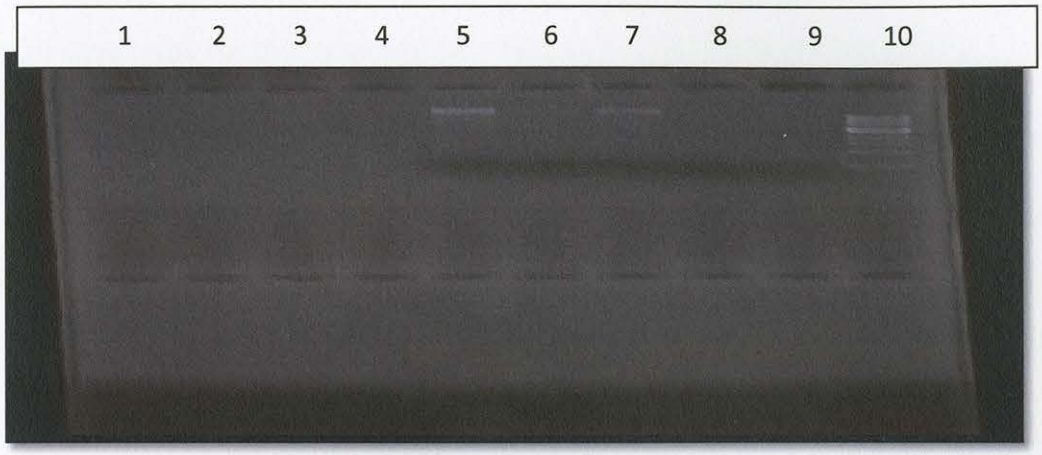

Figure 2. 1% agarose gel containing 2µl of recovered DNA samples from the organic differential extraction. Samples are as follows: 5-OD10, 6-OD7, 7-OD5, 8-OD2, 9-OD-1, 10-1kb ladder. (Key: OD= organic differential extraction, and the number following OD is the amount of semen used for extraction)

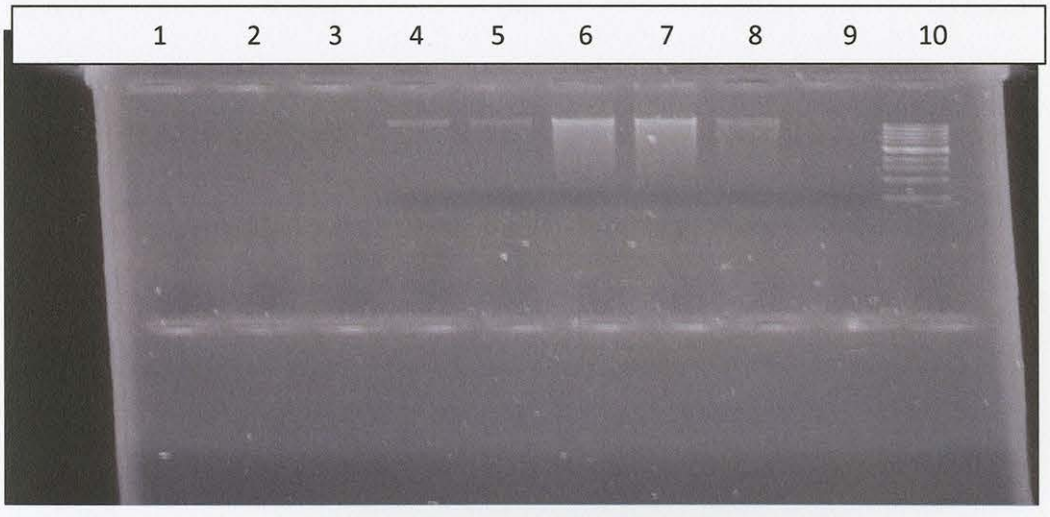

Figure 3. 1% agarose gel containing 2µl of recovered DNA sample from the Differex/ReliaPrep differential extraction. Samples are as follows: lanes 1-3 no samples; 4-DR10, 5-DR7, 6-DR5, 7-DR3, 8-DR2, 9DR1, 10-1kb ladder. (Key: DR=Differex kit and the number following differex is the amount of semen used for extraction)

Once the presence of DNA was confirmed using agarose gels, the DNA samples were evaluated for concentration and purity using more specific quantitation methods. For this, the Quantifiler® Human DNA Quantification Kit

(Applied Biosystems) was used. Two microliters of recovered DNA from each sample was used for this quantitation. Tables 2 and 3 contain the DNA quantitation data for the straight extraction method as well as the Reliaprep gDNA extraction system.

Table 2

DNA Recovery Data for the Different Sample Volumes Using Straight Extraction Method

Sample Name	Blood used for extraction (µl)	DNA vol recovered (µl)	DNA Concentration (ng/µl)	Total DNA Recovered (ng)
1DS0.1	0.1	35	0.0617	2.1595
2DS0.1	0.1	36	0.0841	3.0276
1S0.2	0.2	30	0.0984	2.952
2S0.2	0.2	32	0.206	6.592
1S0.3	0.3	33	0.135	4.455
2S0.3	0.3	36	0.22	7.92
1DS0.5	0.5	36	0.49	17.64
2DS0.5	0.5	30	0.393	11.79
1DS1.0	1	37	0.801	29.637
2DS1.0	1	37	0.581	21.497
1DS2.0	2	36	0.79	28.44
2DS2.0	2	35	0.781	27.335
1DS3.0	3	38	1.21	45.98
2DS3.0	3	35	1.43	50.05

Table 2 (continued).

Sample Name	Blood Used for Extraction (µl)	DNA Vol Recovered (µl)	DNA Concentration (ng/µl)	Total DNA Recovered (ng)
1DS5.0	5	35	2.75	96.25
2DS5.0	5	35	2.66	93.1
1DS7.0	7	37	2.83	104.71
2DS7.0	7	38	4.16	158.08
1DS10.0	10	40	4.51	180.4
2DS10.0	10	39	4.68	182.52

Table 3

DNA Recovery Data for the Reliaprep™Blood Gdna Miniprep System

Sample Name	Blood Used for Extraction (µl)	DNA Vol Recovered (µl)	DNA Concentration (ng/µl)	Total DNA Recovered (ng)
1R 0.1	0.1	100	0.017	1.7
2R 0.1	0.1	100	0.012	1.2
1R 0.2	0.2	100	0.012	1.2
2R 0.2	0.2	100	0.053	5.3
1R 0.3	0.3	100	0.049	4.9
2R 0.3	0.3	100	0.033	3.3
1R 0.5	0.5	100	0.097	9.7

Table 3 (continued).

Sample Name	Blood Used for Extraction (µl)	DNA Vol Recovered (µl)	DNA Concentration (ng/µl)	Total DNA Recovered (ng)
2R 0.5	0.5	100	0.023	2.3
1R 1.0	1	100	0.163	16.6
2R 1.0	1	100	0.101	10.1
1R 2.0	2	100	0.247	24.7
2R 2.0	2	100	0.952	95.2
1R 3.0	3	100	0.946	94.6
2R 3.0	3	100	1.04	104
1R 5.0	5	100	2.97	297
2R 5.0	5	100	1.55	155
1R 7.0	7	100	0.969	96.9
2R 7.0	7	100	2.15	215
1R 10.0	10	100	3.19	319
2R 10.0	10	100	3.34	334

The amount of DNA recovered using the organic differential extraction method, and the differex/reliaprep differential extraction system are given in tables 4 and 5. For clarity, only the sperm fraction DNA recovery is given.

Table 4

Sperm Fraction DNA Recovery Data for the Organic Differential Extraction Method

Sample Name	Initial Sperm Sample Used (µl)	DNA Vol Recovered (µl)	DNA Concentration (ng/µl)	Total DNA Recovered (Sperm Fraction) (ng)
1OD0.1	0.1	18	0.06	1.08
2OD0.1	0.1	40	0.07	2.8
1OD0.2	0.2	28	.05	1.4
2OD0.2	0.2	38	0.11	4.18
1OD0.3	0.3	23	0.05	1.15
2OD0.3	0.3	41	0.15	6.15
1OD0.5	0.5	24	0.21	5.04
2OD0.5	0.5	41	0.23	9.43
1OD1.0	1	21	0.14	2.94
2OD1.0	1	41	0.36	14.76
1OD2.0	2	13	0.3	7.5
20D2.0	2	39	0.68	26.52
1OD3.0	3	25	0.58	14.5
2OD3.0	3	45	2.47	111.15
1OD5.0	5	28	2.53	70.84
2OD5.0	5	43	3.28	141.04
1OD7.0	7	21	4.09	85.89
2OD7.0	7	42	4.41	185.22

Table 4 (continued).

Sample Name	Initial Sperm Sample Used (µl)	DNA Vol Recovered (µl)	DNA Concentration (ng/µl)	Total DNA Recovered (Sperm Fraction) (ng)
1OD10.0	10	26	5.65	146.9
2OD10.0	10	35	9.69	339.15

Table 5

Sperm Fraction DNA Recovery Data for the Differex/ Reliaprep Extraction Method

Sample Name	Initial Sperm Sample Used (µl)	DNA Vol Recovered (µl)	DNA Concentration (ng/µl)	Total DNA Recovered (Sperm Fraction) (ng)
1DR 0.1	0.1	100	0.022	2.2
2DR 0.1	0.1	100	0.019	1.9
1DR 0.2	0.2	100	0.029	2.9
2DR 0.2	0.2	100	0.076	7.6
1DR 0.3	0.3	100	0.132	13.2
2DR 0.3	0.3	100	0.067	6.7
1DR 0.5	0.5	100	0.187	18.7
2DR 0.5	0.5	100	0.06	6
1DR 1.0	1	100	0.614	61.4

Table 5 (continued).

