Comparison of Extraction Kits PrepFiler® and DNA IQ™ for STR Analysis of Contact DNA Samples

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Comparison of Extraction Kits PrepFiler® and DNA IQ™ for STR Analysis of Contact DNA Samples

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ABSTRACT

Contact DNA evidence is becoming a common occurrence at crime scenes and is often collected and analyzed for human identification. Current Short Tandem Repeats (STRs) techniques are still limited in Low Copy Number (LCN) DNA analysis due to contamination and stochastic effect. Increasing DNA yield from the extraction step is a potential benefit for many investigations. One hundred and fifty samples collected from five female individuals on five commonly-used items were extracted by two forensic extraction systems: DNA IQ™ and PrepFiler Kit® with subsequent genotyping by PowerPlex® 16 HS system. Results determined that these extraction systems are not suitable for LCN DNA samples. Only two complete STR profiles were produced without contamination. These findings indicated that further improvements are required in order to utilize STR analysis for LCN DNA.

TABLE OF CONTENTS

TABLE OF FIGURES

TABLE OF TABLES

CHAPTER ONE

LITERATURE REVIEW

For almost one hundred years, the technology of forensic science has developed rapidly and today's evidence can be identified from minute samples of biological materials such as contact deoxyribonucleic acid (DNA). Contact DNA is defined as small amounts of DNA on an item after it has been touched or handled (Bode Technology, n.d.). Locard's exchange principle states that any contact by individuals and/or items will result in the exchange of material between those individuals and items (Thornton, 1974). Locard's exchange principle is fundamental to the field of forensic science analysis (Thornton, 1997). Whether biological, chemical, or impression-based evidence, materials once in contact may display evidence of this past interaction. Evidence found at a crime scene, on a victim, or on a suspect has been shown to provide a link between suspect, victim and crime scene which can aid the investigation. The analysis of small amounts of biological evidence continues to attract the attention of forensic science researchers and practitioners.

Common sources of biological evidence include hair, skin cells, spermatozoa, and blood, all of which can be transferred in minute or large quantities depending on the circumstances of the event. No matter the quantities or the biological nature of the evidence, DNA contained within the cell can be used in an attempt to obtain a DNA profile.

A crime scene is not the ideal place to preserve these small amounts of biological evidence. Environmental factors such as humidity, heat, and ultra-violet light can damage the DNA within biological samples. In instances of small or trace amounts of a biological sample,

a robust protocol for DNA collection and analysis is required. Forensic scientists are seeking solutions to overcome the difficulties of evaluating contact or low copy number (LCN) DNA.

Difficulties in contact DNA sample analysis

The extraction and analysis of contact DNA from biological evidence has become an increasingly used technique in forensic DNA laboratories all around the world. DNA profiling technology continues to advance, becoming more sophisticated, and requiring less genetic material. Current amplification techniques have been shown to amplify DNA from less than 17 diploid cells and still yield a full genetic profile (Butler & Hill, 2010). Seventeen diploid cells contain approximately 100 picograms (pg) of DNA. As the area of low level DNA analysis continues to gain in popularity, new terms are required in order to accurately communicate specific details about the analytical procedures. The term LCN DNA is used to describe a sample that contains less than 100 pg of DNA. LCN DNA sample has become interchangeable with other terms including trace or contact DNA (van Oorschot & Jones, 1997).

Contact DNA is typically extracted from skin cells. Skin cells are constantly shed by individuals and are often unpredictable in quantity. Although approximately tens of thousands of skin cells fall from one individual every day (Bode Technology, n.d.), the number of skin cells that can be recovered from casually touched objects varies from person to person and time to time (Lowe et al., 2002). Due to a yet unexplained phenomenon, different individuals have different skin shedding rates. For example, the same individual can shed significantly less skin cells after hand washing (Lowe et al., 2002). Both factors,

individual and time, may cause unpredictable results when performing DNA analysis on contact samples, especially when the sample size is below 17 cells.

LCN DNA samples are difficult to amplify during DNA analysis. Before current techniques in LCN DNA analysis became available in forensic human identification, contact samples were processed and analyzed the same way as large quantity biological samples originating from blood, semen, or saliva (Lowe et al., 2002; Petricevic et al., 2006; Phipps & Petricevic, 2007). LCN DNA samples are challenging when analyzed using traditional procedures. Factors such as stochastic fluctuations, contamination, and artifacts have been observed in LCN DNA samples that would not be seen in the more common biological samples (Butler & Hill, 2010). When a sample size is too small, the selection of a portion from that sample occurs randomly. This phenomenon is commonly referred to as stochastic effect. In LCN DNA analysis, the stochastic effect causes unequal sampling between loci within the DNA profile and heterozygous alleles within those loci. Instances of contamination have also been elevated in LCN DNA analysis. DNA contamination from the examiner or other items of evidence may exceed that of the evidence sample of interest (Gill, et al., 2000). LCN DNA analysis should be performed with extreme care to avoid contamination. Ultra-violet (UV) radiation has also been examined for its ability to eliminate contamination, and UV light did not significantly affect subsequent DNA analysis when sterilizing swabs and tubes (Pang & Cheung, 2007a; Shaw et al., 2008). Artifacts include such things as stutter peaks and allelic drop-in. Stutter peaks are artifacts generated by strand slippage during amplification and appear as smaller peaks when compared to true allele peaks (Butler & Hill, 2010). Allele drop-in peaks are incorrect allele peaks caused by high

background noise of the instrument**.** Stutter and allele drop-in peaks result in difficulty in identifying true allele peaks and cause uncertainty in interpretation of a DNA profile.

In order to yield reliable DNA profiles, specific procedures for the collection and analysis of LCN DNA samples must be examined and improved. DNA can be collected from both porous and non-porous surfaces. These surfaces vary in their ability to yield sufficient DNA samples. Collection methods have been developed to accommodate these differences and include cutting, swabbing, tape-lifting, and scraping (Bode Technology, n.d.). For example, cutting is a better practice for collecting DNA samples from cloth, whereas swabbing is preferred on glass or plastic. Tape-lifting and scraping are also used to collect DNA samples from porous and soft material. Tape-lifting and scraping can sample a large surface area and increase the chance of obtaining DNA samples (Bode Technology, n.d.). However, cutting, tape-lifting, and scraping could damage the evidence after collection. Another collection method involves a double swab technique, which is a wet swab followed by a dry swab on the object. The double swab technique has been shown to recover more DNA than regular swabbing and does less damage on the object than cutting, tape-lifting, or scraping (Pang & Cheung, 2007b).

Forensic scientists are also attempting to produce an extraction method for increasing DNA recovered from contact samples. An optimized LCN DNA extraction method can increase DNA template recovery and benefit amplification (Schiffner et al., 2005). Forensic scientists have also tried increasing the number of amplification cycles from 28 to 34 in order to obtain a greater number of PCR products**.** As a result of amplification

enhancement techniques the instances of allele drop-in and stutter peaks have increased as well as true alleles (Petricevic et al., 2010).

History of forensic DNA analysis

Modern forensic DNA analysis techniques were developed in the early 1990s (Butler, 2005), originally from "DNA fingerprinting" in 1984 by Dr. Alec Jeffreys (Jeffreys et al., 1985a; Jeffreys et al., 1985b). DNA profiling is a technique used to identify genetic information by electrophoresis as a means of DNA separation. Fragments of DNA molecules are pushed by the electromotive forces. These fragments migrate through the gel matrix at different rates, based on their relative size, resulting in a pattern that can be utilized for the identification of individuals. DNA analysis for use in human identification requires a comparison between reference and unknown samples. The electrophoresis patterns of unknown samples are compared against a known reference to determine the degree of similarity.

Advances in DNA technology have occurred rapidly and replaced the earliest formation. Law enforcement agencies began collecting and analyzing DNA evidence in the late 1980s. After 1990, the development of PCR analysis with small amounts of genetic material was common in law enforcement investigations. From the late 1990s to today, LCN DNA samples have been examined to discover the potential utility for this technique; however, the application of using and interpreting results yielded from LCN DNA samples are still in debate (Budowle et al., 2009).

Forensic DNA identification is viewed positively by the public, but LCN DNA analysis has the potential to countermine the entire field if not properly examined and reviewed. Research has shown more than 8 in 10 Americans (85%) considers DNA evidence as either completely (27%) or very (58%) reliable (Gallup, 2005). However, LCN DNA analysis has been shown to require extreme care to avoid contamination, a complex interpretation procedure, and still produces low success rates (Budowle et al., 2009). These drawbacks are contrary to public opinion of DNA analysis, and if mishandled could result in the loss of confidence by the public.

Biochemistry of DNA

DNA is the nucleic acid macromolecule that contains the genetic information of all known living organisms. Unique organisms have an equally unique genome, and detectable variations are observed within the members of the same species. DNA is a polymer of nucleotide monomers. Each nucleotide contains three components, a 2'-deoxyribose sugar, a phosphate group, and a nitrogenous base (See Figure 1). The 2'-deoxyribose sugar is a monosaccharide containing five carbon atoms and a single oxygen atom in a ring form. The nitrogenous bases of nucleotides contain nitrogen within the rings, and are classified based on the structure of the rings. The nitrogenous bases are classified into adenine (A), guanine (G), cytosine (C), thymine (T). These four nitrogenous bases differentiate the nucleotides and result in the variation of DNA molecule sequence.

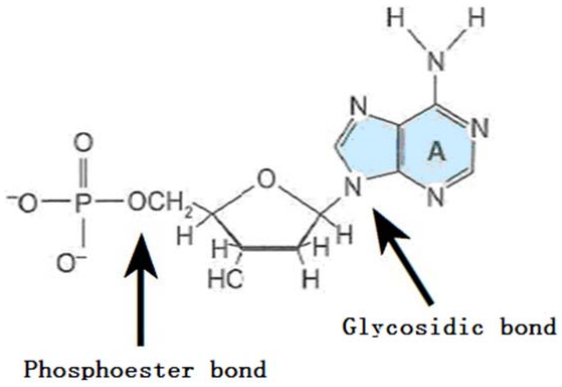

Figure 1. General structure of deoxyribo nucleotide. The phosphoester bond between the 2'-deoxyribose sugar and phosphate group, and the glycosidic bond between the nitrogen base and the 2'-deoxyribose sugar are shown. (Creecy J.)

The nitrogenous bases of the nucleotide are covalently bound to the carbon backbone of the 2'-deoxyribose by a glycosidic bond (Figure 1). The junction carbon between the nitrogenous base and the 2'-deoxyribose is labeled as the 1'- carbon. The bond between the phosphate group and the 2'-deoxyribose at the 5'-carbon is the phosphoester bond. The bond between the phosphate group and the 2'-deoxyribose from another nucleotide at the 3'-carbon is the phosphodiester bond. The DNA backbone links adjacent nucleotides forming a polymer of alternating sugar and phosphate groups as a single strand (Figure 2). Each single strand of DNA runs antiparallel to the complementary strand on the opposing side of the double helix. The nitrogenous bases of the nucleotides are positioned within the helix, stacking one on top of the other forming a highly repetitive and conserved structure. The amount of adenine is equal to the amount of thymine, and the amount of guanine is equal to the amount of cytosine in a double standard DNA molecule. Two connected nucleotides on the complementary DNA strand are named base pairs (bp). Adenine is paired with thymine via two hydrogen bonds, and guanine is paired with cytosine via three hydrogen bonds (Figure 2). Glycolytic bonds,

phosphoester bonds, and hydrogen bonds contribute to the structural stability of the DNA molecule.

