QUANTITATION OF HUMAN CHROMOSOMAL DNA IN FINGERPRINTS AND HAIR ROOTS USING THE AMELOGENIN LOCUS

By

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NOMENCLATURE

bp	base pair
CV	coefficient of variation
DNA	deoxyribonucleic acid
Fingerprint	residue left on glass slide due to contact between it and a finger
ng	nanogram
PCR	polymerase chain reaction
pg	picogram
Q-TAT	quantitative amplification of the Amelogenin locus
RFLP	restriction fragment length polymorphism
RFU	relative fluorescence unit
SD	standard deviation
STR	short tandem repeat
VNTR	variable number of tandem repeat

1. Introduction

Criminal investigators use multiple types of physical evidence for identifying possible suspects in crime-related cases. Common types of physical evidence include: blood, semen, saliva, documents, drugs, explosives, fibers, fingerprints, firearms and ammunition, glass, hair, impressions, organs and physiological fluids, paint, petroleum products, plastic bags, plastic, rubber, and other polymers, powder residues, serial numbers, soil and minerals, tool marks, vehicle lights, wood and other vegetative matter (Saferstein, 2004). From this extensive list, fingerprints are the most widely recovered and the most unique.

The uniqueness of fingerprints is determined by their ridge characteristics (or minutiae). These characteristics are divided into three types: loops, whorls, and arches. All fingerprints can be classified by these characteristics and unless skin damage such as severe burns or cuts occur (penetrating 1-2 mm beneath the surface), ridge characteristics remain unchanged throughout a person's lifetime (Saferstein, 2004). There are two main layers of skin: the outer layer (epidermis) and inner layer (dermis). Between the epidermis and dermis lies an interface consisting of dermal ridges (also known as the dermal papillae) and epidermal ridges (Figure 1). These ridges, along with hair follicles and sweat and sebaceous glands, form the irregular shape of the interface. This determines the form and pattern of the ridges and grooves seen in fingerprints (Gartner and Hiatt, 1997).

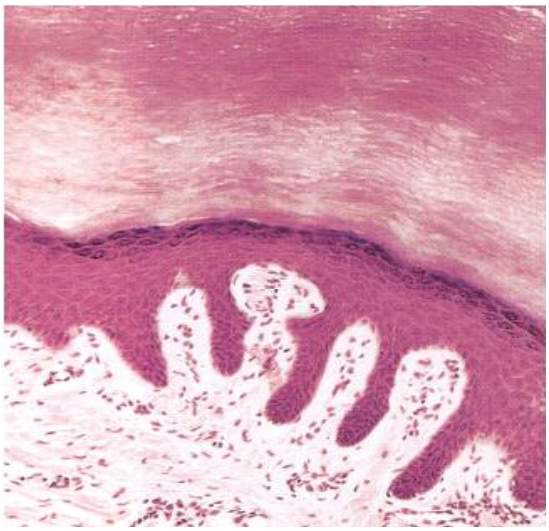

Figure 1. Histology of epidermis (outer layer), dermis (inner layer), and dermal / epidermal ridge interface (Gartner and Hiatt, 1997).

The epidermis is mostly composed of epithelial cells known as keratinocytes that are arranged in five layers (stratum basale, stratum spinosum, stratum granulosum, stratum lucidum, and stratum corneum). The dermis is composed of two layers (papillary and reticular). Keratinocytes are constantly renewed through the process of mitosis. When new cells are formed, the stratum basale layer of mitotically active, cuboidal to columnar-shaped cells is forced up into the stratum spinosum layer, containing mitotically active polyhedral to flattened cells. These in turn are forced up into the stratum granulosum layer, the most external layer in which cells contain clearly visible nuclei. The cells are flattened. The cells then are forced up into the stratum lucidum layer (present only in the palms of the hands and soles of the feet). These cells lack visible nuclei and organelles. The cells are forced up into the stratum corneum layer, containing flattened keratinocytes that also lack visible nuclei and organelles. The keratinocytes die as they reach the surface and are sloughed off. Sweat glands run from

the dermis to the epidermis to sweat pores on the surface of the skin, causing perspiration (Gartner and Hiatt, 1997) (Figure 2). When contact occurs between a finger and a substrate, the sloughed off skin cells, perspiration, other oils and debris within the ridges are transferred to the substrate, causing a "fingerprint."