Sample Name	Initial Sperm Sample Used (µl)	DNA Vol Recovered (µl)	DNA Concentration (ng/µl)	Total DNA Recovered (Sperm Fraction) (ng)
2DR 1.0	1	100	0.971	97.1
1DR 2.0	2	100	2.64	264
2DR 2.0	2	100	0.596	59.6
1DR 3.0	3	100	1.64	164
2DR 3.0	3	100	2.32	232
1DR 5.0	5	100	1.75	175
2DR 5.0	5	100	2.25	2.25
1DR 7.0	7	100	2.34	234
2DR 7.0	7	100	2.30	230
1DR 10.0	10	100	3.33	333
2DR 10.0	10	100	4.94	494

Table 6 shows the comparison of average DNA recovery using the organic extraction method and the ReliaPrep kit method and table 7 shows the comparison of average DNA recovery using the organic differential extraction method and the Differex/ReliaPrep kit extraction methods.

Table 6

Average DNA Recovery for the Straight Extractions Using Both Organic Extraction and the Reliaprep Extraction Kit

Vol. of Blood for Extraction	Total DNA Recovered (Organic Extraction.)	Total DNA Recovered (ReliaPrep Extraction)
0.1µl	2.59	1.45
0.2µl	4.77	3.25
0.3µl	6.18	4.1
0.5µl	14.72	6
1.0µl	25.42	13.35
2.0µl	27.89	59.95
3.0µl	48.02	99.3
5.0µl	94.68	187.5
7.0µl	131.4	226
10.0µl	181.46	326

Table 7

Average Recovery of DNA for the Differential Extractions Using Both the Organic Differential Extraction Method and the Differex Differential Extraction System

Vol. of Semen Used	Total DNA Recovered (Differential Extraction-organic)	Total DNA Recovered (Differex/Reliaprep)
0.1µl	1.94	2.05
0.2µl	2.79	5.25
0.3µl	3.65	9.95
0.5µl	7.24	12.7
1.0µl	8.85	79.4
2.0µl	17.01	176.8
3.0µl	62.83	198
5.0µl	105.94	200
7.0µl	135.56	232
10.0µl	243.01	413.5

The average amount of DNA recovered from the blood samples extracted using organic extraction and the ReliaPrep system are shown in figures 4 and 5.

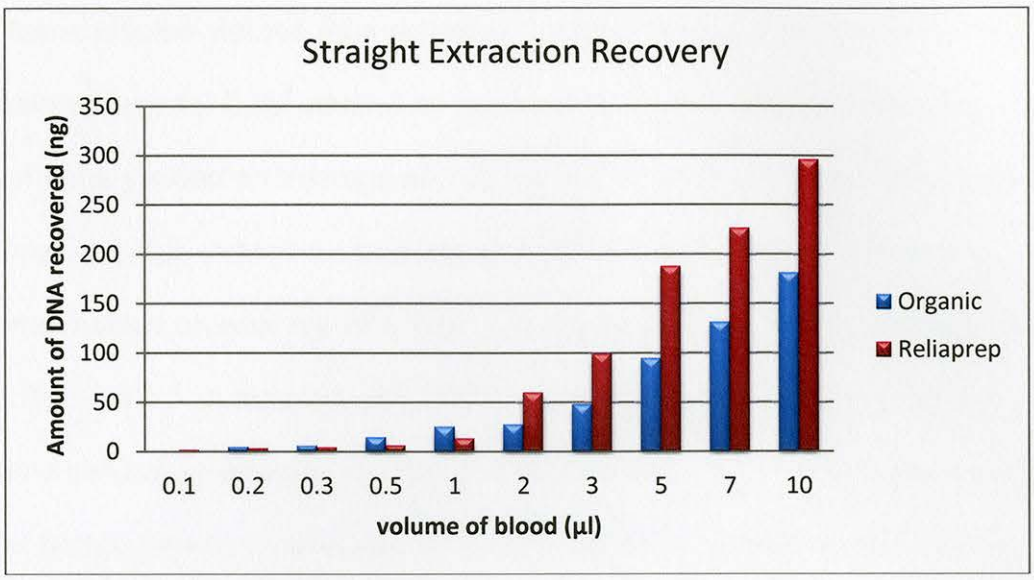

Figure 4. Histogram Depicting the amount of DNA recovered from organic extraction and the ReliaPrep system.

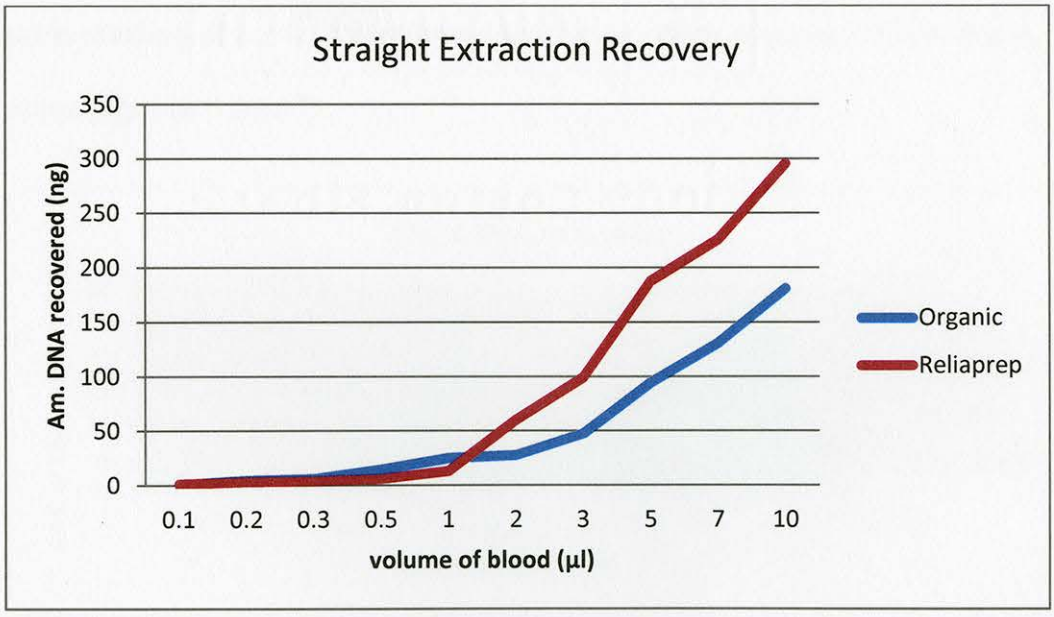

Figure 5. Line Graph displaying the relationship of DNA recovery and the amount of blood used for DNA extraction.

Straight Extractions

The amount of DNA recovered from straight organic extraction for blood sample volume of 0.1µl yielded an average of 2.59ng, while the ReliaPrep for

the same volume yielded an average of 1.45ng. The organic extraction for sample volume for 0.2µl yielded an average of 4.77ng, while the ReliaPrep for this volume yielded an average of 3.25ng. The organic extraction for sample volume for 0.3µl, yielded an average of 6.18ng, and the ReliaPrep for this volume yielded an average of 4.1ng. The organic extraction for sample volume for 0.5µl yielded an average of 14.72ng, while the ReliaPrep for the same volume yielded an average of 6ng. The organic extraction for sample volume of 1µl of blood yielded an average for 25.42ng while the ReliaPrep yielded an average of 13.35ng. Both methods of extraction yielded an increase in recovery of DNA as the amount of blood used for extraction increased; however, the Organic method of extraction allowed for the recovery of more DNA in these samples (figures 6 and 7).

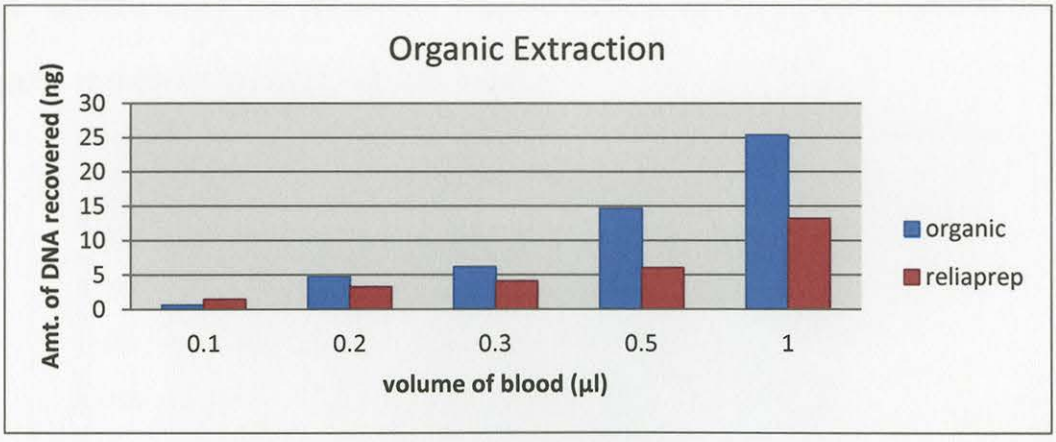

Figure 6. Histogram depicting the DNA recovery results from the small volume samples used.

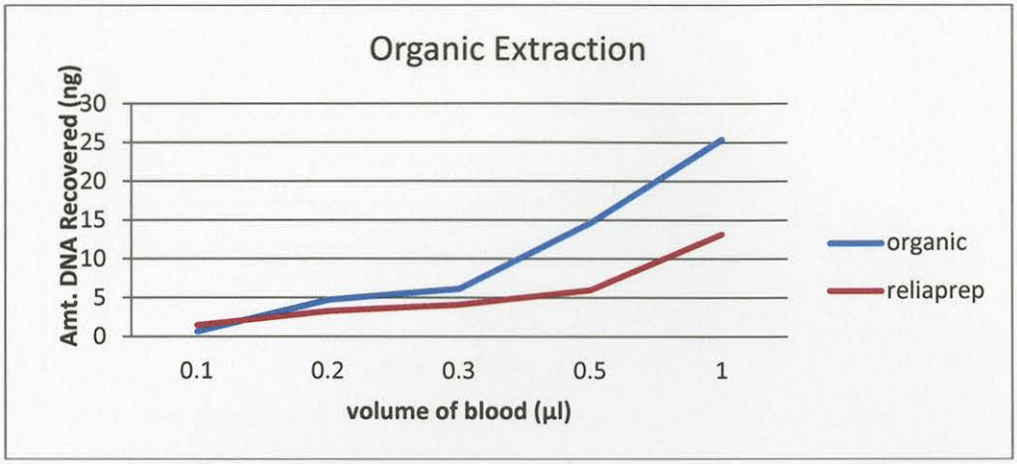

Figure 7. Line graph depicting the DNA recovery results from the small volume samples used.