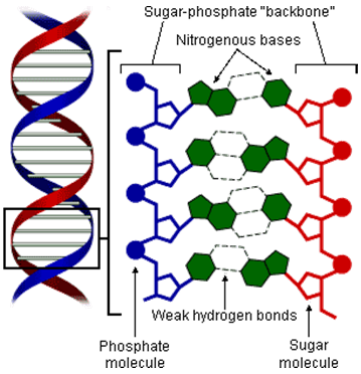

Figure 2. DNA structure with nitrogenous bases, phosphate molecule and sugar molecule. Green color is labeled as the four bases, pentagon identifies pentose sugar, and cycle is labeled as phosphate group. Hydrogen bases are formed between nitrogenous bases. (http://evolution.berkeley.edu/evosite/history/images/dna_structure.gif)

The DNA molecule is stable when preserved in a proper environment, but can be irreversibly damaged by environmental agents like UV radiation and X-rays. Sunlight is the nature source of both X-rays and UV radiation, both of which are prevalent at many crime scenes. The double bonds in the nitrogenous bases of DNA are capable of absorbing UV radiation. The absorption of this harmful energy results in the creation of thymine dimers between adjacent thymine base pairs. Damaged DNA cannot be used in PCR amplification and genotyping. Most detrimental to a DNA sample are X-Rays. X-rays can cause damage to the DNA molecule's phosphate backbone resulting in double-strand breaks. Double-strand breaks result in cleavage of the DNA molecule and are irreversible. Due to the nature of LNC DNA samples, both UV and X- Ray radiation can disproportionally affect LCN DNA samples when compared to traditional DNA samples (Pearlman et al., 1985).

Molecular genetics

Nuclear DNA is located in the nucleus of most cells, and is often referred to as the nuclear genome of an organism. The human nuclear genome is linear and possesses a high level of sophistication. The human nuclear genome is comprised of 22 pairs of autosomal chromosomes and a pair of sex chromosomes. The size of the human genome is approximately 3.2 billion base pairs, which includes intergenic regions, genome-wide repeats, genes and gene related sequences (NHGRI et al., 2003).

Genes, genome-wide repeats, and intergenic DNA regions have different functions and uses to forensic science. Genes are stretches of functional DNA for coding proteins and include exons and introns. Exons are directly transcribed and produce functional proteins, and introns are not functional and are removed during transcription (Figure 3) (Gilbert, 1978). The genetic information within exons has a high degree of similarity between humans, therefore exons are not utilized for forensic human identification. As mentioned above, the genetic information within introns is not transcribed. The nature of introns allows for genetic mutations to arise resulting in significant genetic variances between individuals, and therefore they are commonly used for human identification using current DNA technology.

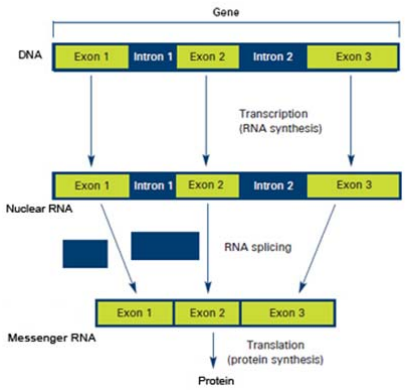

Figure 3. Genes contains stretches of DNA making a protein (exon, green) and stretches of spacer DNA that are not translated (intron, blue). Exons have a high degree of similarity between individual, where as introns have highly possibility to mutate and cause genetic variance. (Gilbert, 1978)

Genome-wide repeats consist of more than 100 bps repetitive fragments. They are dispersed throughout the genome, and can possess multiple copies in tandem, such as *Alu* elements. *Alu* elements are primate-specific repetitive fragments found throughout the nuclear genome that can be used to determine ancestry. Genome-wide repeats are complicated in genetic interpretation. The intergenic region is a stretch of DNA sequences including regulatory regions, MicroRNAs (miRNAs) and microsatellites, which are located between genes. Regulatory regions and miRNAs regulate transcription. miRNAs are used for body fluid identification by a quantitation screening technique (Zubakov et al., 2010). Microsatellites are sequences of DNA nucleotides and are also named Short Tandem Repeats (STRs). Microsatellites are widely dispersed in genomes, highly variable among populations and easy to amplify. Therefore, the majority of DNA regions used for human identification are microsatellites. Eight intergenic loci and five intron repeats were selected for the FBI Combine DNA Index System (CODIS) Core STR Loci (Figure.4) (Butler, 2005).

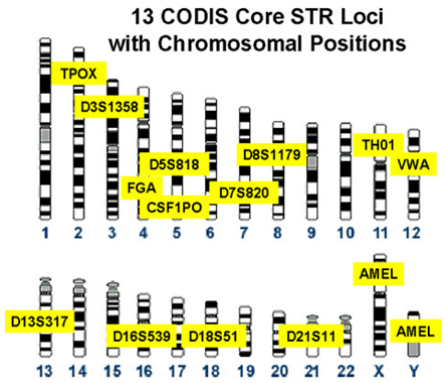

Figure 4. 13 CODIS core STR loci selected for human identification. These 13 CODIS core STR loci and the Amelogenin are standard used by forensic community in the United States. These STR loci are highly variable among the Population in the United States. (http://www.cstl.nist.gov/strbase/fbicore.htm)

STR markers

STRs are short DNA fragments containing 2-5 bp tandem repeats, and typical 100 to 400 bp in total length. The nomenclature of STRs is based on the number of the repeats in the markers. For example, four bp tandem repeats are named tetranucleotides. Tetranucleotides are the most common STRs used in forensic identification. These loci have lower stutter rate and are more dependable than other STR loci (Butler, 2005).

Repeat units lacking one or more nucleotides are referred to as microvariants. Microvariants provide greater genetic variance compared to complete repeat units (Butler, 2005). STR repeats are different not only in the number of repeats, but also the repeat pattern. The pattern can be simple, complex, or compound. Simple repeats are identical in length and sequence. Compound repeats are two or more adjacent simple repeats. Complex repeats are repeats containing varying block of nucleotides. All three types of STRs are used in forensic human identification (Urquhart et al., 1994).

Forensic STR markers require three characteristics: high heterozygosity, distinguishable alleles, and robust amplification. High heterozygosity and distinguishable alleles are necessary to ensure the high discrimination power among a population. The discrimination power in a population among individuals is extremely great when multiple STR loci are analyzed (Butler, 2005; Butler et al., 2003). Simple repeat patterns reduce the difficulty in interpreting the result. STR markers selected for forensic use must be robust in amplification. Therefore, small quantities of DNA samples may be analyzed, such as LCN DNA samples.

Applied Biosystems (Foster City, California) and the Promega Cooperation (Madison, Wisconsin) both developed commercial STR kits that amplify the13 CODIS core STR loci, and both are able to perform multiplex analyses. Current commercial STR kits require one nanogram or less of DNA sample to carry out amplification, but LCN DNA samples are still problematic for the current technique.

DNA extraction

DNA extraction isolates whole genomic DNA from proteins, lipids or other contaminates, and produces purified DNA template for quantification and amplification. Cellular membrane lysis can be enzymatic and osmotic. The PrepFiler® and DNA IQ™ DNA extraction products utilize lysis buffers containing formulations of proteinases, detergents and chaotropic salts to isolate DNA from other biological material (Applied Biosystems, 2008; Promega, 2009a). Commonly included in lysis buffers are proteinase K and sodium dodecylsulfate (SDS) which digest the proteins and lipids of the cellular membranes, respectively. The chaotropic salts present increase the osmotic pressure within the cells of the sample. The combination of high

osmotic pressure and fragile cellar membranes results in the liberation of the genomic DNA. The chemical components of the lysis buffer have no adverse chemical or physical effects on the DNA molecules, therefore intact genomic DNA can be obtained.

The PrepFiler® and DNA IQ™ extraction systems both employ a silica bead based method of DNA purification. The DNA within a given sample becomes affixed to the silica beads. The silica binding technique was first developed to recover DNA fragments in agarose gel by using the silica in glass fiber (Vogelstein & Gillespie, 1979). The silica method has become widely applied to a variety of different nucleic acid recovery, such as plasmid purification (Marko et al, 1982), genomic DNA purification (Yamada et al., 1990), RNA purification (Yamada et al., 1990), and virus DNA and RNA purification (Boom et al., 1990). The silica binding technique has been proven as an efficient and effective method to recover DNA from solution.

There are two hypothesized mechanisms for silica-DNA binding. The cation binding hypothesis describes a cation bridge formation between DNA molecules and the silica surface when the solution contains chaotropic salts (Depasse, 1978). The cation bridge naturalizes the negative charge of DNA molecules and the silica surface and forms electrostatic shielding, which causes the DNA molecules and the silica surface to bind together (Figure 5). The bonds between the DNA molecules and the silica surface are named the silanol group (Depasse, 1978).

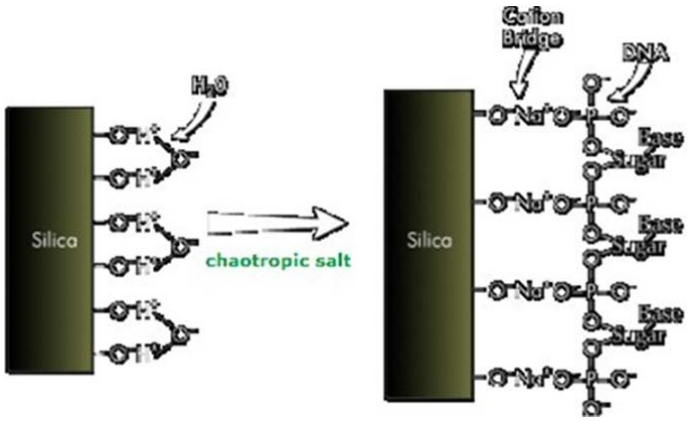

Figure 5. The silica surface possesses hydroxyl group. A Cation bridge forms assisted by the sodium ion in the lysis buffer. DNA molecule backbone binds the silica surface via the bonds provided by the cation bridge.

Silica-DNA binding has also been hypothesized to occur as the result of the formation of hydrogen bonds between the DNA phosphate groups and the silica surface silanol group (Figure 6). Although a single hydrogen binding force is relatively weak, the large number of hydrogen bonds formed during the extraction process allows the DNA molecules to securely attach to the silica surface (Mao et al., 1994).

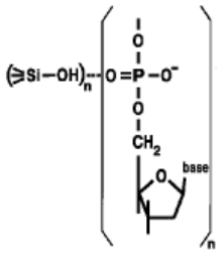

Figure 6. The hydrogen bonds are created between Silica surface and the backbone of DNA molecule. This binding force makes the DNA molecule attached to the silica surface. Hundred of thousand bonds make this force stable. (Mao et al., 1994)

The washing step of both DNA extraction techniques removes remaining lipids, proteins, and contaminants from the solution, and is performed when the DNA molecules are bound to the silica beads. The washing buffers of both PrepFiler® and DNA IQ™ are ethanol based and easily evaporate in an open air environment. Proteins, lipids and other biological

materials, which are soluble in ethanol, are washed away and discarded (Applied Biosystems, 2008; Promega, 2009a).

Once the DNA within the sample has been purified, the elution step releases the DNA molecules from silica beads. The elution solution contains water and 2-Amino-2-hydroxymethyl-propane-1, 3-diol (Tris buffer) which changes the pH environment. The change in pH causes the chemical bonds and hydrogen bonds between the DNA molecules and the silanol to break. DNA molecules are released from the silica surface and back to the elution solution where it is collected and used for additional analysis. (Applied Biosystems, 2008; Promega, 2009a). Figure 7 is for a diagram of the DNA extraction steps.

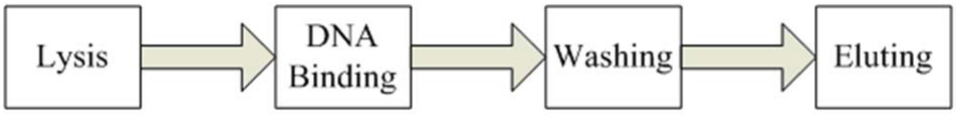

Figure 7. Flowchart of the DNA extraction process for PrepFiler® and DNA IQ™.