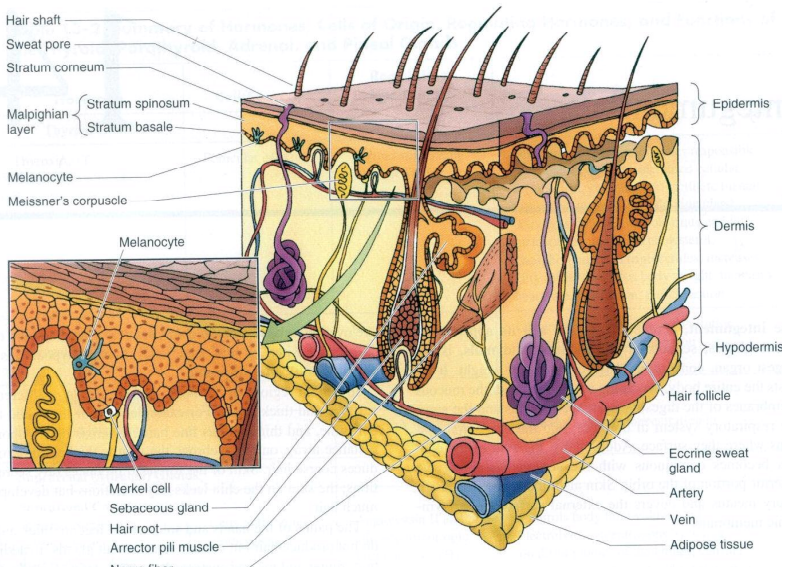

Figure 2. Cross-section of skin (Gartner and Hiatt, 1997).

Besides skin, hair is also formed from keratinocytes and grows out of the epidermis. Hair is found on most of the human body in two forms. Vellus hairs are soft, fine, short and pale (found over the eyelids). Terminal hairs are hard, large, coarse, long, and dark (found on the scalp, eyebrows, etc.). Hair develops from structures known as hair follicles. Together with hair roots (the expanded ends of hair follicles), they form what are known as hair bulbs. The hair roots conform to the shape of the dermal papillae (Gartner and Hiatt, 1997) (Figure 3).

Figure 3. Hair follicle, hair root and papilla (Gartner and Hiatt, 1997).

Most of the cells contained within hair roots are considered the matrix. The matrix is similar to the stratum basale layer of the epidermis because the cells are being constantly renewed, causing the hair to grow. The layers of the hair follicle are similar to the layers of epidermal skin. The outer layers form what is known as the external root sheath, consisting of one layer of hair bulb and several layers near the skin's surface. The next layer is the internal root sheath, consisting of a row of cuboidal cells (Henle's layer), one or two layers of flattened cells (Huxley's layer), and the internal root sheath cuticle. The internal root sheath terminates where the sebaceous gland duct attaches to the hair follicle (Figure 4). As the matrix cells within the hair root proliferate and move upward, they develop into the hair shaft. As the process continues, the keratinocytes accumulate and harden, causing hair to appear the way it does. Pulling the hair shaft out of its follicle is then difficult, due to the hair cuticle cells and internal root sheath cuticle cells

interlocking. Pulled hairs retain the shape of the hair roots. Whether hair is pulled out or falls out naturally, the hair follicle forms a new hair (Gartner and Hiatt, 1997).

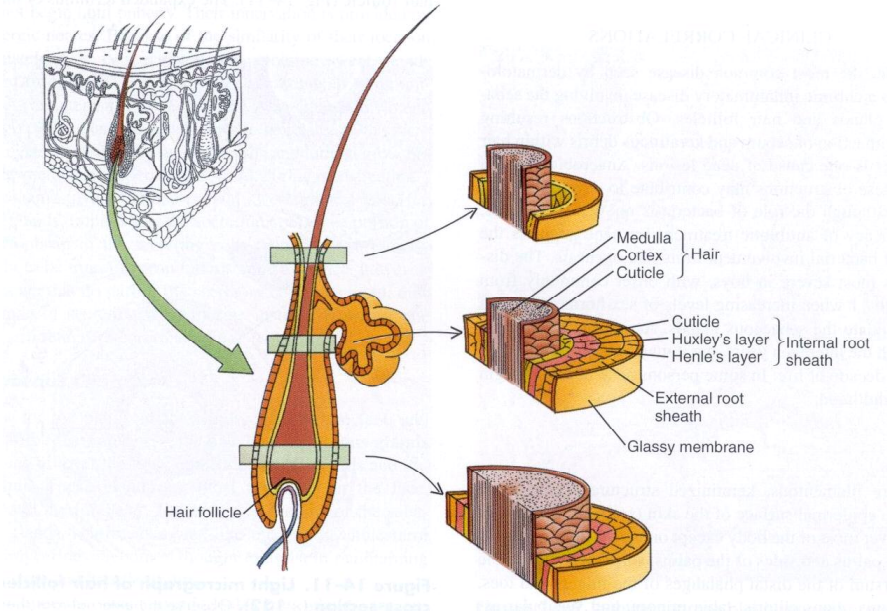

Figure 4. The hair follicle (Gartner and Hiatt, 1997).