The average amount of DNA recovered from the organic extraction and the ReliaPrep extraction kit for the blood sample containing sample volumes of 2, 3, 5, 7, and 10µl are given in table 6. Both methods of extraction yielded an increase in DNA as the blood sample volume increased; however with the larger initial sample sizes, the ReliaPrep method recovered more DNA than that of the organic extraction method (figures 8 and 9).

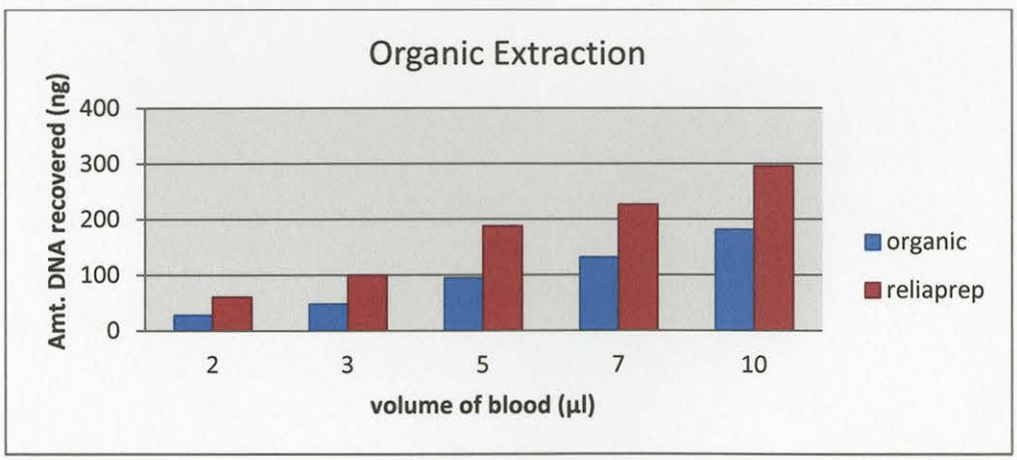

Figure 8. Histogram depicting the amount of DNA recovered in the larger volume sample set (2 – 10 µl).

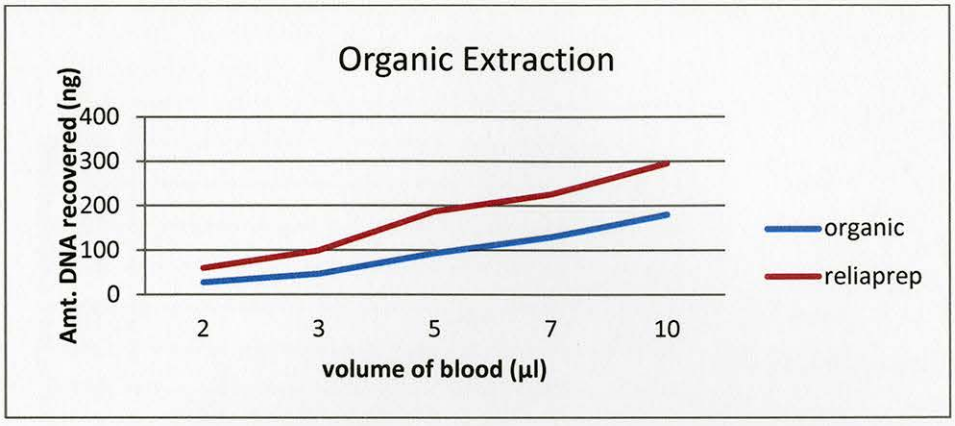

Figure 9. Line graph illustrating the increase in DNA recovery as the initial volume of blood used increases.

Differential Extraction

The average amounts of DNA recovered from both the organic differential extraction as well as the Differex/ReliaPrep differential extraction methods are illustrated in figures10 and 11.

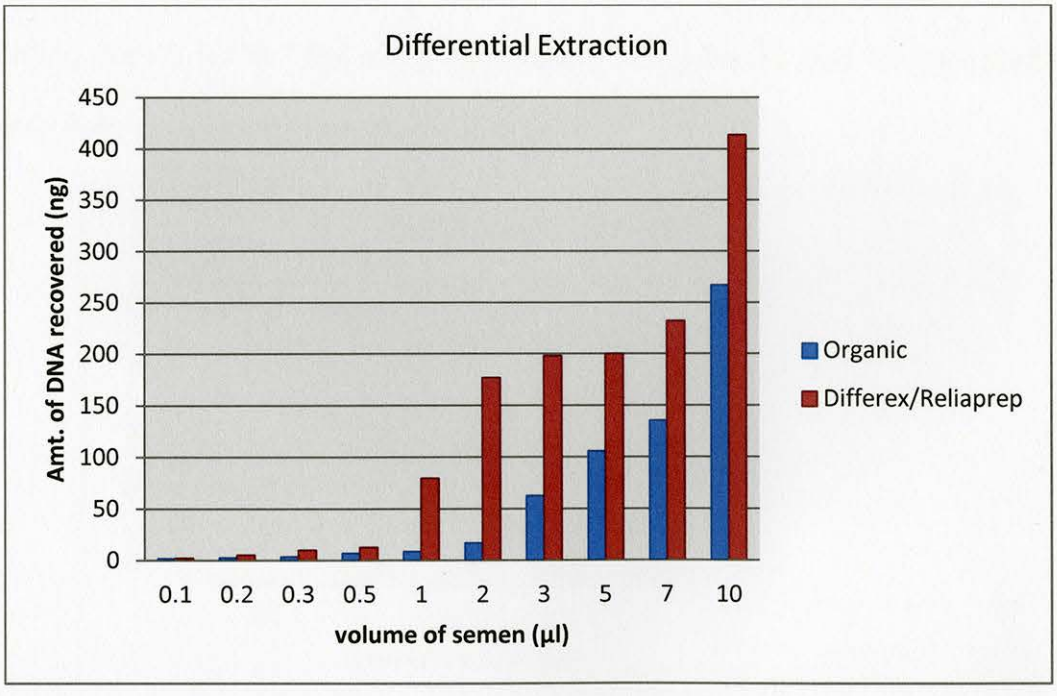

Figure 10. Histogram depicting the amount of DNA recovered from the organic differential extraction method as well as the Differex/RelliaPrep extraction method.

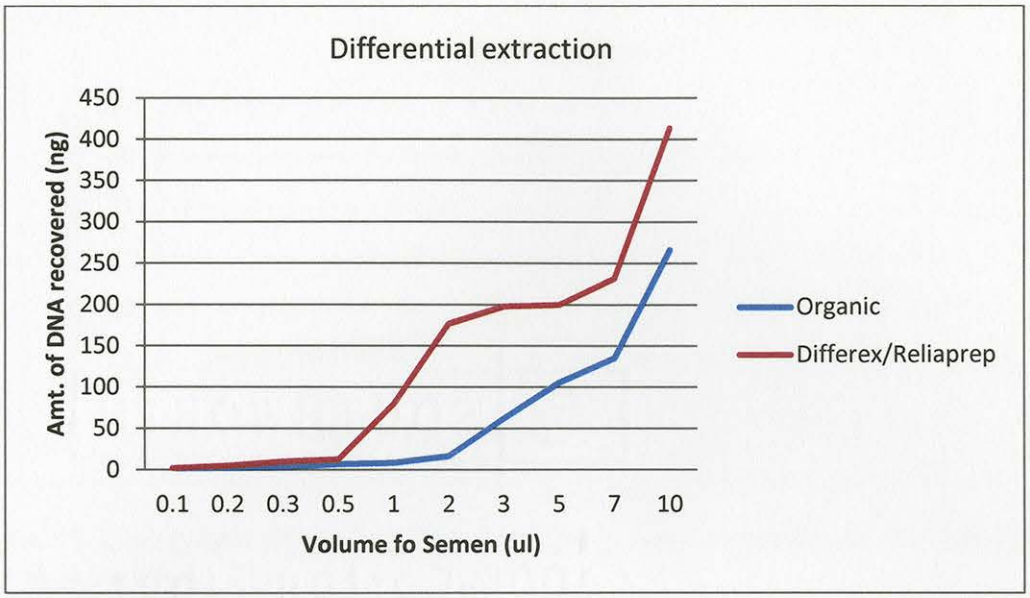

Figure 11. Line graph illustrating the increase in recovery of DNA for the two methods of differential extraction.

The average amount of DNA recovered from the organic differential extraction as well as the Differex differential extraction protocol for the semen volumes ranging from 0.1µl to 1.0µl are given in table 7. Figures12 and 13 represent the same data using histogram and line graphs.