The polymerase chain reaction

The Polymerase Chain Reaction (PCR) is an enzymatic reaction that uses a naturally occurring DNA polymerase to create new DNA strands. The PCR process of DNA replication adds deoxyribonucleotides onto a preexisting 3'-OH group of the parent DNA strands. Thermus aquaticus *(Taq)* polymerase is one of the most important enzymes to initiate the PCR reaction and was discovered by Thomas Brock in 1965 (Brock, 1978). The PCR reaction was developed to amplify copies of specific DNA sequences in a short time using thermal cycling technique in 1985 (Mullis et al., 1986). The PCR technique has become a

common and essential technique used in a variety of DNA analyses due to the strength of producing sufficient replicates of samples (Bartlett & Stirling, 2003).

The PCR reaction requires three steps denaturation, primer annealing, and elongation. Denaturation results in the double-stranded structure of DNA separating into two single strands. This is achieved when temperatures are higher than 94℃. The primer will bind to the parent template at the primer binding site prior to the target STRs sequence at 60℃ （Promega, 2011). *Taq* DNA polymerase will bind to the primer-template junction and extend at 75℃, forming a new DNA strand complementary to the parent DNA template. The cycle will return to the denaturation step to start over again. The temperature changes performed during the PCR reaction are known as thermal cycles. The thermal cycling protocol has been adopted by forensic laboratories (Mullis et al., 1986).

Scientists have attempted to optimize the PCR reaction. Regular denature temperature may result in primer templates attached to each other, which is named primer dimer. A higher starting temperature can effectively minimize the chance of primer dimer occurrence. This higher temperature initiation technique is referred as hot start PCR. The PowerPlex® 16 HS system adopted the hot start PCR thermal cycling technique using a modified *Taq* polymerase which is named the AmpliTaq Gold® polymerase. The AmpliTaq Gold® polymerase can be active only after a 95℃ incubation (Innis & Gelfand, 1999). The PCR protocol for the PowerPlex®16 HS system is performed in two parts. The first 10 cycles are initiated with a regular temperature, while the next 22 cycles are initiated with a lower temperature to preserve AmpliTaq Gold® polymerase activity. The detailed thermal cycling protocol for the PowerPlex®16 HS system is divided into five steps as shown in Figure 8.

Protocol for the GeneAmp® PCR System 9700 Thermal Cycler[1]
96°C for 2 minutes, then:
ramp 100% to 94°C for 30 seconds ramp 29% to 60°C for 30 seconds ramp 23% to 70°C for 45 seconds for 10 cycles, then:
ramp 100% to 90°C for 30 seconds ramp 29% to 60°C for 30 seconds ramp 23% to 70°C for 45 seconds for 22 cycles, then:
60°C for 30 minutes
4°C soak

Figure 8. The thermal cycling protocol for the PowerPlex®16 HS system. (Promega, 2011)

The PCR process has been standardized through the use of commercial kits. The Promega PowerPlex® 16 HS system is a commercially available amplification kit. The PowerPlex®16 HS system inherited the advantages of previously validated PowerPlex®16 system, including components such as internal standard (ILS600), allelic ladder, primers, deoxynucleotide triphosphates (dNTPs). In addition, hot start polymerase has been integrated into the premix solution (Figure 9). The PowerPlex®16 HS system has been engineered to overcome inhibited, challenging, and LCN DNA samples, therefore making it an ideal amplification chemistry for analyzing contact samples. During developmental validation, Promega reported full and interpretable DNA profiles could be obtained from as low as 0.1ng DNA, and samples lower than 0.1ng of DNA could be amplified to yield STR profiles (Promega, 2011).

Pre-amplification Components Box (Blue Label)	
1 × 500µl	PowerPlex® HS 5X Master Mix
1 × 250µl	PowerPlex® 16 HS 10X Primer Pair Mix
25µl	9947A DNA (10ng/µl)
5 × 1,250µl	Water, Amplification Grade
Post-amplification Components Box (Beige Label)	
1 × 25µl	PowerPlex® 16 HS Allelic Ladder Mix
1× 150µl	Internal Lane Standard (ILS) 600

Figure 9. Products components of the PowerPlex®16 HS system. (Promega, 2011)

PCR is a dynamic technique and allows for more than one target DNA sequence to be amplified simultaneously (Edwards & Gibbs, 1994), this is referred as multiplex PCR. The primers for multiplex PCR need to be designed with enough complexity to anneal target DNA sequence and to be optimized through validation studies. The PowerPlex®16 HS system is one of the multiplex PCR systems with optimized primers for the loci to be amplified, including 13 CODIS STR markers, Amelogenin and two highly discriminating pentanucleotide STR markers. All sixteen loci are able to be amplified simultaneously in a single injection (Promega, 2011).

DNA quantification

Current STR quantification is based on a modified PCR technique referred to as Real-time or quantitative PCR. Real-time PCR is a development of PCR technology that relies on the detection of fluorescent signal generated from amplified DNA sequence during each cycle of a PCR reaction. The most commonly used analytic method is the TaqMan® assay. The TaqMan® probe is labeled with a florescent dye. The probe anneals on the target DNA sequence between two PCR primers (Ong & Irvine, 2002). The reporter (R) dye is

attached at the 5'-end and the dye quencher (Q) are attached at the 3'-end of the probe. Strand synthesis will break down TaqMan® probes which have hybridized on the target DNA sequence during the PCR reaction. The reporter dye begins to fluoresce after being release from the quencher dye. The quantity of the TaqMan® probe that has been broken determines the strength of the fluorescent signal (Figure 10).

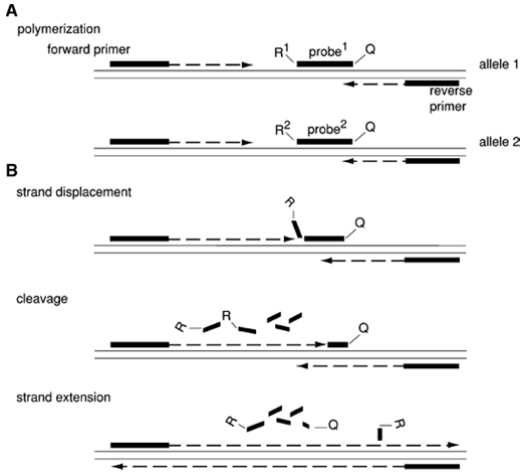

Figure 10. Schematic of TaqMan® assay. The TaqMan® probe anneals the target DNA sequence. The reporter day cannot florescent due to the connected quencher. When the new strand grows, the probe is broken. The reporter becomes fluorescent, when it is separated from the quencher. (Hui et al. 2008)

The Quantifiler® Human DNA Quantification Kit (Applied Biosystems) is one of the validated quantification kits using the real-time PCR technique. An internal PCR control (IPC) is included for each reaction to monitor if the analyses are working correctly. The Quantifiler® kit has been used for forensic analyses on various types of samples, is easily compatible with common extraction techniques, and has a broad dynamic range including the accurate detection of LCN DNA samples (Applied Biosystems, 2010).

Genotyping

Capillary electrophoresis (CE) is a method used to separate DNA fragments based on their size. The DNA fragments in question are the previously amplified STR loci. The florescent dye labeled primer anneals on the target DNA strand during the PCR reaction. These primers are incorporated into the PCR products. The florescent dye on the DNA fragment is excited by a laser that passes through the capillary during electrophoresis, and the excited signal is captured by a charge-coupled device (CCD). The captured signal is analyzed and the corresponding allele peaks of the DNA fragments are generated by the accessory software. The allele peaks represent a human STR genotype for forensic identification. The process for separation and detection of DNA fragments is named genotyping (Figure 11).

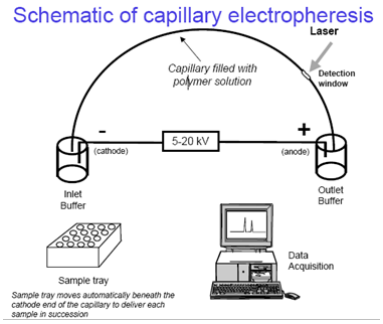

Figure 11. Schematic of capillary electrophoresis instruments used for DNA analysis. The capillary is a narrow glass tube. A polymer buffer is filled inside the capillary as a sieve. The DNA fragments are separated after a high voltage is applied cross the capillary. (Butler, 2005)

ABI 3130 Genetic Analyzer is a commonly used four capillary electrophoresis for forensic genotyping. DNA fragments migrate from the cathode to the anode through the capillary under the influence of an electric field. The POP4 polymer is filled in the capillary as a sieve to separate the DNA fragments. The size of DNA fragments determines the time of retention. One size reference, internal lane standard (ILS), is added into each sample. The ILS

600 provided by Promega consists of 22 bands ranging in size from 60 bp to 600 bp and is used to assign sizes to the DNA fragments separated by CE (McLaren et al., 2008). Four florescent dyes, Fluorescein, JOE, TMR and CXR from the PowerPlex®16 HS system are labeled on the DNA fragments during the PCR reaction. The CCD of the ABI 3130 Genetic Analyzer captures the four florescent dyes near the end of the capillary. The signal is analyzed by the accessory software GeneMapper® ID version 3.2. The allele peaks from different DNA fragments are generated in four color channels (Figure 12) (Promega, 2011).

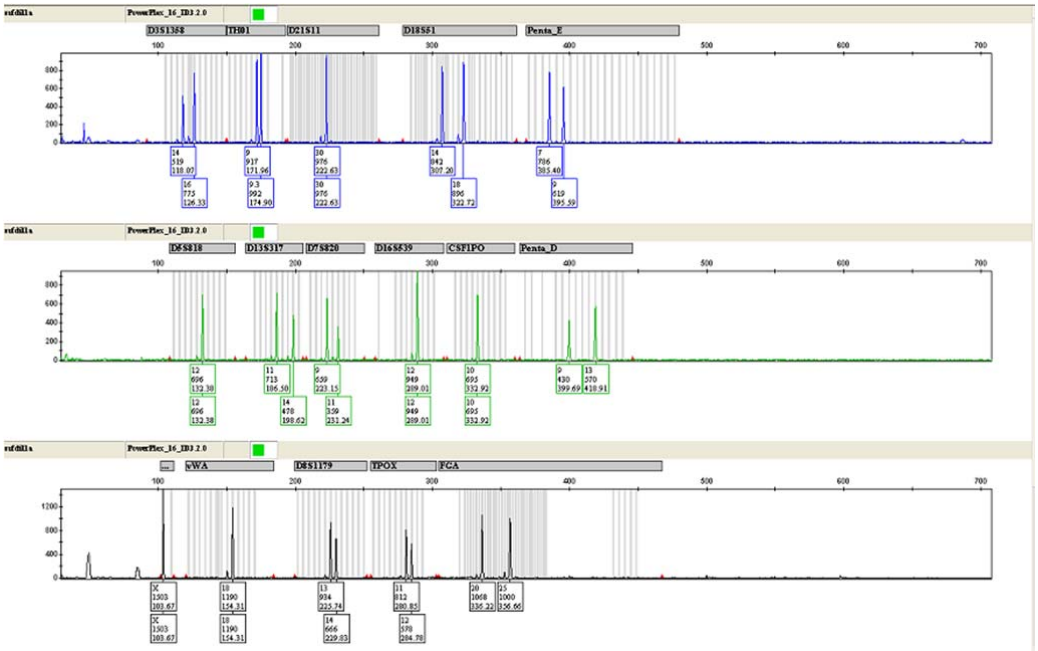

Figure 12. Example of GeneMapper ID® STR data. The blue channel is the fluorescent dye CXR labeled on D3S1358, TH01, D21S11, D18S51 and Penta E loci. The green channel is fluorescein labeled on D5S818, D13S317, D7S820, D16S539, CSF1PO and Penta D loci. The black channel is JOE labeled on Amelogenin, vWA, D8S1179, TPOX, FGA Loci. The red channel is TMR labeled on allelic ladder which is not included here. The allelic ladder is not part of STR profile. (Promega, 2011)

Known extraction issues from previous studies

The DNA IQ™ system from Promega has been a common extraction method for several years in many forensic laboratories. New products, such as the PrepFiler® forensic DNA

extraction kit from Applied Biosystems, continue to come to the market claiming better performance (Applied Biosystems, 2009). Both extraction methods have adopted the silica-binding technique with a simplified process and are able to provide quality DNA samples directly to amplification (Applied Biosystems, 2008; Promega, 2009a). A scientific research study from Applied Biosystems indicated that the PrepFiler® kit can yield higher quantity DNA products than other methods on various types of DNA samples (Figure 13) (Applied Biosystems, 2009). Although the total amount of DNA is higher, this research did not attempt to reflect all commonly encountered sample types, and in particular contact samples or LCN samples.