For criminal investigators, detecting fingerprints at a crime scene can be very helpful, especially if the fingerprint is among the thousands in a database such as AFIS (Automated Fingerprint Identification Systems). However, often only partial fingerprints are found, which may not contain enough discriminatory features to match against entries in the database. Other times, whole fingerprints are found but they are smeared or smudged. In these circumstances, it may not be possible to match fingerprints from a scene, especially if no suspects have been identified. The same goes for hair. Without a suspect, it is often impossible to find a match, based solely on the physical characteristics of the hair.

In the past 20 years, another means of criminal identification has made it into the spotlight: DNA typing. Matches of DNA profiles are weighted in terms of probabilities. The lower the probability of a random match, the greater the chance an individual is the source of DNA evidence left at a crime scene.

DNA molecules carry genetic material from one generation to the next, one-half from the mother and one-half from the father. DNA is composed of two chains in a series of repeating nucleotides (consisting of a sugar molecule, a phosphate group, and a base) running in opposite directions. Specific pairs of bases bind together (or hybridize). For instance, adenine (a purine base) binds with thymine (a pyrimidine base) and guanine (a purine base) binds with cytosine (a pyrimidine base). DNA is present in all nucleated cells, as well as in mitochondria. 22 pairs of autosomal chromosomes and one pair of sex determining chromosomes make up a person's genome. Males have one X chromosome and one Y chromosome. Females have two X chromosomes (Butler, 2005).

In 1985 Alec Jeffreys first described DNA typing. He discovered regions of DNA that consisted of repeated sequences (Jeffreys et al, 1985). Because the number of repeats varied between individuals, Jeffreys and his collaborators found it possible to distinguish individuals at the DNA level (Jeffreys et al, 1985 a & b). He labeled the sequences variable number of tandem repeats (VNTRs) and adapted restriction fragment length polymorphism techniques for the visualization of DNA profiles (Jeffreys et al, 1985 a & b).

Since Jeffreys' discovery, other DNA typing methods have been developed. Even though the RFLP procedure is useful, it is very time consuming and labor intensive. The polymerase chain reaction (PCR) procedure, originally described in 1985 by Kary Mullis, is useful for DNA typing of forensic samples because it is common to find only a very small amount of biological material remaining at a crime scene. Using PCR, it is possible to amplify VNTR markers, producing millions of copies of the genetic markers of interest (Hammond et al, 1995). Using PCR, multiple short tandem repeat (STR) loci located on distant chromosomes are amplified together, or multiplexed, to produce highly discriminating DNA profile results in a short amount of time (Butler, 2005).

As mentioned above, fingerprints found at crime scenes are an excellent tool for identification, as long as there are reference samples to compare to. The same is true for DNA. If there are reference DNA profiles to compare to, DNA evidence recovered from crime scenes can provide conclusive proof of the source of forensic evidence. Besides the typical sources of DNA found at crime scenes, such as blood, semen, saliva, or hair, fingerprints are also likely sources of DNA. Creating DNA profiles from intact fingerprints found at crime scenes gives investigators twice the chance of identifying a possible suspect, since both the fingerprint databases and DNA databases can be searched. And, if a suspect is identified, fingerprint evidence validates the DNA profile further than just DNA evidence alone, and vise versa. If, however, crime scene fingerprints are smeared, and there is no way to identify a suspect by them, there is still the possibility of identifying a suspect through DNA typing.

Every individual deposits DNA on contact with other individuals or objects (Van Oorschot and Jones, 1997). Researchers have found that even brief contact with an object can transfer enough DNA for successful STR typing (Van Oorschot and Jones, 1997). STR DNA profiles have been retrieved from latent fingerprints found on objects such as the interior of latex gloves (Pizzamiglio et al, 2000), drinking glasses (Van Renterghem et al, 2000), clothing (Schultz and Reichert, 2000), plastic (Van Oorschot et al, 2003), and standard sheets of white paper (Balogh et al, 2003). Also, from previous work conducted in this laboratory, Joslin (2004) demonstrated that it is possible to obtain STR DNA profiles from fingerprints found on pre-fired and post-fired bullet casings.

The amount of DNA deposited in a fingerprint varies depending on the individual, the area of contact, history of previous touches, material being touched, moisture levels, and presence of fingerprint powder (Van Oorschot et al, 2003). It is possible that latent fingerprints contain a very small number of epithelial cells and that objects touched only briefly contain only nuclei-free corneocytes (Balogh et al, 2003). These cells in turn only contain a very small amount of DNA, quite commonly less than 100 picograms. This small amount of DNA has been named "low copy number DNA" or LCN DNA (Gill et al, 2000). PCR amplification of LCN DNA increases the chance of allelic dropout (when one allele of a heterozygote locus is not amplified), stutter (or false alleles), and contamination and other unexplained artifacts visualized in an STR profile (Gill et al, 2000).