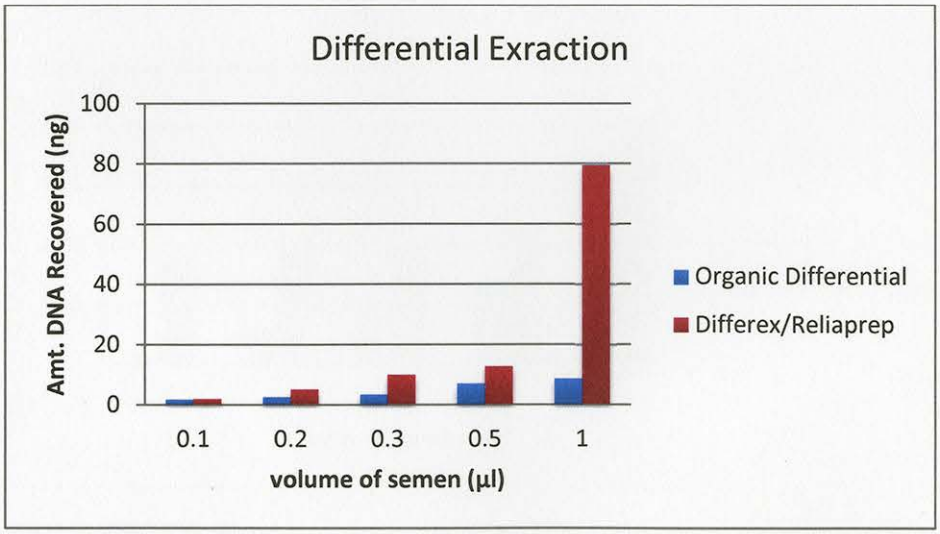

Figure 12. Histogram depicting the amount of DNA recovered from the organic differential extraction and the Differex/ReliaPrep extraction methods for small sample volumes.

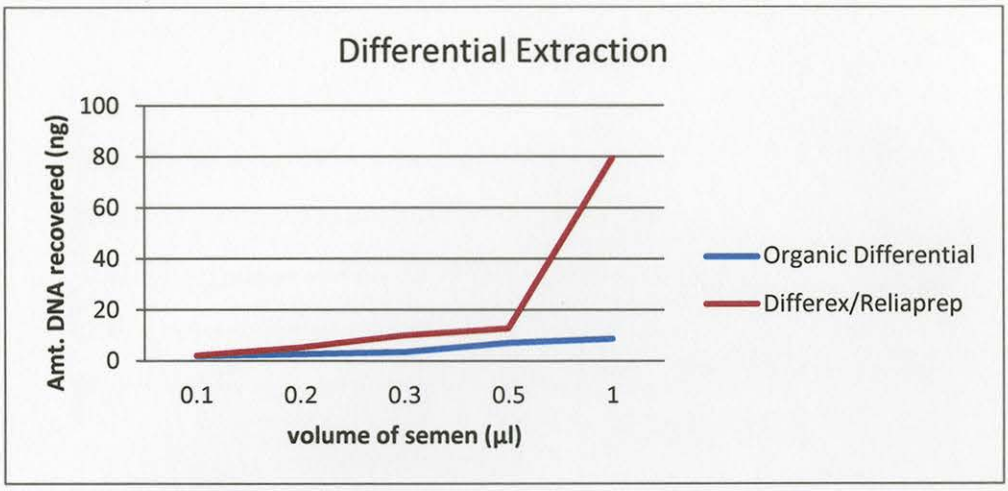

Figure 13. Line graph illustrating the increase in DNA recovery as the sample volume increases.

The amount of DNA recovered from the organic differential extraction for the semen volumes of 2, 3, 5, 7, and 10 µl were17.01, 62.83, 105.94, 135.56, and 267.25 ng respectively while the amount of DNA recovered for the same volume of semen using the Differex system are 176.8, 198, 200, 232, and 413.5 ng respectively (figures 14 and 15).

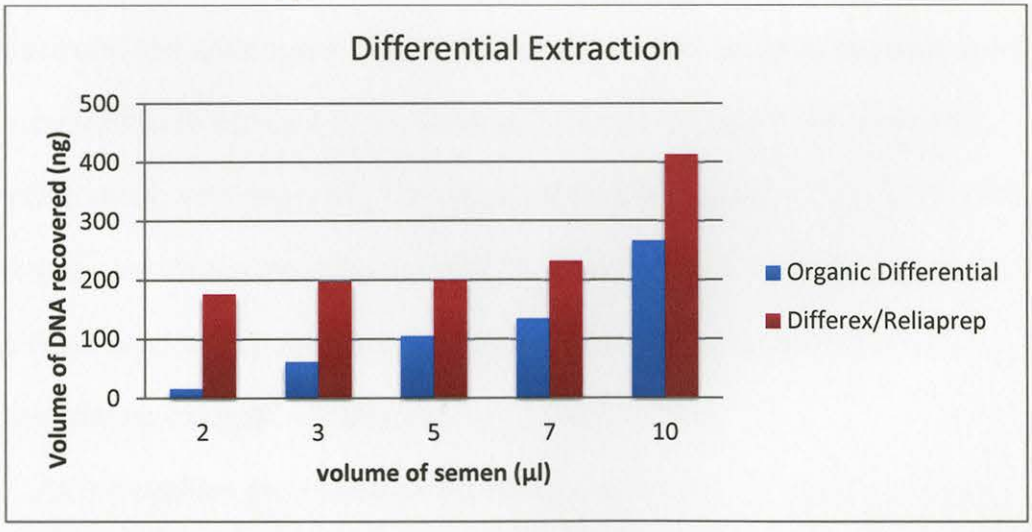

Figure 14. Histogram depicting the amount of DNA recovered from the organic differential extraction as well as the Differex/ReliaPrep extraction method for the larger sample volumes.

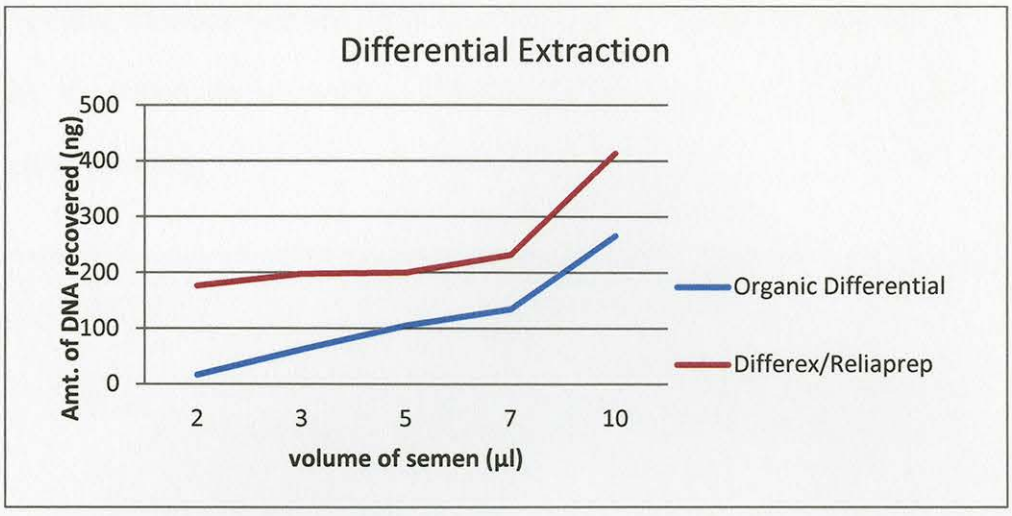

Figure 15. Line graph illustrating the increase in DNA recovered for both differential extraction methods as the initial volume of semen increases.

In order to determine the quality and quantity of the DNA recovered from both straight and differential extractions, few selected DNA samples were amplified using the AmpfISTR Identifiler human identification kit and the Amplicons were analyzed in an ABI 310 genetic analyzer with genemapper ID software. Approximately 0.5 -1 ng of DNA was used to amplify the 15 STR markers and the amelogenin locus. This analysis was done to determine if there was any inhibitors present in the DNA samples that might have been co-extracted along with the DNA. Example electropherograms of the DNA profiles generated are displayed in figures 16-23. These figures illustrate the quality of DNA recovered from both the organic extraction methods as well as the commercial kit extraction methods.

All the profiles generated using straight extraction of blood samples produced a complete female DNA profile as expected except in one sample where there were allelic drop outs in two loci. The sperm fraction DNA that were

used for amplification generated only a male specific DNA profiles which suggest the complete separation of male and female cells during the differential extraction process.