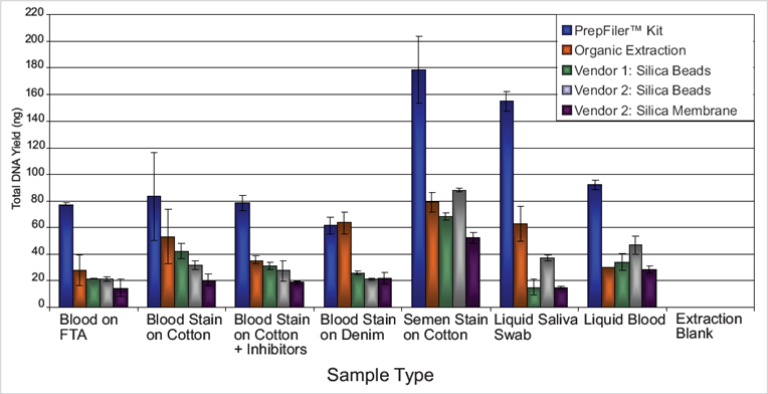

Figure 13. PrepFiler® extraction versus three other vendors and organic extraction The Y-axis is the amount of DNA product after extraction. The X-axis indicates the sample types (Applied Biosystems, 2009).

In the developmental validation study of the PrepFiler® Extraction kit, the researchers performed case-type sample studies on various objects (Figure 14). The researchers quantified the extraction products using a Quantifiler® Human DNA Quantification kit in a forensic laboratory. The results indicated the PrepFiler® kit might not be able to yield sufficient DNA from the LCN DNA samples (Brevnov et al., 2009). An evaluation of

PrepFiler® kit and DNA IQ™ system on LCN DNA samples should also estimate the quality of the STR profile, not only the quantity of DNA.

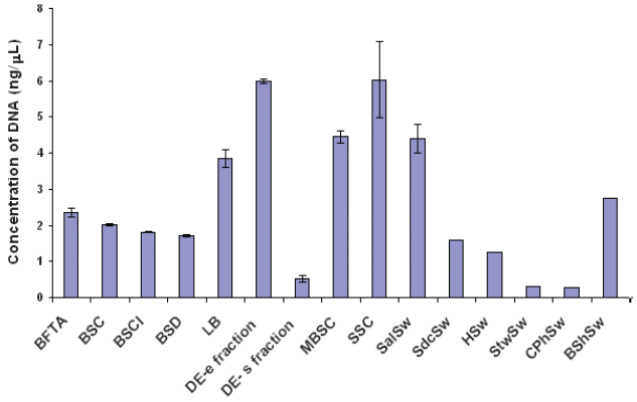

Figure 14. SdcSw, HSw, StwSw, CPhSw & BShSw were contact evidence samples. SdcSw, swab of a soda can; HSw, swab of a hat; StwSw, swab of a steering wheel; CPhSw, swab of a cell phone; BShSw, swab of blood stain on shoe. Others represent regular amount of DNA biological evidence (Brevnov et al., 2009)

.

Scientific evaluation methods

The Analysis of Variance (ANOVA) is a collection of statistical models that have been used to compare the quantity of DNA extraction products in several studies (Viltrop et al., 2010; Cheng et al., 2010). The ANOVA is a statistical test of whether or not the means of several groups are all equal. Doing multiple t-tests would result in an increased chance of committing a type I error. The ANOVA is a useful tool in pair wise comparing two, three or more groups and with several factors. The ANOVA is based on a linear model. The linear regression model is used to determine the relationship between two variables. Given a value for one variable, the value of the other variable is automatically determined (Triola, 2007).

The purpose of an ANOVA either rejects a null hypothesis or fails to reject. In our experiment, the null hypothesis (H_0) states the mean DNA concentrations of different groups are all equal. The F value is calculated to estimate the variance between samples divided by

the variance within samples. The F value is used to determine the P value, and reject or fail to reject the null hypothesis. If the P value is smaller than 0.05, then the null hypothesis is rejected (Triola, 2007).

Standards of validation

Validation is a requirement of good forensic laboratory practices. The validation process establishes the accuracy, precision, and reproducibility of a scientific procedure (SWGDAM, 2003). Validation can help ensure the consistency of DNA yield and the concordance of the STR profile from DNA extractions.

A linear regression study is required by SWGDAM Guideline 2.3, which can estimate the strength of the relationship between the input DNA amount and the extraction products (SWGDAM, 2003). In addition, a linear model is required for the ANOVA analysis. A precision study is also required by SWGDAM Guideline 2.9 to obtain the information of stochastic effect on different samples in different concentration range (SWGDAM, 2003). A repeatability study is required by SWGDAM Guideline 2.5 to evaluate the variation of results from different samples analyzed by the same instrument and operator (SWGDAM, 2003).

A mock case samples study evaluates the combination of all variations such as contamination, stochastic effect, individual difference, and other unknown factors. All samples simulate real forensic evidence (SWGDAM Guideline 2.6, 2003). Because the LCN sample is vulnerable to contamination and other chemical components, which can make the result very difficult to interpret, the cross contamination study by (SWGDAM Guideline 3.6, 2003) and stability studies (SWGDAM Guideline 2.4, 2003) may not be suitable for this

study. This study evaluates the performance of PrepFiler® and DNA IQ™ systems, and illustrates the problem from stochastic effect and contamination with LCN DNA sample interpretation.

CHAPTER TWO

METHODS AND MATERIALS

Contact DNA samples and reference samples from volunteers were collected in the crime scene bay of the Forensic Science Institute (FSI) at the University of Central Oklahoma (UCO). Work spaces were cleaned with a 10% concentration bleach solution followed by pure alcohol wipes. Contact items and tools were also sterilized in the same manner. Sterilization was conducted before and after the collection of biological material from the contact items. Laboratory coat, mask, and gloves to prevent self-contamination were required throughout all sample collection and analysis. Following collection, samples were preserved in a refrigerator at 4℃.

DNA extraction, quantification, amplification, and genotyping were conducted in the Biology Department Molecular Biology Laboratory at UCO. Work spaces, tools, and pipettes were sterilized by pure alcohol wipes. Consumables such as tubes and pipette tips were sterilized by UV radiation prior to use. Sterilization was conducted before and after each step in order to provide a contamination-free environment.

Contact DNA samples and reference samples collection

Volunteers were recruited from the student population at UCO. All five volunteers were female, and their ages ranged from approximately 20 to 30 years of age. Previous studies indicated gender or age was not a contributing factor of an individual's ability to shed DNA on a given item (Phipps & Petricevic, 2007). Volunteers were required to sign a consent form

prior to participating in the project, and the IRB Application (# 10010) was approved before sample collection began.

Sample collections from volunteers were conducted at different times in order to prevent cross-contamination. Volunteers were requested to wash their hands fifteen minutes before contact with any of the items. This protocol was maintained for all contact items which included a wooden baseball bat, a plastic knife handle, a plastic bag, clothing, and paper. These items were selected in an attempt to simulate evidence commonly encountered in case work. Volunteers made contact with one item following the instruction designed by the researcher (Table 1).

Detailed directions for uniform contact by volunteers were provided with each item. The directions were as follows: (1) The volunteer used both hands, held the baseball bat with strong force, and swung it five times; (2) The volunteer used one hand, and grabbed the knife handle with strong force for 30 seconds; (3) The volunteer used one hand and grabbed the plastic bag with moderate force for 30 seconds; (4) The volunteer used one hand and grabbed the clothing with strong force for 30 seconds; (5) The volunteer wrote 20 words on a piece of paper and kept the wrist and hand side touching the surface of the paper (Table 1).

Table 1. Directions for the volunteers to contact with the items for mock case study

Items	Relative force used	Hand contact area	Action	Duration
baseball bat	strong	both palms	swing	five times
knife handle	strong	one palm	grab	thirty seconds
plastic bag	moderate	one palm	grab	thirty seconds
clothing	strong	one palm	grab	thirty seconds
paper	moderate	wrist and hand side	write	twenty words

Items indicates which item was used. Hand contact area indicates major part of hands contacting the item. Action indicates which action was used on that item.

DNA samples were collected from each item using a pair of wet swabs. The swabs were preserved in two uniquely labeled evidence bags, thereby generating two presumed identical samples. Another pair of dry swabs was then used on the previously swabbed site of the item to collect the remaining DNA which may have been left by the wet swab. The dry swabs were separated and preserved. One evidence bag was given an odd identification number, and the other an even identification number. At the conclusion of a collection phase, two evidence envelopes would be generated. Each evidence bag contained one wet and one dry swab. Contact items were sterilized by bleach followed by alcohol wiping after collection. The contact, collection, and sterilization process was repeated for a total of three times. All samples evidence bags were preserved at 4°C.

Buccal swab samples were taken from each volunteer as a reference for comparison and quality assurance purposes. The buccal swabs were put into different evidence bags indicating reference samples and preserved in the refrigerator at 4°C. Total sample numbers for the mock case study were 150 evidence samples and five reference samples, which

coincide with the number of samples for an internal validation study recommended by SWGDAM Guideline 3.0 (SWGDAM, 2003).

DNA IQ™ extraction

Contact DNA evidence from the evidence bags with odd numbers was extracted using the DNA IQ™ System (Promega, Madison, WI, part#DC6700). DNA extraction methods followed the protocols suggested by the manufacturer (Promega, 2009a). One pair of swabs from a single evidence bag was placed into a 2 mL centrifuge tube (USA Scientific, part#1620-2700). A reagent blank was also created at this time. Reagent blank tubes were treated to all the same procedures as the sample tube. To the swabs, 400 µL of lysis buffer was added to the appropriate sample tube. Sample tubes were incubated for 30 minutes at 70°C in a Dri-bath (Thermolyne Type 16500, #229920807548). Swabs were carefully removed from the tubes and inserted into a spin basket (Promega, part#V1221). The spin basket was inserted back into the original 2 mL centrifuge tube. Sample tubes containing the spin baskets were centrifuged for two minutes at 10000 revolutions per minute (RPM). Once the swabs were dry, the spin baskets and swabs were discarded.

At this time, the DNA that was originally collected on the two swabs should be located within the liquid remaining in the 2 mL centrifuge tube. To the remaining liquid sample, 7.0 µL of resin solution was added to each sample tube. The tube was vortexed for 10 seconds and placed on the magnetic stands (Promega, part#Z5332) for five minutes. During the five minute incubation time, each tube was vortex for ten seconds every minute and placed back

on the magnetic stand. The magnetic beads separated from the liquid portion of the sample instantly.

Following incubation, the liquid component was removed and discarded, leaving only the magnetic particles in the tube. The magnetic particles and the DNA bound to them were washed with 100 µL of lysis buffer and vortexed for 10 seconds. The tube was placed back on to the magnetic stand. All lysis buffer was removed from the tube and discarded. The magnetic beads were washed three more times using 100 µL of 2X Wash buffer, vortexed for 10 seconds, and returned to the magnetic stand each time. Each time the liquid solution was discarded. After the final wash, the buffer was removed from the sample tube and the lid was left open for five minutes in order to air dry.