Depending on the amount of DNA deposited, Lowe et al (2002) labeled individuals as "good shedders" and "poor shedders." Good shedders are individuals who deposit an adequate amount of DNA on the object they touch to determine their DNA profile. Therefore, reliable DNA profiles can be obtained. On the other hand, poor shedders (or non-shedders) are individuals who deposit DNA on objects they touch in amounts too low to produce an STR profile using typical DNA typing methods. Therefore, a reliable DNA profile cannot be obtained (Lowe et al, 2002). Correlations could exist between shedder type and the relative proportions of chemicals that make up skin secretions, an individual's personal hygiene, and stress (Lowe et al, 2002).

Researchers have also confirmed the observation that secondary transfer of DNA exists. Secondary transfer occurs when DNA from one person is transferred to a second person, and then subsequently transferred to an object the second person touches. Preliminary findings have shown that the quantity and quality of DNA profiles produced from DNA deposited through secondary transfer is dependent upon the particular individuals involved in the transfer process. Therefore, secondary transfer is seen more when DNA from a "good shedder" is found on an object a "poor shedder" just touched after coming in contact with the "good shedder" (Lowe et al, 2002). Secondary transfer also occurs when DNA is transferred from other parts of a person's body to his / her hands, and then subsequently transferred to an object the person touches.

Currently, regardless of the sample and how much DNA it might contain, it is important for forensic scientists to be able to quantitate the amount of DNA recovered

from a sample. Quantitation is especially important when dealing with PCR. Amplification of too little DNA template can result in allelic dropout. Amplification of too much DNA template can result in an imbalance of alleles within loci and between loci in the profile. This causes results to be difficult to interpret. It is also important to be able to differentiate between human genomic DNA and DNA from other organisms, and to differentiate between human male and female DNA if mixed samples are found. Also, it is important for forensic labs to follow the standards set forth by the accreditation agencies under which they operate. One of these standards requires forensic labs to quantitate the amount of human genomic DNA recovered from samples. Two methods for human DNA quantitation that are currently used by forensic scientists include using Quantiblot assay kits and real-time PCR assays.

Quantiblot® is a human DNA quantitation kit available from Applied Biosystems (Foster City, CA). It is based on the quantitative hybridization of human DNA probes to extracted DNA samples immobilized on a nylon membrane as "slot blots" or "dot blots" (Walsh et. al, 2003). The comparison of serial dilutions of known standards (they come with the kit) to serial dilutions of unknown test samples allows the forensic scientist to obtain either colorimetric or chemiluminescent quantitative results providing an estimate of the quantity of DNA in a sample. This method allows for the rapid quantitation of large numbers of DNA samples. However, Van Oorschot et al (2003) found that the accuracy of Quantiblot is limited and that when using Quantiblot, sample quantities are often underestimated. Also, this method is based on visual comparison, which is very subjective and does not differentiate between male and female DNA.

The second method of DNA quantitation, real-time PCR (Applied Biosystems, Foster City, CA), has several advantages over the Quantiblot method. It is extremely fast, accurate and sensitive, because it quantifies the accumulation of human DNA product as amplification occurs, cycle by cycle, thereby eliminating all post-PCR processing. Because it eliminates all post-PCR processing, it also minimizes the risk of contamination from the analyst compared to regular PCR. It allows the forensic scientist to target specific genetic loci such as *Alu* sequences in human DNA, or the X and Y chromosomes. It also gives an estimate of the proportion of input template from each sample in mixed male / female samples, such as are common in sexual assault evidence. However, not many labs are able to use this technology. The cost of the equipment is large. Also, it takes time and money to train technicians using the complex technology. Since real-time PCR is still relatively new, costs will likely go down, in time, and more laboratories will be able to employ it.

Being that Quantiblot assays only give a rough estimate of the amount of human DNA in a sample and do not differentiate between male and female human DNA, and that real-time PCR assays are accurate and allow differentiation, but are expensive for most forensic labs to utilize, an obvious problem exists. Forensic labs need a DNA quantitation method that is accurate, sensitive, reasonably inexpensive, less time-consuming than other methods, and yet will allow a lab to meet accreditation requirements. A possible method that fulfills all of those criteria was developed in this lab and is known as "quantitative amplification of the Amelogenin locus" (or Q-TAT). In cases of sexual assault or mass casualty disasters, it is important to distinguish whether

a sample originated from a male or female. The Amelogenin gene allows for this distinction. It distinguishes the X chromosome from the Y chromosome based upon a 6 base pair (bp) difference in the sizes of amplification products produced from each chromosome (Butler, 2005). After extracting and amplifying DNA from a sample using a sex typing kit, the amount of fluorescence in X and / or Y amplicons produced from unknown samples is compared to fluorescence in products amplified from known inputs of genomic DNA used to create a standard curve. The processes involved include PCR and capillary electrophoresis, two processes in widespread use currently in forensic labs.

Clear advantages of quantitating human DNA using the Amelogenin locus are that it has a well-defined methodology, it uses existing technology, and it is a lot less expensive than real-time PCR. In addition, this quantitation method uses the same equipment and reagents as regular STR typing methods. Thus, there is no special training or quality assurance needed for Q-TAT that is not already in place in support of regular STR typing methods.