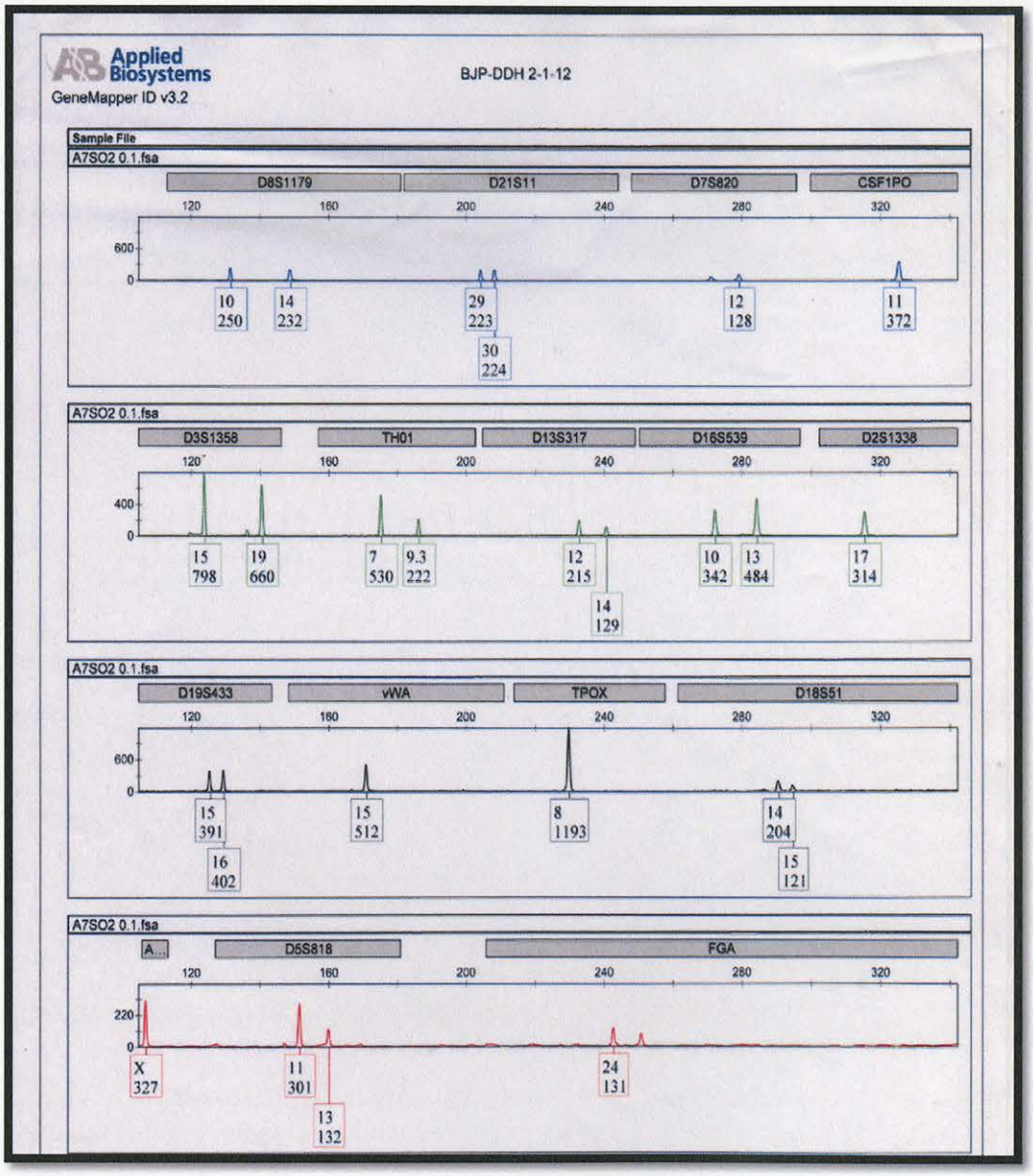

Figure 16. Clean female DNA profile obtained from DNA recovered in the straight organic extraction of 0.1µl blood sample. 0.4ng was used to amplify the 15 STR loci. Allelic drop outs were identified only in two loci, D7S820 and FGA.

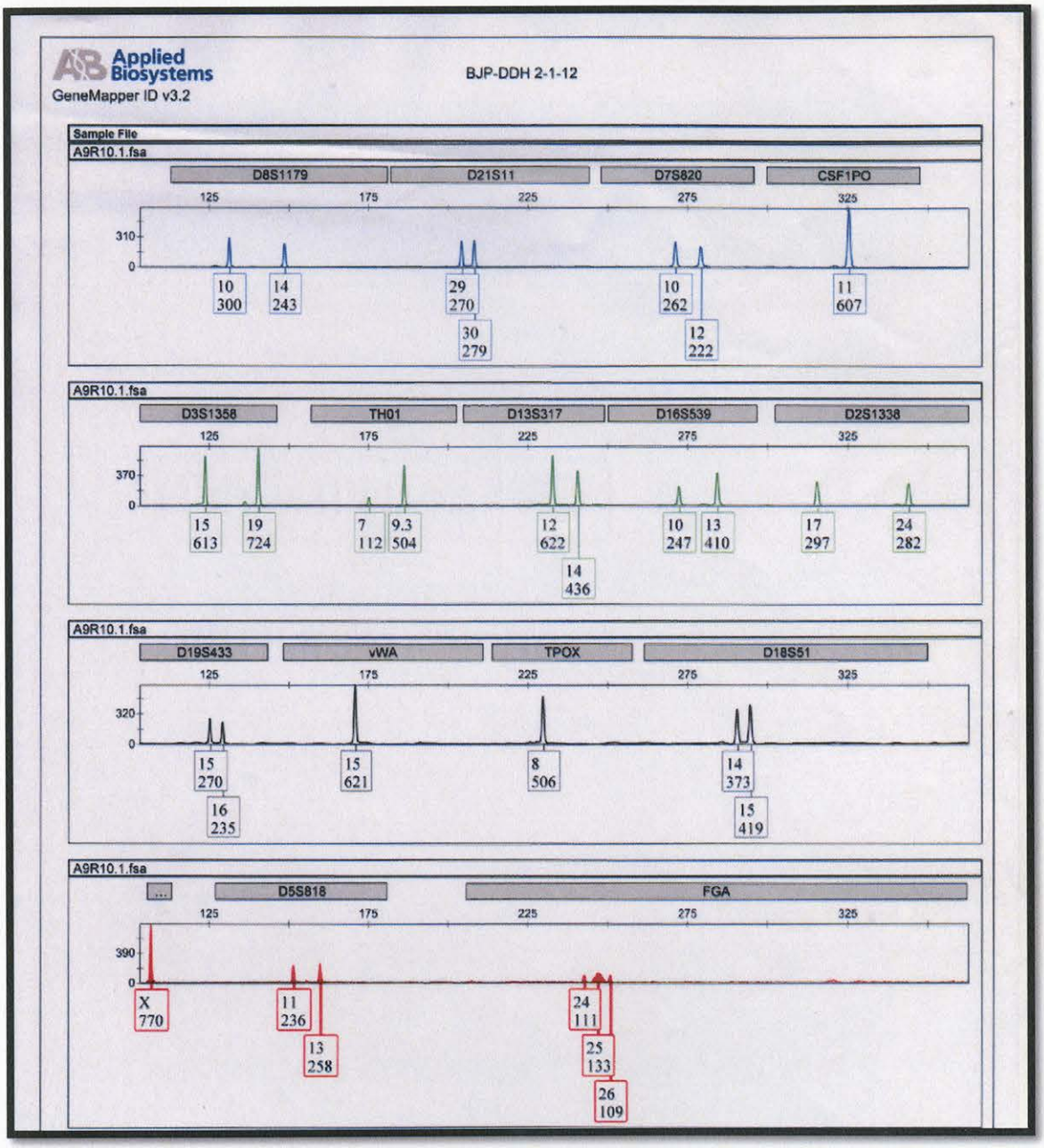

Figure 17. Complete female DNA profile obtained from DNA recovered using the ReliaPrep extraction kit from 0.1µl blood sample. 0.1ng DNA was used to amplify the 15 STR loci.

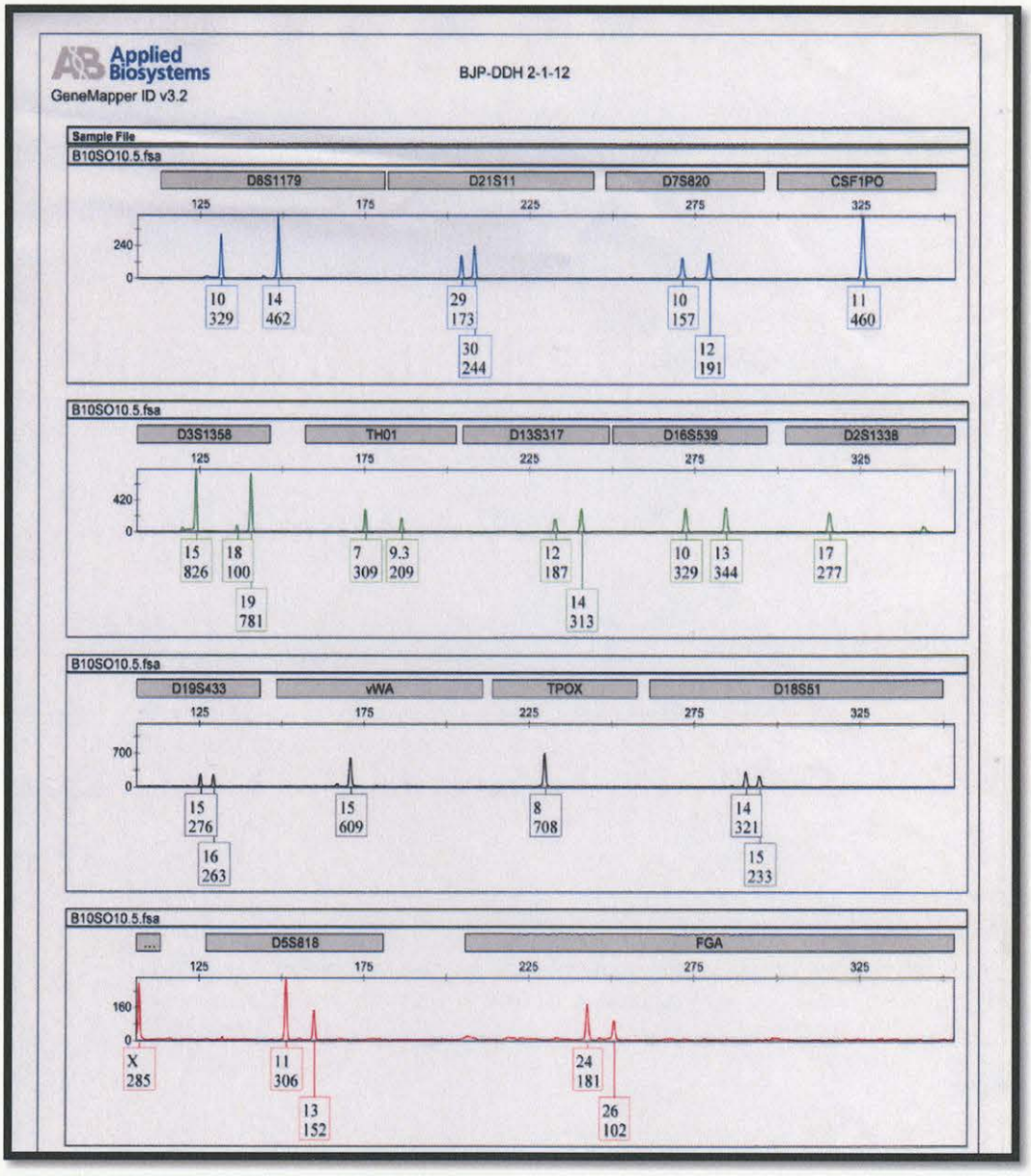

Figure 18. Complete and clean female DNA profile obtained from DNA recovered using the straight organic extraction of 0.5µl blood sample. 0.5 ng of DNA was used to amplify the 15 STR loci.