To the dried magnetic beads, 50 µL of elution buffer was added. The contents of the tube were vortexed for 10 seconds, and placed on the hot water bath to incubate for five minutes at 65 °C. The tube was taken out of the water bath, vortexed for 10 seconds and placed immediately on the magnetic stand. The eluate that remained was transferred to a new centrifuge tube and preserved in the freezer at -20°C.

PrepFiler® extraction

Samples from the evidence bags with even numbers were extracted using the PrepFiler® Forensic DNA Extraction Kit (Applied Biosystems, Carlsbad, CA, part# 4392852). DNA extraction methods followed the protocols suggested by the manufacturer (Applied Biosystems, 2008). An empty tube served as the reagent blank and was also collected at this

time. The reagent blank tube was treated to the same procedure as the sample tube. Lysis buffer, at a volume of 400 µL per sample, was added to each tube, vortexed for five seconds, and centrifuge for 10 seconds. The tube was then incubated for 40 minutes at 70 °C, and centrifuged for 10 seconds following incubation. The swabs were removed from the tube and put into a filter column. The filter column was inserted into the corresponding tube and centrifuged for two minutes at 14,000 rpm. The filter column and the swabs were discarded following centrifugation. From the magnetic particle stock solution, 15 µL was pipetted into the sample tube, vortexed for 10 seconds, and centrifuged for another 10 seconds. Isopropanol binding solution at a volume of 180 µL, was added to each tube, vortexed for five seconds, centrifuged for 10 seconds at top speed, and then 10 minutes at low speed 1000 rpm. Each sample was centrifuged once more for 10 seconds at a low speed and placed on the magnetic stand (Applied Biosystems, part# 43928). Separation of the magnetic partials and remaining liquid component started to occur instantly and was completed in one to two minutes. All remaining liquid was removed and discarded. To each sample tube, 300 µL of 1X Wash buffer was added. The tube was removed from the magnetic stand, vortexed for five seconds, centrifuged 10 seconds and placed back in the magnetic stand for 30 to 60 seconds. Again all liquid was removed and discarded. This described washing process was repeated a total of three times. The tube lid was opened and air dry for 10 minutes. Following incubation 50 µL of elution buffers was added to each sample tube. The tube was incubated for five minutes at 70°C, vortexed for five seconds, centrifuged for 10 seconds, and placed back on the magnetic stand. The eluate was transferred to a new microcentrifuge tube and preserved in the freezer at -20°C.

Quantification

All extracted samples were quantified using the Quantifiler® Human DNA Quantitation Kit (Applied Biosystems, part#4343895). DNA quantification methods followed the protocols suggested by the manufacturer (Applied Biosystems, 2010). Eight standard solutions were prepared by performing a serial dilution of the Human genome standard solution provided to achieve the following concentrations: 50 ng/µL, 16.7 ng/µL, 5.56 ng/µL, 1.85 ng/µL, 0.62 ng/µL, 0.21 ng/µL, 0.068 ng/µL, and 0.023 ng/µL. The required real-time PCR reagents were prepared by combining 10.5 µL of primer mix and 12.5 µL reaction mix for each sample well. To each well of the 96-well plate, 23 µL of the prepared reagent was added to 2 µL of diluted DNA standard or unknown DNA sample. The plate was sealed with an adhesive cover and placed in the 7500 Real-time PCR system (Applied Biosystems, part#4351104). The data were analyzed using 7500 System SDS Software v1.2.3 (Applied Biosystems, part#4351104). The concentrations of samples were obtained from the accessory computer and the data provided were used for subsequent DNA analysis procedures (Applied Biosystems, 2010).

Amplification

All contact samples were amplified at the CODIS STR loci using the PowerPlex® 16 HS System (Promega, part# DC2101) kit. DNA amplification methods followed the protocols suggested by the manufacturer (Promega, 2011). Total DNA input for each of the samples was approximately 0.7 ng per PCR amplification. If the contact DNA sample contained an insufficient quantity of template, a total of 17.5 µL of DNA sample was added to the sample

tube. In accordance with quality assurance guidelines, an amplification positive control and a negative control were also created. The PCR amplification mix was prepared as indicated in Table 2 in a 0.5 mL amplification tube. All sample tubes were placed in a 96-Well GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems, part# N8050200). The program protocol for PowerPlex® 16 HS System on GeneAmp® PCR System 9700 is shown in Figure 15.

Table 2. PCR amplification mix for the PowerPlex® 16 HS system

PCR Amplification Mix Component[1]	Volume Per Reaction
Water, Amplification Grade	to a final volume of 25.0µl
PowerPlex® HS 5X Master Mix	5.0µl
PowerPlex® 16 HS 10X Primer Pair Mix	2.5µl
template DNA (0.5–1ng)[2,3]	up to 17.5µl
total reaction volume	**25µl**

The volumes used for amplification were recommended by the guideline provided by the manufacturer. All the component made the final volume to 25µL (Promega, 2011)

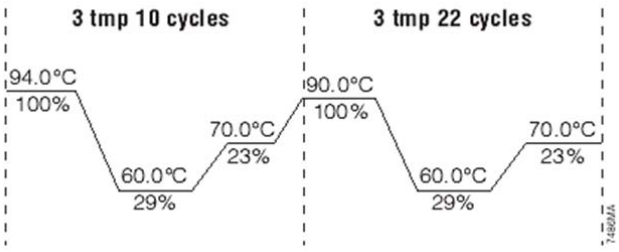

Figure 15. Ramp rates for PowerPlex® 16 HS system. It was recommended by the guideline provided by the manufacturer on 9700 thermal cycle. (Promega, 2011)

Genotyping

Upon completion of STR amplification, all samples were analyzed using the ABI 3130 Genetic Analyzer (Applied Biosystems, part# 3130-01). The genotyping followed the

protocols suggested by the manufacturer (Promega, 2011). To each well on the 96-well plate, 9.5 µL of Hi-Di™ Formamide (Applied Biosystems, part# 4311320) and 0.5 µL of ILS 600 from PowerPlex® 16 HS were added as reaction reagents mix. To the first and the last wells, 1 µL of PowerPlex® 16 HS Allelic Ladder Mix was added. The same volume was added to the positive control, negative control, and amplified samples to the other wells. The plate was sealed with an adhesive cover, and place into the 96-Well GeneAmp PCR System 9700 (Applied Biosystems, part# N8050200) to soak for three minutes at 94 °C, and then placed on ice for five minutes. The prepared plate was placed on the 3130 Genetic Analyzer (Applied Biosystems, part#3130-01). Following the completion of the experimental run, the data obtained was analyzed using GeneMapper® ID v3.2 (Applied Biosystems, part#4338951). All data interpretation was performed by the researcher and verified by the thesis advisor for accuracy.

Sensitivity study and linear regression

The sensitivity study was used to determine the concentration range in which a DNA sample extracted by DNA IQ™ system or PrepFiler® Forensic DNA Extraction Kit would ultimately be able to provide a valid result. The goal of the linear regression study was to quantify the consistency between the yield of DNA obtained from both the DNA IQ™ system and the PrepFiler® Forensic DNA Extraction Kit, and the amount of initial input DNA. A buccal swab taken from volunteer female number two was used as a DNA standard for the sensitivity study and linear regression. The DNA standard was made into serial dilutions at each following concentration: 1.63ng/µL, 0.227ng/µL, 0.0308ng/µL, and 0.0037ng/µL. Each

concentration contained six replicates. Three replicates at each concentration were extracted by DNA IQ™, while the other three replicates at each concentration were extracted by PrepFiler® (Brevnov et al., 2009). After the extraction, the products were quantified using the Quantifiler® Human DNA Quantitation Kit.

For the linear regression and sensitivity study, the independent variable *X* (concentrations of DNA standard) and the dependent variable *Y* (concentrations of DNA standard after extraction) were calculated by SPSS 18 (IBM, Armonk, NY). The equation evaluating the relationship between the two variables was $Y = \alpha + \beta X$. The correlation revealed the consistency of DNA standard input and yield. The concentrations of the DNA standards and dilutions before extraction were treated as the independent variables. The concentrations of the DNA standard and its dilution using DNA IQ™ Systems or PrepFiler® were treated as dependent variables. Linear regression analysis was performed and a slope, intercept, and R square values were generated. Scatter analysis was used to generate a standard curve line graph for both the DNA IQ™ system and PrepFiler® kit. The DNA standard which did not yield a valid result after extraction and quantification was recorded as the low end of the dynamic range.

Precision and accuracy

The precision study was represented by the DNA recovery rate, which revealed the efficiency of the extraction method. The accuracy study was represented by the relative standard deviation (RSD) among the replicates of DNA samples after extraction. Due to the

stochastic effect at LCN levels of DNA input, differences between various concentrations of DNA samples may be observed.

The concentration ranges of 0.5~1.5 ng/μL, 0.05~0.5 ng/μL, 0.005~0.05 ng/μL and 0~0.005 ng/μL were classified as groups one, two, three and four, respectively. Triplicates of the stock DNA were made to provide the average and stable result at different concentration ranges. The mean and standard deviation of overall DNA yield was determined for all three replicates extracted (Promega, 2009b). The concentration of the generated DNA standard prior to the second extraction was divided by the concentration of the DNA standard following the second extraction using the DNA IQ™ system or PrepFiler® Forensic DNA Extraction Kit. The resulting value was multiplied by 100% in order to resolve the recovery rate for a single sample (Equation 1).

Equation1: *Single Sample Recovery Rate ＝DNA standard concentration ／DNA standard concentration after extraction ×100 %*

The corrected recovery rate equals mean of single sample recover rate plus or minus the relative standard deviation. This formula evaluated precision and accuracy of the replicate samples at the same concentrations (Equation 2).

Equation 2: *Corrected Recovery Rate ＝Mean of Single Sample Recovery Rate ±relative standard deviation*

Reproducibility

The reproducibility study was used to compare the difference between the yield of DNA extracted from the DNA IQ™ and PrepFiler® kits on different days. The DNA standard was the same from the sensitivity study. The DNA standard was aliquot into 18 samples to two equal groups, one identified as the DNA IQ™ group, and another as the PrepFiler® group. Each group was again divided into triplicates for each day. Each set of triplicates were extracted on three different days using the standard protocols discussed above. Following DNA extraction, the samples were quantified using Quantifiler® Human DNA Quantitation Kit. Concentrations from each extraction method were analyzed using SPSS 18. Mean concentrations for the three different days were calculated. The standard deviations of the mean concentrations were calculated using the software.

Mock case concentration quantity

The mock case study was used to compare the difference between the concentrations of different extraction methods on different items. Samples labeled with odd numbers were extracted using the DNA IQ™ System. Samples labeled with even numbers were extracted using the PrepFiler® Forensic DNA Extraction Kit. All extracted samples were once more quantified using the Quantifiler® Human DNA Quantitation Kit. The resulting concentrations from each sample were analyzed using SPSS 18. The extraction methods and items sampled were indicted for future comparison. General linear model and univariate analysis were performed on the quantitation data. The concentrations obtained were treated as the

dependent variable, and the item of origin and DNA extraction methods were the fixed factors. A confidence level of 95% was established. A graph was generated using the multiple lines method within the software. The mean of concentrations was left as the variable. The category axis was set as the type of item sampled from. The lines were then defined by the DNA extraction methods (University of Waterloo, 1998).

Mock case profile quality

The mock case profile quality study was used to compare the profile completeness and contamination levels of the mock case samples extracted by different methods. The mock case samples, which yield valid concentration values after extraction were amplified and genotyped using the PowerPlex® 16 HS System on a 96-Well GeneAmp PCR System 9700 and 3130 Genetic Analyzer. Alleles were recognized following the guideline of the UCO PowerPlex® 16 HS internal validation study (Forbes, 2011). The numbers of contaminated, complete profile, incomplete profile, and no profile samples of each extraction system were recorded. The average numbers of true alleles yielded from different types of samples were recorded.