For any DNA quantitation method to be effective in a forensic laboratory, validation is needed that demonstrates the technique to be effective with representative forensic samples. Perhaps the most difficult samples to quantitate accurately are those containing trace amounts of DNA. The specific goal of this study was to **quantitate the amount of human DNA recovered from typical forensic samples like fingerprints and hair roots**. Individual goals, and research questions, of this study were:

1. Determine the amount of DNA recovered from three classes of fingerprint donors.
    - Is there a significant difference in the amount of DNA recovered from the non-dominant hand and the dominant hand?
    - Is there a significant difference between the amount of DNA recovered from males and females?
    - Does the DNA contained within a fingerprint decompose over time?
2. Determine the amount of DNA recovered from three different types of hair.
    - Is there a significant relationship between DNA recovery and region of the body from where the hair was plucked?
    - Is there a significant difference in the amount of DNA recovered between male and female head hairs, male and female pubic hairs, and male and female limb hairs?
    - Is there a significant relationship between DNA recovery and apparent hair root size (visualized microscopically)?
    - Is there an overall significant relationship between DNA recovery and body region within each individual donor?

The importance of this research is that in time forensic scientists will be able to use the Q-TAT method to obtain a highly accurate estimate of DNA recovery from forensic unknowns using equipment and methodologies routinely used in the forensic laboratory.

2. Materials and Methods

This project involved a group of 51 volunteers and focused on evaluating a method to quantitate human chromosomal DNA in trace evidence using primers directed against the Amelogenin locus located on the X and Y chromosomes (Allen and Fuller, in press). The Amelogenin locus encodes a component of tooth enamel and the gene exhibits a length polymorphism on the X and Y chromosomes (Butler, 2005). PCR products amplified from the locus on the X chromosome exhibit 210 base pairs (bp) in size while those amplified from the Y chromosome are 216 bp long (Butler, 2005). Thus, the Amelogenin locus can be used to distinguish males and females (Butler, 2005).

For purposes of this study, the term "fingerprint" was defined as the residue left on a glass microscope slide due to contact between a finger and the substrate. Fingerprints as defined by this study would not necessarily be suitable for pattern-based identification. 50 volunteers consented to provide fingerprints on clean glass microscope slides. The collection and processing of samples evolved as new information was obtained from preliminary results. Initially, three fingers on both hands were used to collect fingerprints, but due to the discovery early on that more DNA is found on the non-dominant hand, only the non-dominant hand was used in later phases of the project.

In addition to DNA contained within fingerprints, another common item of forensic evidence is hair. Therefore, another goal of this project was to quantitate the recovery of DNA from hair roots. 20 volunteers, most of whom had also been used to collect fingerprints, consented to provide hair samples, with the root present, representing

three different types of hair: head, pubic, and limb. Collection of all samples took place either at the OSU-CHS campus or in the privacy of the volunteer's home.

2.1. Identification of DNA Shedder Status

Prior studies from this laboratory (Joslin, 2004 and Gulick, 2005) demonstrated that individuals differ in the amount of DNA deposited in a fingerprint and could be generally grouped into one of three categories: good shedder, moderate shedder, or poor shedder. Determining shedder status consisted of the volunteers lightly pressing their thumbs, forefingers, and middle fingers on clean glass microscopic slides, thereby creating visible fingerprints. Depending on the amount of DNA recovered from the volunteers' fingerprints, as reflected in the relative amount of fluorescence contained in Amelogenin amplicons, individuals were classified as light shedders, intermediate shedders, or heavy shedders as generally defined by Gulick (Thesis of Melisa Gulick, submitted to OSU in Spring 2005).

2.2. DNA Extraction

DNA was extracted from all samples with widely used extraction procedures involving Proteinase K (200 μg/mL) and 0.5% SDS (w/v) in TNE (10 mM Tris-Cl pH 8.0 + 200 mM NaCl + 1 mM EDTA). Proteinase K and SDS were diluted into TNE from stocks of 20 mg/mL and 20% (w/v) in dH_2O respectively. Fingerprints were transferred from glass microscope slides to sterile Dacron swabs moistened with TE^{-4} buffer (10 mM Tris-Cl pH 8.0 + 0.1 mM EDTA). The swabs were cut from the applicator stick and placed (tip facing up) in individual 0.65 mL microfuge tubes, each properly labeled with

the sample number and date. Prior to extraction of DNA from hair, each was viewed under a dissecting microscope to visually estimate a root size. Depending on the size of the root, a rating between 1 and 3 was assigned to each hair. A rating of 1 indicates a root is present but was very small and barely visible. A rating of 2 denotes a root that was easily seen. A rating of 3 denotes a root that was quite large. An appropriate amount of extraction buffer (200 µL for hair roots and 300 µL for fingerprints) was added to all tubes, including a tube with no swab or hair in it (this serves as a negative control or extraction blank). The tubes were vortexed to ensure immersion and incubated for two hours at 65°C or overnight at 37°C.