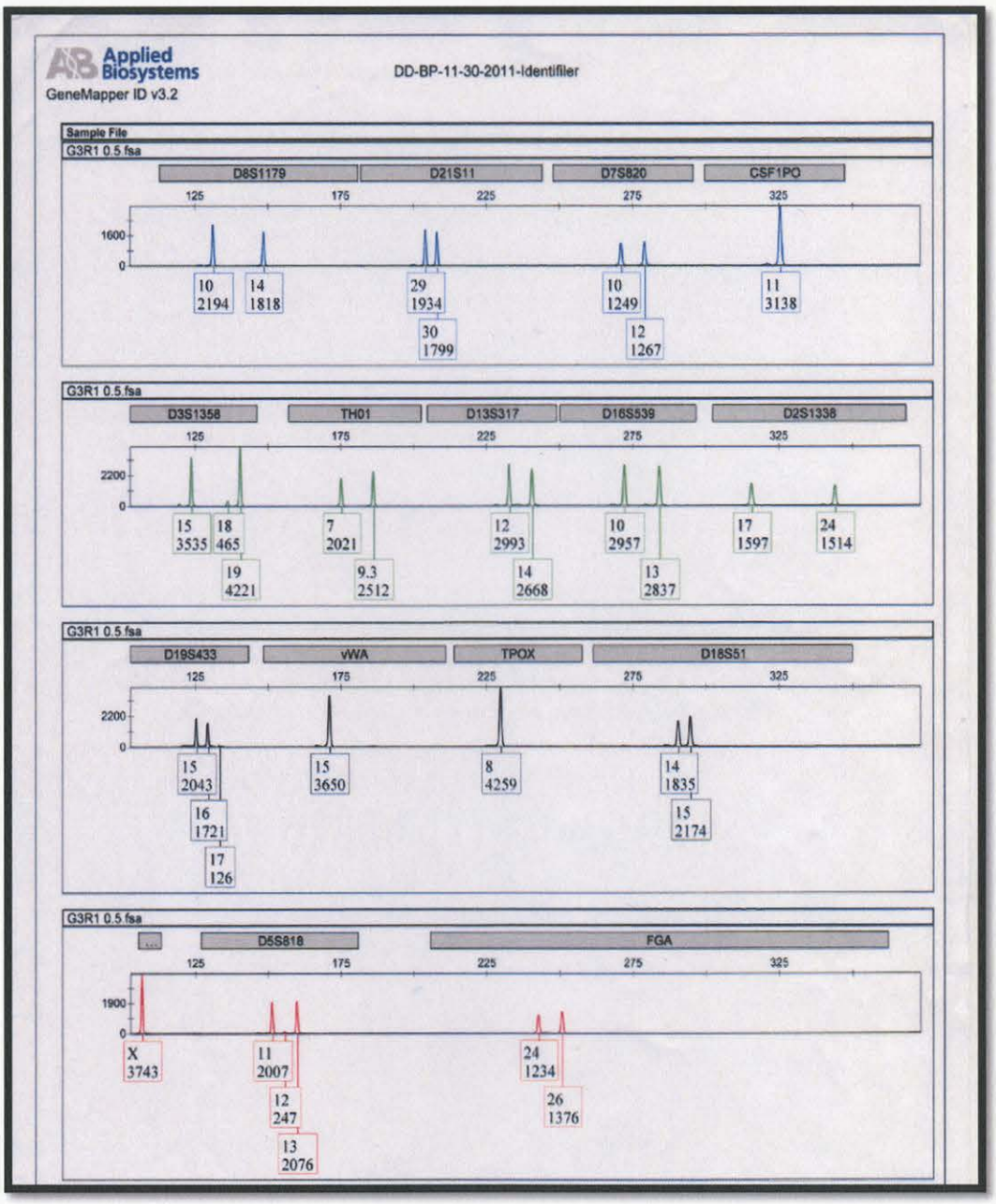

Figure 19. Complete and clean female DNA profile obtained from DNA recovered using the ReliaPrep extraction of 0.5µ blood sample. 0.5 ng of DNA was used to amplify the 15 STR loci.

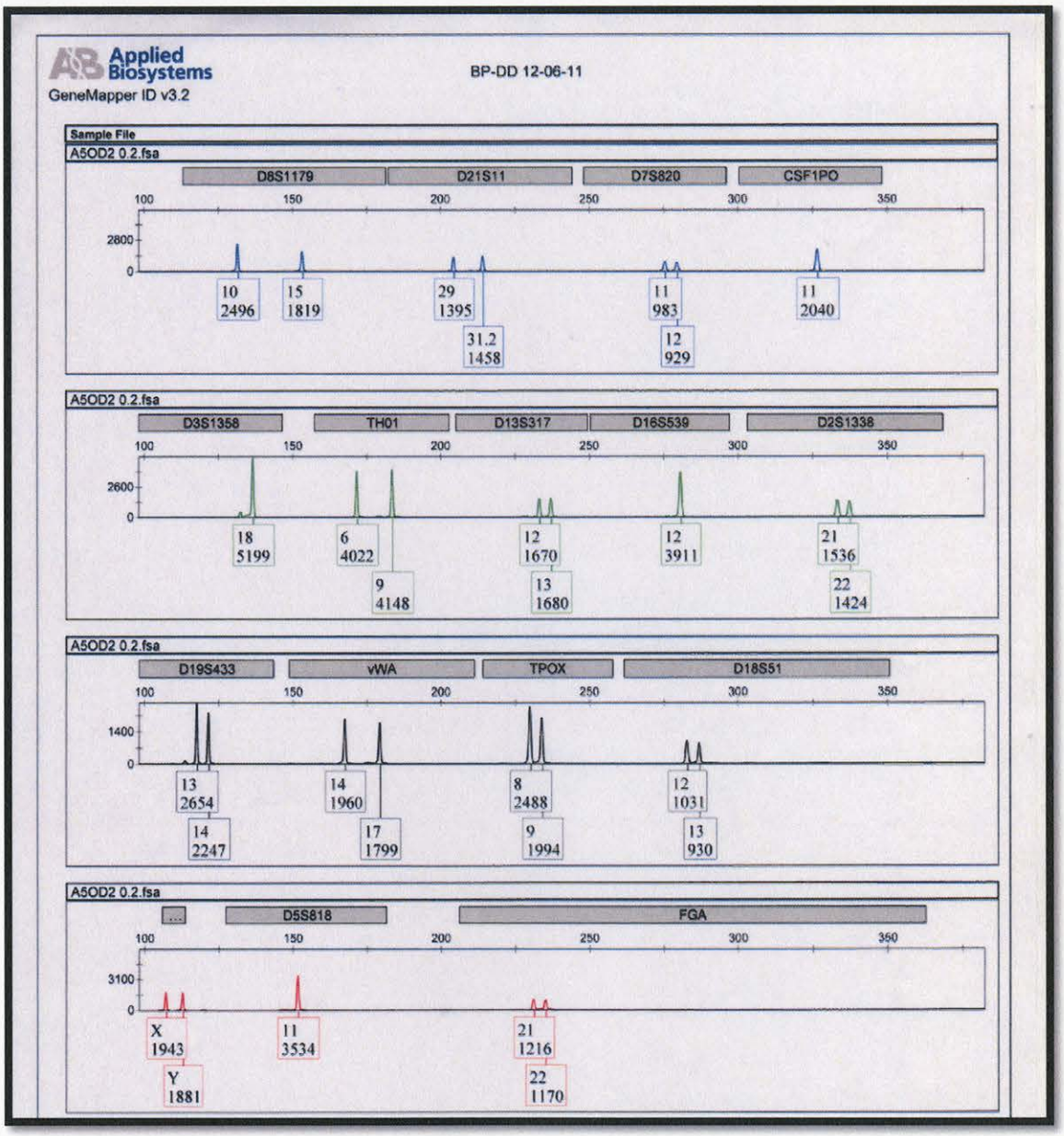

Figure 20. Complete and clean male DNA profile obtained from DNA recovered using the organic differential extraction of 0.2µl semen sample. 0.5 ng of DNA was used to amplify the 15 STR loci. The male profile only data indicate the complete separation of the female epithelial cells from that of the male sperm cells.