CHAPTER THREE

RESULTS

Sensitivity and linear regression

Twenty-four samples were examined to determine the sensitivity and linear regression. Table 3 depicts the initial DNA concentrations acquired from the first round of analysis using the 7500 System SDS Software v1.2.3. Once the 7500 System SDS Software could not detect any DNA product, an undetected mark was entered instead of the concentration. At the concentration 0.0037 ng/μL, it was observed that the analysis technique was no longer able to detect a sufficient concentration of DNA (See Table 3). This concentration was viewed as the detection threshold for the 7500 quantification system. Because most undetected results from quantification were not typable (Lewis, 2006), the use of undetected results was limited. Samples with undetected results were used for amplification and genotyping in our experiment. Because the undetected concentration samples contained no DNA product recognized by the software, the undetected results were considered as 0 ng/μL in the following statistical calculations.

Table 3. The concentrations for both extraction kits at 7500 quantification system for the sensitivity study (Input volume 2µL)

Sample Name	DNA concentration before extraction (ng/µL)	DNA dilutions after DNA IQ™ extraction (ng/µL)	DNA dilutions after PrepFiler® extraction (ng/µL)
dil1r1	1.6300	0.9830	0.9470
dil1r2	1.6300	1.2200	1.2500
dil1r3	1.6300	1.1000	1.2400
dil2r1	0.2270	0.0487	0.1270
dil2r2	0.2270	0.1060	0.1210
dil2r3	0.2270	0.0973	0.1530
dil3r1	0.0308	0.0070	0.0141
dil3r2	0.0308	0.0158	0.0172
dil3r3	0.0308	0.0098	0.0169
dil4r1	0.0037	Undetected*	0.0058
dil4r2	0.0037	0.0018	Undetected*
dil4r3	0.0037	0.0019	0.0039

"dil" was the abbreviation of "dilution". "r" was the abbreviation of "replicate". "dil1r1" was serial dilution concentration range one, replicate one. The elution volume for DNA extraction was 50 µL.

*Undetected results indicated the concentration cannot be acquired by the 7500 System SDS Software v1.2.3.

The R square value, X intercept, and slope for the linear regression function for both the DNA IQ™ system and PrepFiler® kit assays were calculated. The resulting linear regression function for the analysis of the DNA IQ™ system extraction (Y) verses the known DNA standard samples (X) was $Y=0.688X-0.026$, $R^2=0.985$ ($P<0.05$), using DNA concentrations ranging from 0.0037 ng/µL to 1.6300 ng/µL (Figure 16). The resulting linear regression function for the analysis of the PrepFiler® Forensic DNA Extraction Kit (Y) verses the known DNA standard samples (X) was $Y=0.706X-0.01$, $R^2=0.978$ ($P<0.05$), using DNA concentrations ranging from 0.0037 ng/µL to 1.6300 ng/µL (Figure 17). Both R square values indicate good strength (above 95%) of the relation between the quantity of DNA sample input and quantity of DNA extraction products. However, the linear regression equation did not

perfectly correspond with the input DNA concentration and the DNA extraction products at the LCN DNA concentration range due to the stochastic effect.

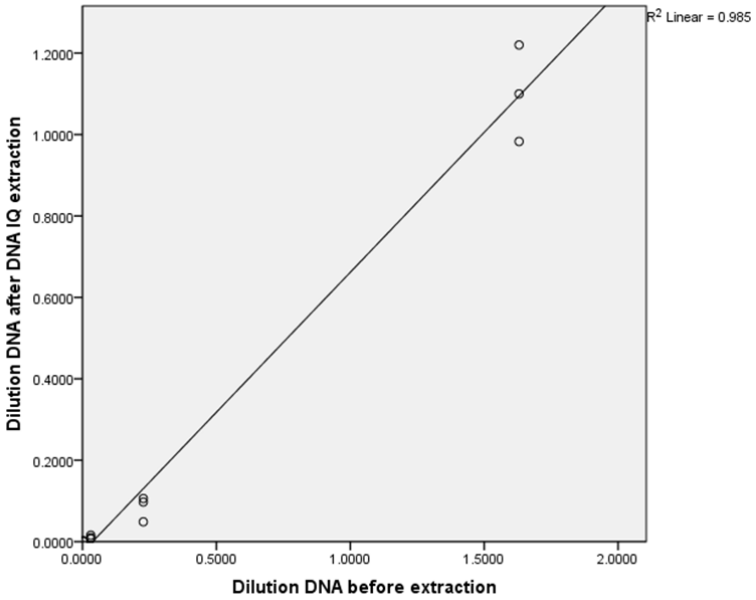

Figure 16. Spots indicate the concentrations of samples. The regression equation and graph were generated by the Coefficients and R squared. The Y axis was the concentrations (ng/µL) of DNA dilution samples extracted using the DNA IQ™ system. The X axis was the concentrations (ng/µL) of the DNA dilution samples without extraction.

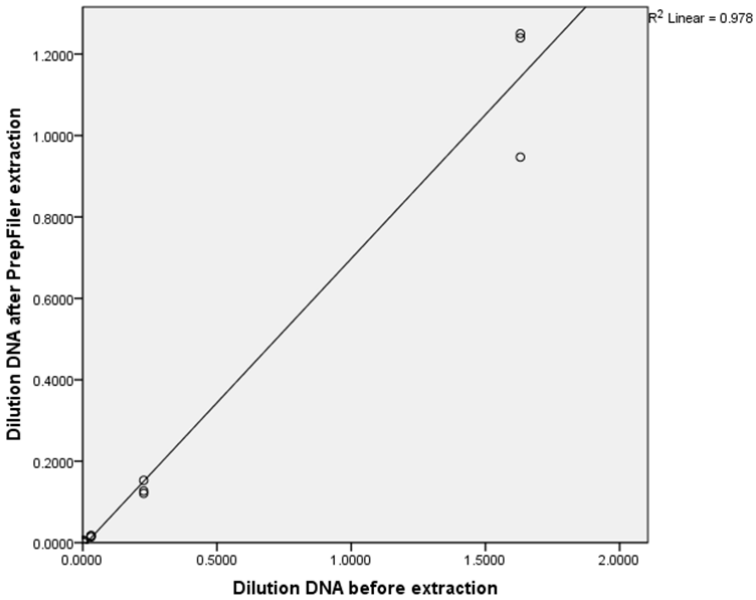

Figure 17. Spots indicate the concentrations of samples. The regression equation and graph were generated by the Coefficients and R squared. The Y axis was the concentrations (ng/µL) of the DNA dilution samples extracted using the PrepFiler® Forensic DNA Extraction Kit. The X axis was the concentrations (ng/µL) of the DNA dilution samples without extraction.

Precision and accuracy

The precision study was used to indicate the consistency of DNA yield from the extraction and quantification experiments in a single day performed by the same researcher. The accuracy, represented by recovery rate, demonstrates the efficiency of each extraction method studied. The result reported here is the corrected recovery rate which was calculated using the mean value of the recovery rates plus or minus the relative standard deviation of the recovery rates of each of the replicated DNA samples. The corrected recovery rates of DNA IQ™ system and PrepFiler® Kit are shown below (Table 4).

Table 4. Recovery rates of both extraction methods at 7500 quantification system for the accuracy and precision studies

DNA input concentration levels (ng/μL)	DNA IQ™ corrected recovery rate (%)	PrepFiler® corrected recovery rate (%)
0.5~1.5	67.55±7.27	70.29±10.56
0.05~0.5	37.00±13.61	58.88±7.50
0.005~0.05	35.28±14.60	52.16±5.55
0~0.005	33.33±28.90	87.39±79.91

A Tukey's honest significance test has been performed. The recovery rate was the mean of recovery rate corrected with relative standard deviation. The frontal number represented the accuracy, and the latter number represented the precision.

Reproducibility

Reproducibility was the variation in measurements performed by a single person on the DNA samples under the same extraction method performed on different days. Eighteen concentrations of DNA extraction products were yielded (See Appendix A). The standard deviation for the DNA IQ™ extraction was 0.18448 and 0.29052 for the PrepFiler® extraction (Table 5). None of the replicates for either chemistry resulted in a concentration value above or below two standard deviations of the mean yield concentrations over the three days (Figure 18). Both extraction methods provided reproducible results. Based on the

previous precision and accuracy study, the standard deviations between the concentrations of LCN DNA samples were not consistent with the means. Therefore, DNA samples at LCN concentrations were not attempted in the reproducibility study.

Table 5. DNA sample replicates extracted in three different days by both extraction kits and quantified by Quantifiler® for the reproducibility study (ng)

Extraction methods on a same sample	Mean concentration extracted in day 1	Mean concentration extracted in day 2	Mean concentration extracted in day 3	Mean concentration extraction in 3 days	Standard deviation
DNA IQ™	4.08	4.33	3.97	4.13	0.18448
PrepFiler®	4.29	4.61	4.03	4.31	0.29052

The standard deviation was calculated from these concentrations of the replicates from different days. No outlier was observed. The number of replicates in each day was three.

Mock case concentration quantity

The mock case DNA concentration quantity study evaluated the totality of each extraction's efficiency on different types of the contact items. Quantification yielded 62 out 150 (41.3%) valid DNA samples with a concentration suitable for further DNA analysis. Among the 62 samples, 40 samples were extracted using the DNA IQ™ system, whereas 22 samples were extracted using the PrepFiler® Forensic DNA Extraction Kit. Ten reagent blanks were also analyzed and each yielded the correct undetected result. The highest yield of DNA from a mock case sample was 0.152 ng/µL and this was obtained using the PrepFiler® kit (See Appendix B).

The differences in DNA concentrations yielded were affected by three main factors: items from which DNA was collected, extraction methods, and items interacting with extraction methods. Figure 18 presents the mean of both extraction methods on different items. The DNA IQ™ extraction showed higher DNA yield on the knife handle, paper and cotton cloth. The PrepFiler® extraction showed higher yield on baseball bat and plastic bag.

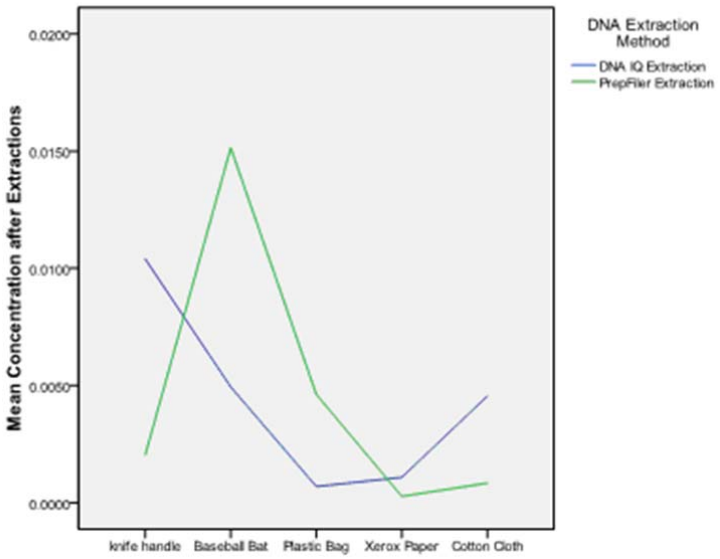

Figure 18. The Y axis was the mean of the concentrations. The X axis was the name of items. The blue line represents DNA IQ™ system. The green line represents PrepFiler® Forensic DNA Extraction Kit. The DNA IQ™ extraction showed higher DNA yield on the knife handle, paper and cotton cloth. The PrepFiler® extraction showed higher yield on baseball bat and plastic bag. Most concentrations were below or close to LCN DNA sample. The DNA concentrations from paper were the lowest among the five items.

An ANOVA comparing the means of the DNA concentrations collected from the different types of items was used to determine the difference in efficiency between the DNA IQ™ system and the PrepFiler® Forensic DNA extraction kit. The null hypothesis stated that the mean DNA concentrations for the samples extracted using the DNA IQ™ system would be the same as those using the PrepFiler® extraction system. On each type of item, there was no statistical difference between the mean concentrations of DNA products extracted using DNA IQ™ system or the PrepFiler® Forensic DNA extraction kit. The P values for the ANOVA test were P=0.107 for the knife handle, P=0.05 for the baseball bat, P=0.448 for the plastic bag, P=0.875 for the paper and P=0.473 for the cotton cloth (See Appendix C). The significance levels for all types of items were above or equaled P=0.05. Therefore, there was

no significant difference in selecting the extraction methods use to isolate DNA samples from the contact samples used in our experiment.