After incubation, each 0.65 mL tube had a hole punched in the bottom with a pushpin and then was placed in a properly labeled 1.8 mL microfuge tube. The tubes were centrifuged at 10,000 x g for 3 minutes at room temperature. This process collected all of the extraction solution from the 0.65 mL tubes into the 1.8 mL tubes. The 0.65 mL tubes containing the swab tips were discarded and extracts were subjected to a phenol:CHCl3:isoamyl alcohol (9:0.96:0.04 v/v) extraction. An equal volume of phenol (200-300 µL) was added to each DNA extract. The mixtures were vortexed until a cloudy emulsion formed then were centrifuged at 10,000 x g for 1 minute at room temperature. Centrifugation was performed to separate the two liquid phases. The top aqueous phase containing DNA was removed and placed into another properly labeled 1.8 mL tube. An equal volume of CHCl3:isoamyl alcohol (24:1) (200-300 µL) was added to all tubes, vortexed until cloudy, and centrifuged at 10,000 x g for 1 minute at

room temperature. The top aqueous phase containing DNA was removed and dispensed in another clean 1.8 mL tube.

A Clean and Concentrator kit from Zymo Research (Orange, CA) was used to recover and concentrate the DNA from the extracts. The kit contains micro columns consisting of silica beads, 2.0 mL collection tubes, a "DNA Binding Buffer," and a "DNA Wash Buffer." The Zymo kit exploits the affinity of DNA for silica in the presence of a high salt environment (Bush and Harvey, 1991). The contents of the buffers are not provided with the kit, but early reports (Bush and Harvey, 1991) of the affinity of DNA for silica indicated that 4-6 M guanidine-HCl promotes the binding, so the kit is likely to contain buffers based on these findings. Thus, the purpose of the "DNA Binding Buffer" is to raise the salt concentration of the DNA extracts to force the DNA to bind to the silica beads during the recovery step. Following the Zymo kit instructions, two volumes of "DNA Binding Buffer" were added to the tubes containing DNA extracts. The mixed solutions were then transferred to properly labeled Zymo columns, which were placed in the collection tubes. The assembly was then centrifuged at 10,000 x g for 10 seconds at room temperature to bind DNA to the silica. The flow-through in the collection tubes was discarded and the columns were washed twice with 200 µL of "DNA Wash Buffer" and centrifuged at 10,000 x g for 30 seconds at room temperature. The flow-through was again discarded. The columns were transferred to new properly labeled 1.8 mL tubes.

The last step of the extraction process was to lower the salt concentration to elute the purified DNA from the silica columns. Initially, the DNA was eluted in 20-30 µL of TE^{-4} at 65ºC. However, DNA was subsequently eluted in dH_2O at 65ºC to avoid any possibility of the EDTA in the TE^{-4} inhibiting PCR amplification of the small amounts of DNA recovered from some samples. 10-15 µL hot TE^{-4} or dH_2O was added to the columns. The tubes containing the columns were placed at 65ºC for 1 minute then centrifuged at 10,000 x g for 10 seconds at room temperature. During centrifugation, the hot TE^{-4} or dH_2O eluted the DNA from the columns and it was collected at the bottom of the tubes. 10-15 µL hot TE^{-4} or dH_2O was added a second time to the columns, followed by heating and centrifugation, leaving an unknown quantity of clean DNA template, ready to be amplified, within the 20-30 µL of TE^{-4} or dH_2O.

2.3. PCR Amplification

Aliquots of clean, recovered DNA from each sample were used as template for the amplification of the Amelogenin locus. Amelogenin primers, UV treated water, Gold ST*R 10X Buffer (500 mM KCl + 100 mM pH 8.3 Tris-HCl + 15 mM $MgCl_2$ + 1% Triton X-100 + 1600 µg/mL BSA + 2 mM of each dNTP) (Promega Corporation, Madison, WI), and Taq Polymerase were used to create a "master mix." Initially, the Amelogenin primers used were part of a sex typing kit purchased from Promega Corporation (Madison, WI). However, fluorescein-labeled primers were subsequently synthesized for this project by Invitrogen, Inc. (Chicago, IL). The primers used were identical in sequence and the presence of a fluorescein label, to the primers included with the sex typing kit. The Invitrogen primers were also validated in house by visually

comparing the X and Y amplicons produced from both sets of primers. There was no significant difference in the qualitative or quantitative characteristics of amplicons produced with either set of primers (Table 1 and Figure 5).