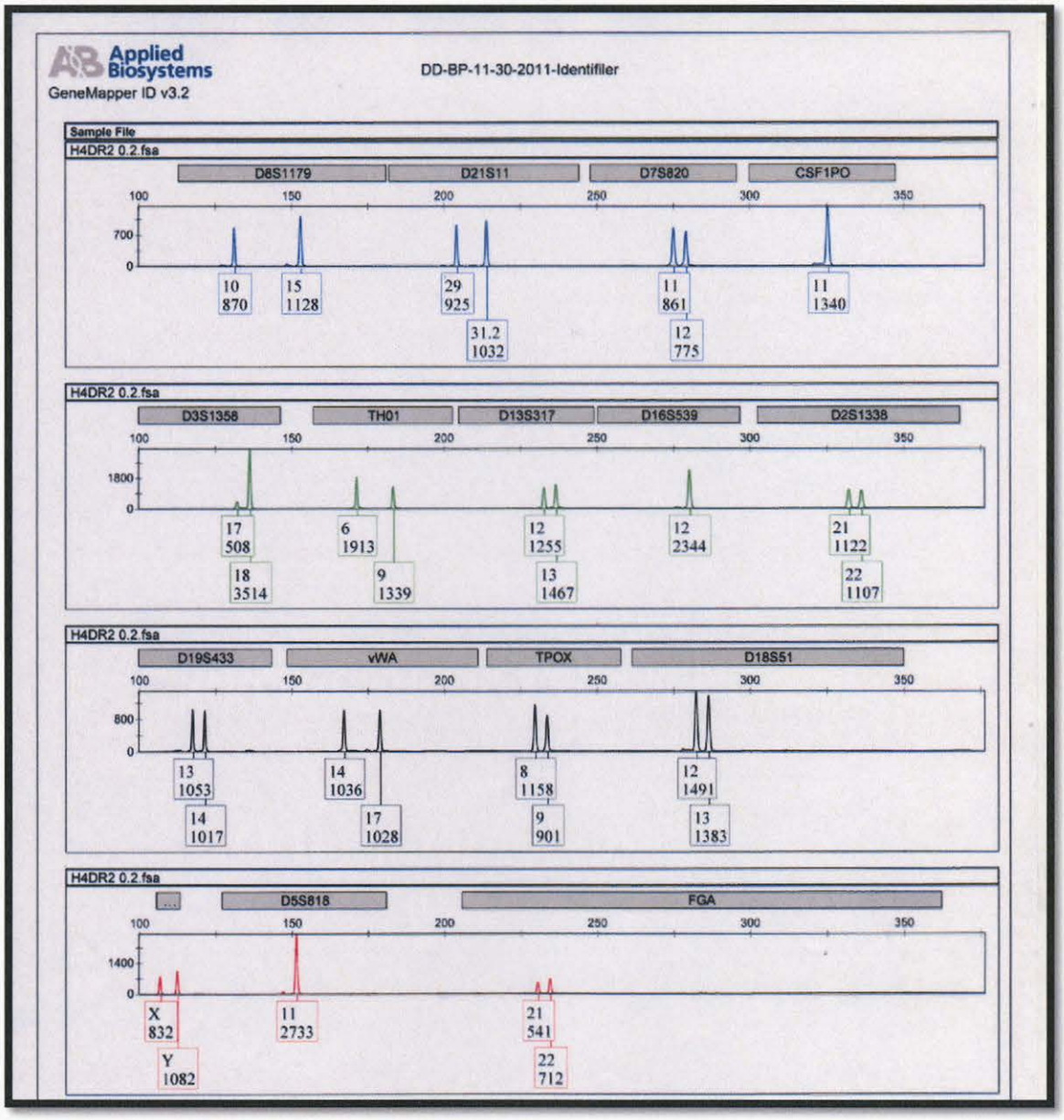

Figure 21. Complete and clean male DNA profile obtained from DNA recovered using the DIfferex/ReliaPrep extraction of 0.2µl semen sample. 0.4 ng of DNA was used to amplify the 15 STR loci.

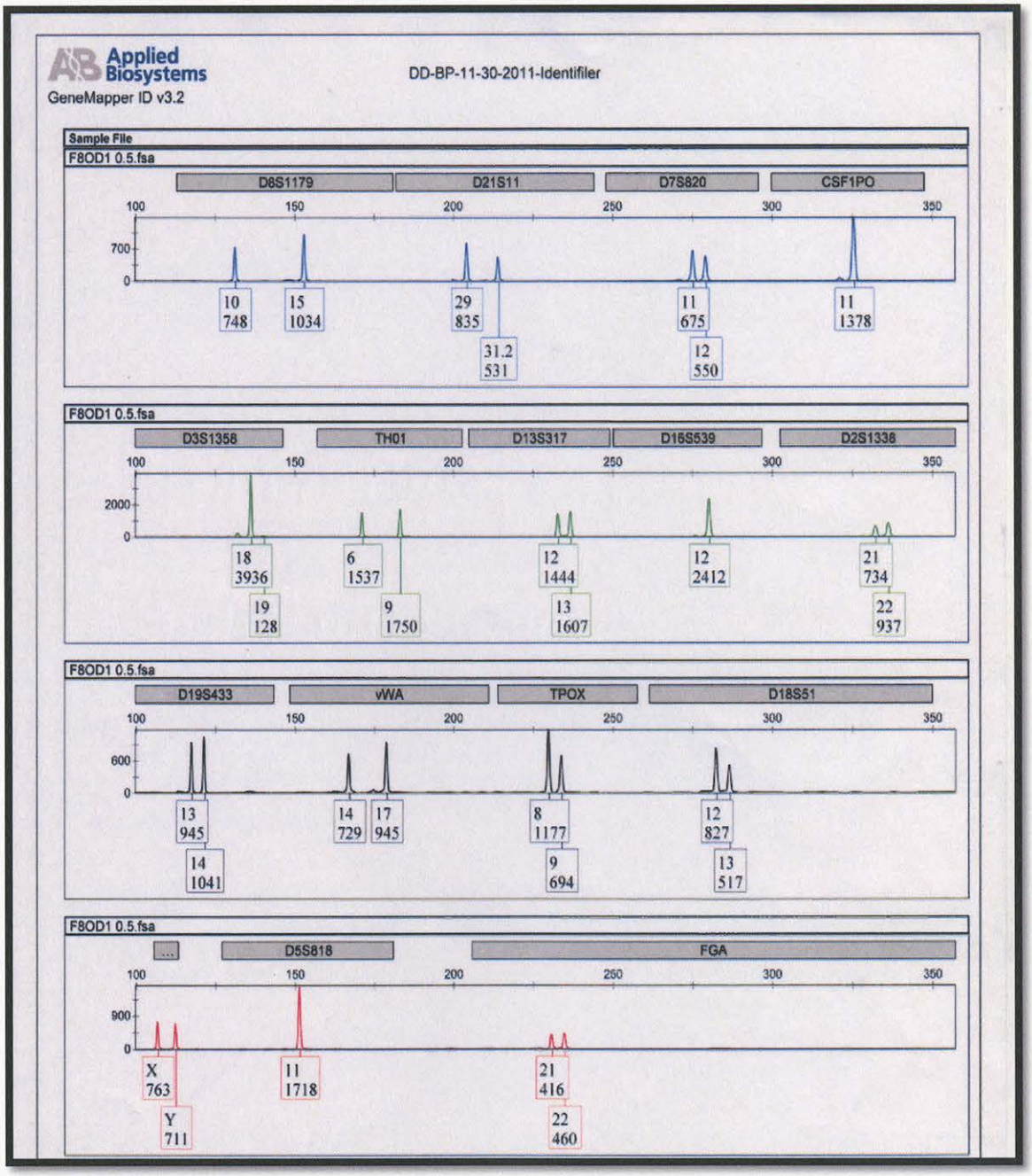

Figure 22. Complete and clean male profile obtained from DNA recovered using the organic differential extraction of 0.5µl semen sample. 0.5ng of DNA was used to amplify the 15 STR loci.

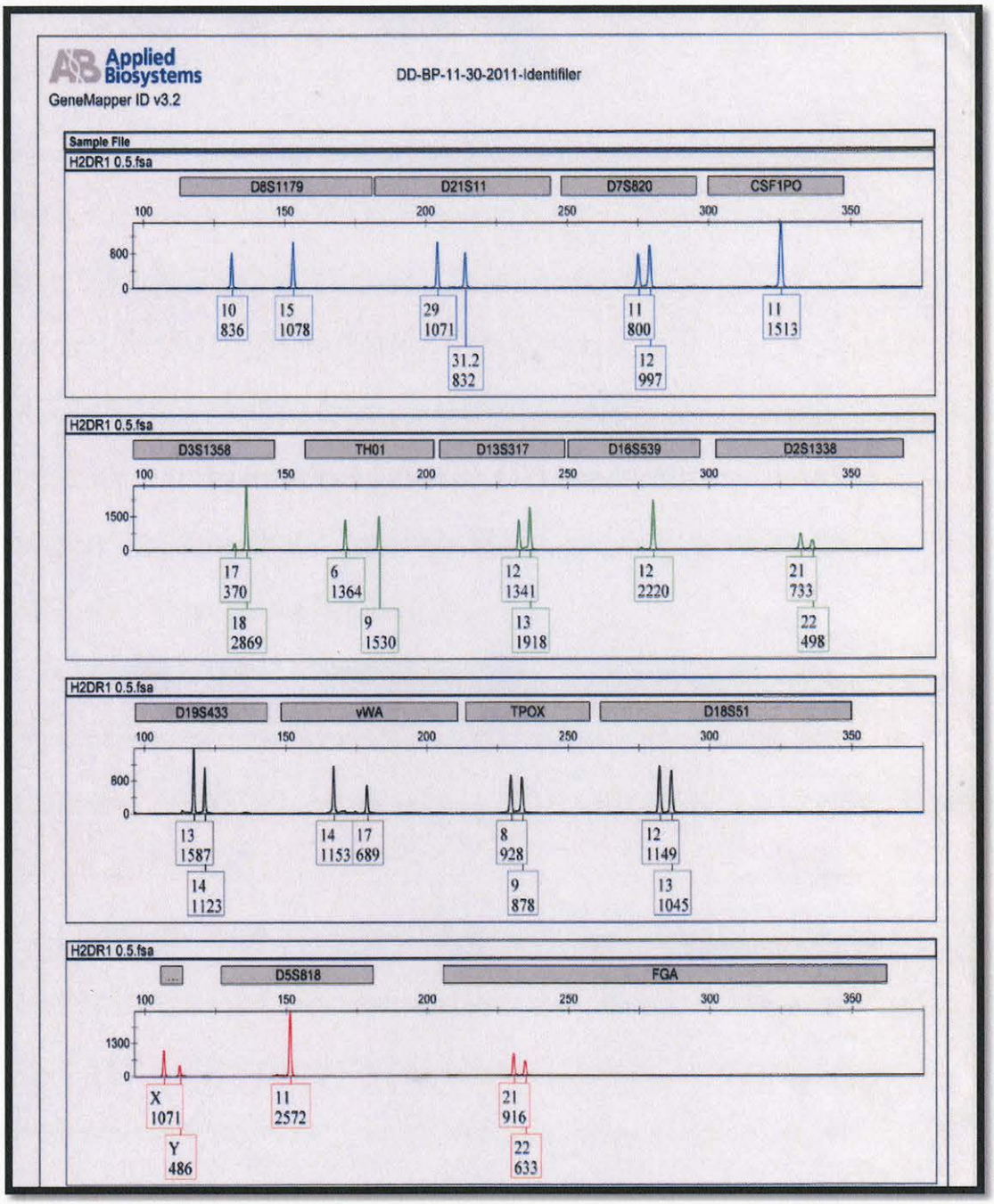

Figure 23. Complete and clean male profile obtained from DNA recovered using the Differex/ReliaPrep extraction of 0.5µl semen sample. 0.5 ng of DNA was used to amplify the 15 STR loci.