An ANOVA analysis was also used to compare the mean concentrations of DNA extraction products extracted from different types of items. The pairwise comparisons included all possible combinations of the contact items used in this experiment. For example, baseball bat versus plastic bag, baseball bat versus knife handle, etc. The null hypothesis stated that the concentrations of DNA obtained from the baseball bat should be the same as the concentrations of DNA obtained from the plastic bag, and so on for each of the combinations purposed above. There was no statistically significant difference in DNA extraction products for all pairwise comparisons ($P < 0.05$) (See Appendix D).

An ANOVA analysis was performed to analyze the mean concentrations of DNA extraction products affected by interaction between collection surface and extraction method. The null hypothesis stated that the mean DNA concentrations yielded by one extraction method on a particular type of item should be the same. The significant level between the means of the DNA concentrations by the extraction methods interacting with items was 0.112, which was above 0.05 (See Appendix E). There was no significant difference the extraction method used on a particular item in our experiment.

Mock case profile quality

Quantification yielded 62 valid concentrations from a total of 150 samples that were suitable for further DNA analysis. Forty samples were extracted using the DNA IQ™ system and 22 samples were extracted using the PrepFiler® Forensic DNA Extraction Kit. The 62

suitable DNA samples were amplified and genotyped using PowerPlex® 16 HS on a ABI 3130 Genetic Analyzer and GeneMapper® ID v3.2 analysis software.

Thirty samples yield incomplete or complete STR profiles, whereas 32 yield no profile. Twenty-nine samples which yielded STR profiles were extracted using the DNA IQ™ system, whereas only one sample which yielded a STR profile was extracted using the PrepFiler® Forensic DNA Extraction Kit (Table 5).

Among the 29 STR profiles yielded by the DNA IQ™ system, only one sample contained the complete DNA profile without allele drop-in and allele drop-out. Twenty-two samples showed signs of contamination, artifacting, stochastic effect, and varying levels of allele drop-in and drop-out. All of these artifacts made the interpretation of these profiles extremely difficult. In addition, five contaminated samples contained alleles similar in appearance to the allelic ladder. The only profile obtained by the PrepFiler® Kit yielded a complete DNA profile without any contamination, allele drop-in or allele drop-out (Table 6).

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Table 6. STR profiles acquired from contact DNA mock case samples extracted by DNA IQ™ and PrepFiler® kit

	DNA IQ™	PrepFiler®		DNA IQ™	PrepFiler®
CXWBBTS1,2	PROFILE	NP	KLKHTS3,4	PROFILE	NP
CXWBBTS3,4	PROFILE	PROFILE	KLKHTS5,6	PROFILE	NP
CXWBBTS5,6	PROFILE	NP	KLPBTS1,2	NP	NP
CXWCTS1,2	PROFILE*	Undet	KLPBTS5,6	Undet	NP
CXWCTS3,4	PROFILE*	Undet	KLPTS3,4	PROFILE	Undet
CXWCTS5,6	PROFILE*	NP	KLPTS5,6	PROFILE	Undet
CXWKHTS1,2	PROFILE*	Undet	MFBBTS1,2	PROFILE	NP
CXWKHTS3,4	Undet	NP	MFBBTS3,4	NP	NP
CXWKHTS5,6	NP	Undet	MFCTS1,2	PROFILE	Undet
CXWPBTS1,2	PROFILE	NP	MFCTS3,4	NP	Undet
CXWPBTS3,4	PROFILE	NP	MFCTS5,6	PROFILE	Undet
CXWPBTS5,6	NP	NP	MFKHTS1,2	PROFILE	Undet
CXWPTS5,6	NP	Undet	MFKHTS3,4	PROFILE	Undet
ERCTS1,2	PROFILE	Undet	MFKHTS5,6	NP	NP
ERCTS3,4	PROFILE	Undet	MFPBTS5,6	PROFILE	NP
KLBBTS1,2	PROFILE	Undet	YXBBTS1,2	NP	NP
KLBBTS3,4	NP	NP	YXBBTS3,4	NP	Undet
KLBBTS5,6	PROFILE	NP	YXBBTS5,6	NP	NP
KLCTS1,2	PROFILE	Undet	YXCTS3,4	PROFILE**	Undet
KLCTS3,4	PROFILE	Undet	YXCTS5,6	PROFILE	Undet
KLCTS5,6	Undet	NP	YXPBTS1,2	PROFILE*	NP
KLKHTS1,2	PROFILE	NP			

The sample name indicates a pair of replicate samples. The odd number was extracted using DNA IQ™ system. The even number was extracted using PrepFiler® kit. “Undet” indicates an undetected result from quantification, and was not genotyped. “NP” indicates a non-STR profile had been acquired. “Profile” with yellow color indicates STR profile with allele drop-in and drop-out. “Profile” with green color indicates a complete STR profile without allele drop-in and drop-out. *STR profile contaminated with suspicious allelic ladder. **STR profile was from one unrelated individual.

CHAPTER FOUR

DISCUSSION

Sensitivity and linear regression

The sensitivity study was conducted in order to identify the DNA concentrations that would reliably provide a quantification result. The lowest concentration obtained was 0.0018 ng/µL which is lower than the dynamic range of the DNA standards used to generate the standard curve (0.023 ng/µL to 50 ng/µL) in the Quantifiler® human DNA quantification kit (Applied Biosystems, 2010). The threshold of detection for the Quantifiler® human DNA quantification kit using the 7500 quantification system equals 3.6 pg, which was much smaller than the definition of LCN DNA sample. Therefore, the Quantifiler® chemistry and real-time PCR analysis demonstrated the ability to detect and analyze DNA samples at and below the quantity defined as LCN level. Undetected results may have resulted from the 7500 Real time PCR instrument's detection ability or possible stochastic effects. No matter which factor caused the undetected result, concentrations close to 3.6 pg were not recommended to be used for the 7500 quantification system.

The DNA IQ™ system and PrepFiler® kit demonstrated a strong linear regression between the input DNA and extraction outcomes. An agreement between this study and the validation study of the PrepFiler® Forensic DNA Extraction Kit was reached (Brevnov et al., 2009). In the PrepFiler® validation study, the linear regression study used volumes of liquid blood and compared the extracted DNA results. In this study, neat DNA was used instead of the liquid blood, and the focus was on the efficiency of the DNA silica binding process. However, the ideal slope value of one was not achieved for both extraction systems. This

result indicated that for both extraction systems there was notable DNA loss during the extraction process.

Precision and accuracy

Accuracy study demonstrated the distance from the expectation, and precision study was focusing on whether there is consistency of the results from replicates. The recovery rate study revealed that the lower the concentration, the lower the accuracy of the DNA IQ™ system and PrepFiler® kit. But the PrepFiler® kit lost fewer DNA molecules when the DNA concentration reached the LCN DNA range, whereas DNA IQ™ system failed to recover half of the amount of DNA at the LCN DNA concentration range. The study indicated the potential capability of the PrepFiler® kit to recover LCN DNA samples.

The PrepFiler® kit had higher precision than DNA IQ™ system. Higher precision provided stable DNA concentration for amplification. According to the internal validation study of PowerPlex 16 HS, consistent DNA concentration would help calculate the ideal DNA volume and yield better STR profiles (Forbes, 2011). The PrepFiler® kit was the recommended extraction system when DNA concentration ranged from 0.005~1.5 ng/μL.

Reproducibility

The reproducibility study indicated the variance within the DNA concentrations obtained over a time period. A set of neat DNA sample replicates were extracted using the DNA IQ™ system or PrepFiler® Kit on three different days. The mean and standard deviation values indicated that the concentration of DNA in the extract for each sample was repeatable on

three different days. The reproducibility study indicated that the chemical components were stable, but also revealed that the samples were stored at the appropriate condition. No observable DNA degradation was indicated during the reproducibility study. DNA samples preserved at 4℃ in the refrigerator for a short term was recommended.

Mockcase concentration quantity

The mock case study focused on the DNA concentrations yielded by differences in multiple factors such as extraction methods and item to item interaction with each extraction method. The DNA IQ™ system and PrepFiler® Kit provided similar statistical results for all 150 samples. The concentrations of most of the DNA extraction products, for both extraction systems, were below the LCN DNA threshold. Because the nature of contact DNA has no indicator of the initial amount, no firm recommendation for either extraction kit could be made based on the overall DNA concentrations without the statistical calculation. According to the internal validation study of the PowerPlex® 16 HS system, only four DNA samples would match the threshold for amplification without the occurrence of allelic drop-out. This study demonstrated that the use of contact DNA samples for STR analysis should be handled with caution due to low DNA yield.

The baseball bat was the best medium for transfer of LCN DNA, while paper yielded the lowest DNA concentration of the five items tested. Different items demonstrated individual capability of transferring and maintaining DNA samples, but there was no statistical difference. As expected, the average amount of DNA yielded from the five items was low or

lower than the definition of LCN DNA. More than half of the samples analyzed yielded no result in quantification.

This study demonstrated that low quantities of DNA are common from contact evidence, with more than half of the samples yielding theoretical no DNA. In addition, most of the samples with low DNA quantity were far below the threshold defined for LCN DNA. Since the use of LCN DNA samples is still problematic with current technology, the application of DNA samples with even lower quantity should not be used for forensic case work.

Mockcase profile quality

The PrepFiler® Kit and DNA IQ™ system provided at least one complete profile out of 150 samples. The success rate was as low as 1.3%. Although the application of using contact DNA samples was feasible, more studies on the validation of other improvements or methods are required. Neither the PrepFiler® Kit nor the DNA IQ™ system was suitable for processing contact DNA samples.

Contamination might occur during the collecting process, even though the sterilization methods were applied during collection, extraction and other experimental methods. Most possible contamination was assumed to have come from the process of mock case sample collection, no DNA contamination was observed in all reagent blanks from the extractions. Another contamination source might have been from sharing the storage space and workspace with other researchers within the laboratory. The results revealed the difficulty dealing with LCN DNA samples. Improved sterilization techniques are recommended when

analyzing LCN DNA samples. Separated laboratory workspace for LCN DNA analysis is recommended to prevent contamination.

Conclusion

The DNA IQ™ system and PrepFiler® Kit were able to provide a linear yield of DNA concentration from the range 0.0037 to 1.63 ng/μL. Both extraction methods provided limited accuracy and precision when used with LCN DNA samples. Although both extraction methods were able to yield DNA from contact samples, the average yield was fairly low. The STR profiles yielded from the contact items were extremely difficult to analyze. Based on this study, neither the DNA IQ™ system nor the PrepFiler® Kit would be recommended for forensic analysis of LCN DNA samples. The success rate was 1.3% of yielding a complete STR profile for both extraction kits.

In the Schiffner et al. study (2005), an optimized LCN DNA extraction procedure resulted in an increased recovery rate over the standard procedure. The optimized procedure demonstrated the ability to facilitate STR genotyping using LCN DNA samples. During our mock case sample study, both extraction kits did not yield adequate amplification quantities of DNA for the majority of samples. The standard procedures recommended by the manufacturer of both kits may not be suitable for LCN DNA extraction. An optimized procedure for the DNA IQ™ and PrepFiler® kits for the extraction LCN DNA samples requires additional research. In addition, the PowerPlex® 16 HS system was not compatible for LCN DNA analysis. A validation study of testing LCN DNA samples using AmpFlSTR® Identifiler® indicated that using the procedure and guideline they have generated, LCN DNA

analysis was reliable and robust (Caragine et al., 2009). Therefore, a quality application of LCN DNA analysis required an optimized procedure for not only the extraction process but also the amplification process. An optimized amplification kit incorporated with a validated extraction method focusing on LCN DNA analysis would facilitate greater quality within STR profiles.