Primers from Sex Typing kit			
Input Male DNA (ng)	Ave. Total Area (RFU)	SD	CV (%)
0	0	0	0
0.03125	2295	2020	88
0.0625	6671	2918	44
0.125	18119	9621	53
0.250	29691	10421	35
0.500	67494	19794	29

Primers synthesized by Invitrogen			
Input Male DNA (ng)	Ave. Total Area (RFU)	SD	CV (%)
0	0	0	0
0.03125	3146	2326	74
0.0625	9016	3858	43
0.125	15786	6951	44
0.250	35174	15193	43
0.500	70917	24224	34

Table 1. Comparison of Standard Curves created from Amelogenin primers purchased with a kit vs. synthesized Amelogenin primers purchased from Invitrogen.

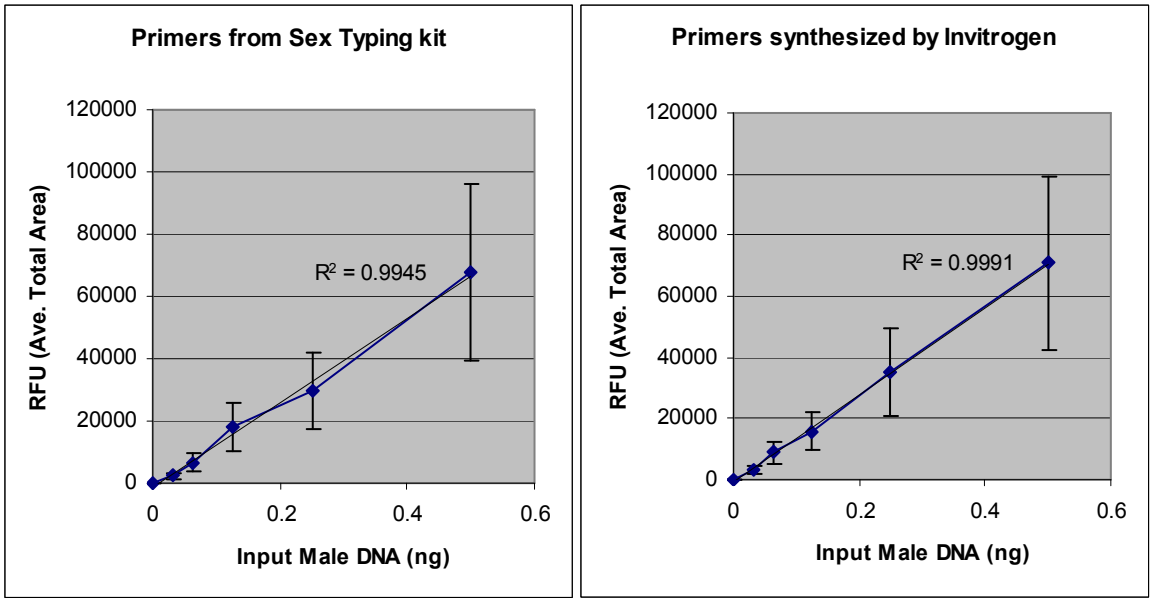

Figure 5. Comparison of Standard Curves created from Amelogenin primers purchased with a kit vs. synthesized Amelogenin primers purchased from Invitrogen.

Male DNA standard with a known concentration of 50 µg/mL was used to create the standard curve produced with each batch of DNA samples to be quantitated. The male DNA standard came from a volunteer donor and was extracted from blood using phenol, ethanol precipitation, and recovery using a loop. The DNA was quantitated using spectrophotometry at 260 nm and also on a yield gel that compared the ethidium bromide fluorescence of sample dilutions against fluorescence of a range of known concentrations of lambda DNA. For this study, male DNA was used to create the standard curve. However, female DNA could just have easily been used. The blank DNA sample extracted (described above) served as negative control. Control female DNA with a concentration of 0.10 µg/mL served as an internal positive control. The control female DNA came from an AmpFl STR kit (Applied Biosystems, Foster City, CA). PCR amplification was performed in 200 µL tubes that were properly labeled.

Each amplification set consisted of unknowns and aliquots of diluted male reference DNA standard. Aliquots of the reference standard (50 µg/mL) and UV treated water were mixed to create a dilution series beginning with either a 1:100 dilution or a 1:500 dilution of the DNA concentrate. Two-fold serial dilutions were then performed in dH_2O to create the following input template amounts in PCR reactions: 0.5 ng, 0.25 ng, 0.125 ng, 0.0625 ng, and 0.03125 ng. PCR reactions contained either 1 µL or 5 µL of each DNA dilution as template added to the master mix to produce the standard curve. 1 µL or 5 µL of DNA recovered from fingerprints or hair roots was added to reaction tubes along with the extraction blank and positive control. Proportions of UV treated water, buffer, primers, and Taq polymerase, depending upon the amount of DNA added, was

combined to create a master mix. Aliquots of the master mix (either 9 µL or 7.5 µL depending upon the volume of DNA amplified) were added to all tubes, making sure the reactants were mixed well and at the bottom of each tube. If the amount of solution was less than 12.5 µL, 1 drop Mineral Oil was added to prevent evaporation during amplification (For clarification, See Figure 6 below). Amplifications were set up in a biological containment hood and amplified using either a PTC 200 Thermalcycler (MJ Research, Reno, NV) or a GeneAmp 9700 Thermalcycler (Applied Biosystems, Foster City, CA) following cycling parameters specified by the instructions supplied with the sex typing kit from Promega Corporation (Madison, WI). The amplification process took approximately two and a half hours to complete.