CHAPTER V

DISCUSSION

DNA analysis is essential to the forensic science community, and DNA typing success relies on the isolation of DNA in sufficient quantity, quality, and purity (DNA IQ Technical Bulletin). When extracting biological materials for forensic DNA typing, it is extremely important to try and avoid degradation of the DNA samples as well as to remove other inhibitors where possible. In addition to this, the specific techniques to be used depend greatly on the type of evidence being examined. There are several methods by which DNA can be extracted from extracellular materials.

Organic phenol chloroform extractions have been used for the longest period of time, and can be performed for both straight and differential extractions. These types of extractions are compatible for both RFLP and PCR typing technologies.

Although viable amounts of DNA can be recovered from organic extractions, this extraction method is extremely time consuming and involves the use of harsh chemicals. Since this is the case, several companies have developed commercial kits that are designed to be less time consuming and more efficient in the extraction of DNA.

In this research, comparison of commercial DNA extractions kits was made with that of traditional organic procedures involving simulated forensic evidence samples. Genomic DNA was extracted, quantitated and compared to test the efficiency of the ReliaPrep™ Blood gDNA Miniprep system (Promega

Corp.) with that of the phenol chloroform organic extraction; in addition, different cell types were mixed and the separation of the two cell types was assessed and compared to test the efficiency of the Differex system (Promega Corp.) with that of the differential phenol chloroform organic extraction.

These comparisons were made in an effort to determine which method provided more extracted DNA in a laboratory setting. In order to assess the sensitivity and efficiency of these methods, blood samples as well as differential samples of different concentrations were extracted using both the organic method as well as commercial kits.

In the first part of the experiment, simulated blood samples in duplicates were extracted using both the organic extraction method as well as the ReliaPrep™ Blood gDNA miniprep system. Once the samples were extracted, the presence and the quality of extracted DNA were verified using 1% agarose gels. After the presence of DNA had been verified, the samples were quantitated using real-time PCR to determine the quantity of human DNA in the samples. The DNA quantities recovered show a considerable difference in the organic extractions vs. the ReliaPrep extractions. The organic extraction of blood samples in the lower volume range (0.1 µl to 1.0 µl) yielded more DNA compared to the ReliaPrep kits (figures 6 and 7). On the other hand the ReliaPrep kit clearly recovered more DNA for the sample volumes ranging from 2 to 10 µl compared to organic extraction procedures (figures 8 and 9).

Although both methods of extraction produced relatively clean DNA profiles, the profile obtained from DNA recovered using organic extraction of 0.1

µl of blood sample contained allelic dropout at two loci, D7S820 and FGA. Allellic dropout refers to the incomplete data available in one or more loci. This usually occurs when the quantity of DNA is low due to limited sample amounts, degradation of DNA or inhibition of PCR by inhibitors that may have been co-extracted with the sample. The profiles of samples originating from other straight extractions generated complete profiles with no allelic dropouts. On the other hand, the DNA profile obtained from DNA extracted from 0.1 µl of blood using the Reliaprep extraction kit showed no allelic dropouts and a complete profile was obtained. This suggests that a cleaner DNA can be extracted using the Reliaprep DNA extraction system. The DNA obtained using both the extraction methods from higher volume of blood showed no allelic dropouts and a complete profile was obtained.

In the second part of the experiment, the differential organic extraction method was compared to that of a combination of the Differex and ReliaPrep kit, both of which were obtained from Promega Corporation. Simulated forensic differential stains in duplicates were extracted using both the organic differential method as well as the Differex/ReliaPrep method. After the samples were extracted the quality, and the presence of DNA were verified using a 1% agarose gel. Once the presence of DNA was verified, the samples were quantitiated using real-time PCR with the Quantifiler Human DNA Quantification kit in order to assess the total human DNA recovered using both the methods. For the differential extraction process, the Differex/Reliaprep kit outperformed

the standard organic extraction procedure for all the sample volumes tested from 0.1 µl to 10 µl of semen (figures10 and 11).

Once the concentration and the quantity of DNA had been determined, few selected DNA samples were amplified using AmpFISTR Identifiler kit for the 15 STR markers and the amelogenin locus to assess the quality and purity of the extracts. DNA recovered from 0.2, 0.3, 0.5, and 1.0µl semen using differential organic extraction as well as Differex differential extraction methods were used for amplification. Approximately 0.5 ng of each sample was used for amplification. All the DNA profiles obtained from the sperm fraction samples listed above generated a complete and clean male DNA profile only. This data proves that both the organic differential extraction and the Differex differential extraction kit are very useful and versatile in separating the female epithelial cells from that of the male sperm cells. These clean single source male profiles indicate that there is no carryover of the female epithelial cell DNA into the male fraction DNA. If there was any carryover of the female epithelial DNA in the sperm fraction DNA, then the resulting genetic profile would be a mixture with both male and female genetic profiles.

There was however some evidence of stutters found in some of the profiles generated by two samples (OD0.5 and DR0.5; figures 22 and 23) at locus D3S1358. Stutter peaks are artifacts that are created during the PCR process and are usually four bases smaller than a true allele and are seen immediately before an allele. These stutter peaks are known artifacts and can be readily distinguished from contamination or peaks due to a second contributor.

In addition to stutters other artifacts may also be seen as evidenced in figure 17 at the FGA locus. These are probably due to dye blobs or other substances that may be present in the sample, and can be distinguished from real alleles.

The efficiency of the organic extraction methods versus the commercial extraction kits can be determined by the recovery and the purity of the DNA samples obtained using each method. The experimental results from this study clearly suggest that the commercial kits provided by the Promega Corporation offer a higher degree of DNA recovery with the wide range of sample volumes except for the low volume range for blood (0.1 – 1.0 µl).

The sensitivity and accuracy of each DNA extraction method is important; however, many other factors like that of cost and time need to be considered in order to select the most practical and efficient methodology, especially in forensic laboratory settings.

The straight organic extraction method takes up to three hours to complete depending on number of samples whereas, the ReliaPrep system can be completed in 45 minutes. Differential organic extraction can take up to four or more hours to complete, whereas, the DIfferex system takes only two hours. Although the Differex system does not take as long to complete it involves several steps in which extreme caution must be taken to ensure the presence of DNA in the final elution. Cost involved in procuring the kits is also a concern in addition to the time required for processing the samples. For instance, organic extractions can be accomplished in laboratories with little money, while the ReliaPrep System costs $235 for a kit that can process 100 samples, and the

Differex system costs $245 for 50 samples in addition to the $80 manual Differex Magnet that must also be purchased in order to use this kit. Although this is the case, in most instances the value of the trace samples outweighs the cost of the experiments. In a forensic laboratory set up, utmost importance is given to trace samples and obtaining an interpretable profile is more important than the cost associated it.

APPENDIX

IRB APPROVAL FORM

THE UNIVERSITY OF SOUTHERN MISSISSIPPI

Institutional Review Board

118 College Drive #5147
Hattiesburg, MS 39406-0001
Tel: 601.266.6820
Fax: 601.266.5509
www.usm.edu/irb

HUMAN SUBJECTS PROTECTION REVIEW COMMITTEE
NOTICE OF COMMITTEE ACTION

The project has been reviewed by The University of Southern Mississippi Human Subjects Protection Review Committee in accordance with Federal Drug Administration regulations (21 CFR 26, 111), Department of Health and Human Services (45 CFR Part 46), and university guidelines to ensure adherence to the following criteria:

- The risks to subjects are minimized.
- The risks to subjects are reasonable in relation to the anticipated benefits.
- The selection of subjects is equitable.
- Informed consent is adequate and appropriately documented.
- Where appropriate, the research plan makes adequate provisions for monitoring the data collected to ensure the safety of the subjects.
- Where appropriate, there are adequate provisions to protect the privacy of subjects and to maintain the confidentiality of all data.
- Appropriate additional safeguards have been included to protect vulnerable subjects.
- Any unanticipated, serious, or continuing problems encountered regarding risks to subjects must be reported immediately, but not later than 10 days following the event. This should be reported to the IRB Office via the "Adverse Effect Report Form".
- If approved, the maximum period of approval is limited to twelve months. Projects that exceed this period must submit an application for renewal or continuation.

PROTOCOL NUMBER: **11063002**
PROJECT TITLE: **Comparison of Commercial DNA Extraction Kits with that of Organic Extraction Procedures Involving Simulated Forensic Evidence Samples**
PROPOSED PROJECT DATES: **07/01/2011 to 06/30/2012**
PROJECT TYPE: **Dissertation**
PRINCIPAL INVESTIGATORS: **Denise Dent**
COLLEGE/DIVISION: **College of Science & Technology**
DEPARTMENT: **School of Criminal Justice**
FUNDING AGENCY: **N/A**
HSPRC COMMITTEE ACTION: **Exempt Approval**
PERIOD OF APPROVAL: **07/21/2011 to 07/20/2012**

Lawrence A. Hosman
Lawrence A. Hosman, Ph.D.
HSPRC Chair

7-21-2011
Date

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