Experimental conditions are different from those encountered in forensic case work. LCN DNA analysis demonstrates the potential uses for contact DNA samples at crime scenes. Cross-contamination will continue to be problematic even as sterilization techniques improve. Even if a STR profile had been generated, the next issue is determining the relevance of the profile. Before national guidelines and procedures for LCN DNA sample analysis are produced, the use of contact DNA analysis should be avoided or used with great caution.

Following studies should focus on improving the techniques associated with LCN DNA analysis prior to conducting research on contact sample. Current quantification systems are capable for LCN DNA analysis, but the extraction systems urgently require greater improves. Possible improvements may include the use of carrier RNA, optimization of SDS concentration in the lysis buffer and a DNA concentration step using Microcon-100s (Schiffner et al., 2005). The effectiveness of current sterilization techniques for LNC DNA analysis should also be examined. A detailed study is recommended to investigate the possibility of introducing contamination during each step of the DNA analysis process. An optimized procedure using improved sterilization technique should be investigated. In addition, other amplification system should be evaluated against the PowerPlex® 16 HS system in LCN DNA analysis scenarios, such as the AmpFlSTR® Identifiler® system

(Caragine et al., 2009). Until the recommended improvements are accomplished, LCN DNA analysis is not recommended for forensic case work.

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APPENDIX

Appendix A. Concentrations of replicate samples extraction in different days for reproducibility study. (ng)

DNA IQ	Day1	Day2	Day3
mfbsd11	3.891	4.853	3.837
mfbsd12	4.252	4.646	3.734
mfbsd13	4.111	3.482	4.345
PrepFiler	Day1	Day2	Day3
mfbsd21	4.463	4.761	3.898
mfbsd22	4.276	4.765	4.167
mfbsd33	4.012	4.304	4.025

Replicate samples were reference samples collected from one individual. The elution volume was 50 µL. The mean of the concentrations in a single day was calculated. The standard deviation was the variance among the means over three different days.

Appendix B. Concentrations of samples defined by extraction methods.

Knife Handle			
DNA IQ Extraction	**Concentration (ng/µL)**	**PrepFiler Extraction**	**Concentration (ng/µL)**
ERKHTS1	Undet.	ERKHTS2	Undet.
ERKHTS3	Undet.	ERKHTS4	Undet.
ERKHTS5	Undet.	ERKHTS6	Undet.
MFKHTS1	0.00167	MFKHTS2	Undet.
MFKHTS3	0.00283	MFKHTS4	Undet.
MFKHTS5	0.00465	MFKHTS6	0.000912
KLKHTS1	0.0365	KLKHTS2	0.0117
KLKHTS3	0.0492	KLKHTS4	0.0145
KLKHTS5	0.0494	KLKHTS6	0.000424
YXKHTS1	Undet.	YXKHTS2	Undet.
YXKHTS3	Undet.	YXKHTS4	Undet.
YXKHTS5	Undet.	YXKHTS6	Undet.
CXWKHTS1	0.00917	CXWKHTS2	Undet.
CXWKHTS3	Undet.	CXWKHTS4	0.0031
CXWKHTS5	0.00261	CXWKHTS6	Undet.

Baseball Bat			
DNA IQ Extraction	**Concentration (ng/µL)**	**PrepFiler Extraction**	**Concentration (ng/µL)**
ERBBTS1	Undet.	ERBBTS2	Undet.
ERBBTS3	Undet.	ERBBTS4	Undet.
ERBBTS5	Undet.	ERBBTS6	Undet.
MFBBTS1	0.0149	MFBBTS2	0.00324
MFBBTS3	0.0025	MFBBTS4	0.00816
MFBBTS5	Undet.	MFBBTS6	Undet.
KLBBTS1	0.00966	KLBBTS2	Undet.
KLBBTS3	0.0071	KLBBTS4	0.00124
KLBBTS5	0.00666	KLBBTS6	0.00127
YXBBTS1	0.0027	YXBBTS2	0.0288
YXBBTS3	0.0162	YXBBTS4	0.0049
YXBBTS5	0.00449	YXBBTS6	0.00415
CXWBBTS1	0.00079	CXWBBTS2	0.00293
CXWBBTS3	0.00184	CXWBBTS4	0.152
CXWBBTS5	0.00733	CXWBBTS6	0.0204

Plastic Bag			
DNA IQ Extraction	**Concentration (ng/µL)**	**PrepFiler Extraction**	**Concentration (ng/µL)**
ERPBTS1	Undet.	ERPBTS2	Undet.
ERPBTS3	Undet.	ERPBTS4	Undet.
ERPBTS5	Undet.	ERPBTS6	Undet.
MFPBTS1	Undet.	MFPBTS2	Undet.
MFPBTS3	Undet.	MFPBTS4	Undet.
MFPBTS5	0.000831	MFPBTS6	0.00407
KLPBTS1	0.000876	KLPBTS2	0.0207
KLPBTS3	Undet.	KLPBTS4	Undet.
KLPBTS5	Undet.	KLPBTS6	0.0239
YXPBTS1	0.00207	YXPBTS2	0.00767

YXPBTS3	**Undet.**	**YXPBTS4**	**Undet.**
YXPBTS5	**Undet.**	**YXPBTS6**	**Undet.**
CXWPBTS1	**0.00193**	**CXWPBTS2**	**0.00589**
CXWPBTS3	**0.00257**	**CXWPBTS4**	**0.00334**
CXWPBTS5	**0.00226**	**CXWPBTS6**	**0.00384**
Paper			
DNA IQ Extraction	**Concentration (ng/µL)**	**PrepFiler Extraction**	**Concentration (ng/µL)**
ERPTS1	**Undet.**	**ERPTS2**	**Undet.**
ERPTS3	**Undet.**	**ERPTS4**	**Undet.**
ERPTS5	**Undet.**	**ERPTS6**	**Undet.**
MFPTS1	**Undet.**	**MFPTS2**	**Undet.**
MFPTS3	**Undet.**	**MFPTS4**	**Undet.**
MFPTS5	**Undet.**	**MFPTS6**	**Undet.**
KLPTS1	**Undet.**	**KLPTS2**	**Undet.**
KLPTS3	**0.0034**	**KLPTS4**	**Undet.**
KLPTS5	**0.013**	**KLPTS6**	**Undet.**
YXPTS1	**Undet.**	**YXPTS2**	**Undet.**
YXPTS3	**Undet.**	**YXPTS4**	**Undet.**
YXPTS5	**Undet.**	**YXPTS6**	**Undet.**
CXWPTS1	**Undet.**	**CXWPTS2**	**Undet.**
CXWPTS3	**Undet.**	**CXWPTS4**	**Undet.**
CXWPTS5	**Undet.**	**CXWPTS6**	**0.00417**
Cotton Cloth			
DNA IQ Extraction	**Concentration (ng/µL)**	**PrepFiler Extraction**	**Concentration (ng/µL)**
ERCTS1	**0.0218**	**ERCTS2**	**Undet.**
ERCTS3	**0.00191**	**ERCTS4**	**Undet.**
ERCTS5	**Undet.**	**ERCTS6**	**Undet.**
MFCTS1	**0.00878**	**MFCTS2**	**Undet.**
MFCTS3	**0.002**	**MFCTS4**	**Undet.**
MFCTS5	**0.00434**	**MFCTS6**	**Undet.**
KLCTS1	**0.0024**	**KLCTS2**	**Undet.**
KLCTS3	**0.00228**	**KLCTS4**	**Undet.**
KLCTS5	**Undet.**	**KLCTS6**	**0.00856**
YXCTS1	**Undet.**	**YXCTS2**	**Undet.**
YXCTS3	**0.00865**	**YXCTS4**	**Undet.**
YXCTS5	**0.00391**	**YXCTS6**	**Undet.**
CXWCTS1	**0.00493**	**CXWCTS2**	**Undet.**
CXWCTS3	**0.00247**	**CXWCTS4**	**Undet.**
CXWCTS5	**0.00493**	**CXWCTS6**	**0.00417**

Raw data of mock case quantity study was indicated in this table. Undetected indicated no DNA product had been observed by 7500 quantification system. Each mock case sample was given a unique id. The id was able to distinguish the extraction method and item sampled from. The odd number samples were extracted using DNA IQ™ kit, whereas the even number samples were extracted using PrepFiler® kit.

Appendix C. Comparison of concentrations from different extraction methods

Sample collected from which surface		Sum of Squares	df	Mean Square	F	Sig.
knife handle	Contrast	.001	1	.001	2.626	.107
	Error	.028	140	.000		
Baseball Bat	Contrast	.001	1	.001	3.906	.050
	Error	.028	140	.000		
Plastic Bag	Contrast	.000	1	.000	.579	.448
	Error	.028	140	.000		
Paper	Contrast	4.986E-6	1	4.986E-6	.025	.875
	Error	.028	140	.000		
Cotton Cloth	Contrast	.000	1	.000	.518	.473
	Error	.028	140	.000		

This table was generated by SPSS 18. “Sig.” column indicates the significance of different extraction methods Used. If the significance was less than 0.05, then reject the null. There were no significant difference between DNA IQ™ and PrepFiler® kit, no matter which item sampled from.

Appendix D. Multiple comparisons of concentrations after extraction from different items

(I) Sample collected from which surface	(J) Sample collected from which surface	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
knife handle	Baseball Bat	-.003820	.0036474	.833	-.013900	.006260
	Plastic Bag	.003557	.0036474	.866	-.006523	.013637
	Paper	.005537	.0036474	.553	-.004544	.015617
	Cotton Cloth	.003518	.0036474	.871	-.006562	.013598
Baseball Bat	knife handle	.003820	.0036474	.833	-.006260	.013900
	Plastic Bag	.007377	.0036474	.261	-.002703	.017457
	Paper	.009356	.0036474	.083	-.000724	.019436
	Cotton Cloth	.007338	.0036474	.266	-.002742	.017418
Plastic Bag	knife handle	-.003557	.0036474	.866	-.013637	.006523
	Baseball Bat	-.007377	.0036474	.261	-.017457	.002703
	Paper	.001979	.0036474	.983	-.008101	.012059
	Cotton Cloth	-.000039	.0036474	1.000	-.010120	.010041
Paper	knife handle	-.005537	.0036474	.553	-.015617	.004544
	Baseball Bat	-.009356	.0036474	.083	-.019436	.000724
	Plastic Bag	-.001979	.0036474	.983	-.012059	.008101
	Cotton Cloth	-.002019	.0036474	.981	-.012099	.008061
Cotton Cloth	knife handle	-.003518	.0036474	.871	-.013598	.006562
	Baseball Bat	-.007338	.0036474	.266	-.017418	.002742
	Plastic Bag	.000039	.0036474	1.000	-.010041	.010120
	Paper	.002019	.0036474	.981	-.008061	.012099

The table demonstrates the differences between different items side by side with all different combinations ($P < 0.05$). No significant value was below 0.05. For example, the P value equaled 0.833 between knife handle and baseball bat, and it was indicated that same amount of DNA was extracted from knife handle and baseball bat. There was no statistically significant difference in DNA extraction products for all pairwise comparisons

Appendix E. The significance of concentrations with different factors.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.003[a]	9	.000	1.766	.080
Intercept	.003	1	.003	14.978	.000
Item Surface	.002	4	.000	2.060	.089
Extraction Method	2.281E-6	1	2.281E-6	.011	.915
Item Surface * Extraction Method	.002	4	.000	1.911	.112
Error	.028	140	.000		
Total	.034	150			
Corrected Total	.031	149			

The table demonstrates the significance between the factors of item surface, extraction method and item surface interacting with extraction method. “Sig.” column was the significance level. This table indicated that there was no significant difference when the item sampled from interacting with extraction methods, because the significant value equaled 0.112.