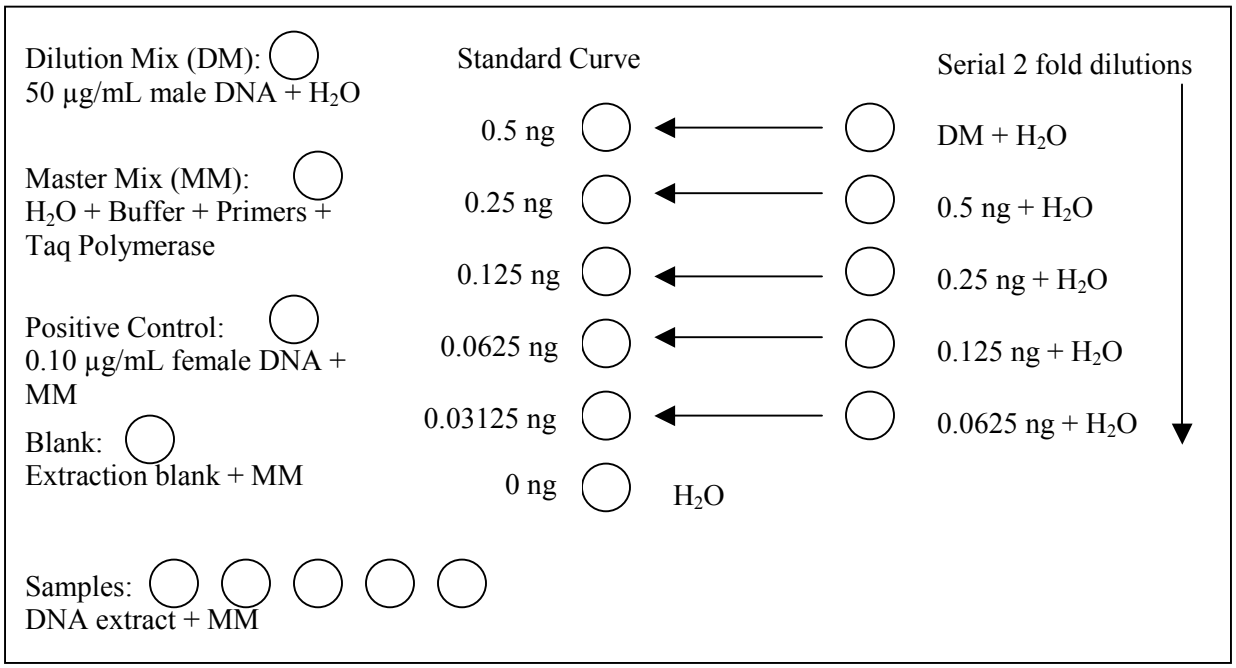

Figure 6. Representation of a PCR amplification setup. Each circle represents one 200 µL amplification tube.

2.4. Capillary Electrophoresis on the 310 Genetic Analyzer

Once amplifications were complete, comparable aliquots of PCR products (1-1.5 μL) were mixed with 25 μL of formamide (Hi-Dye, Applied Biosystems, Foster City, CA) containing an internal size standard (GS 350, Applied Biosystems, Foster City, CA). The internal size standard consists of 10 DNA fragments whose sizes are known and are labeled with the fluorescent dye ROX, which emits fluorescence at a red wavelength easily distinguished from the fluorescence emitted by fluorescein. Samples were placed in a 310 Genetic Analyzer (Applied Biosystems, Foster City, CA) that consists of a capillary, two buffer vials, two electrodes connected to a high-voltage power supply, a laser excitation source, a fluorescence detector, a sample tray, and an integrated computer (Butler, 2005). The 310 utilizes capillary electrophoresis to separate DNA on the basis of size, and collects and stores electrophoresis data from a single sample in the attached computer. The genetic analyzer was pre-programmed to electrophorese each sample at 60ºC at 15 kV for 24 minutes. Electrophoresis occurs within a narrow capillary filled with a polymer that exerts a sieving action on molecules of different size. As fragments move past a detection window in the capillary, a laser and a charged-coupled device (CCD) camera excite the fluor attached to the fragment thereby illuminating and capturing the fluorescent emissions from the fragments. A Macintosh computer collects and stores the data produced from each injection for subsequent analysis (Figure 7).

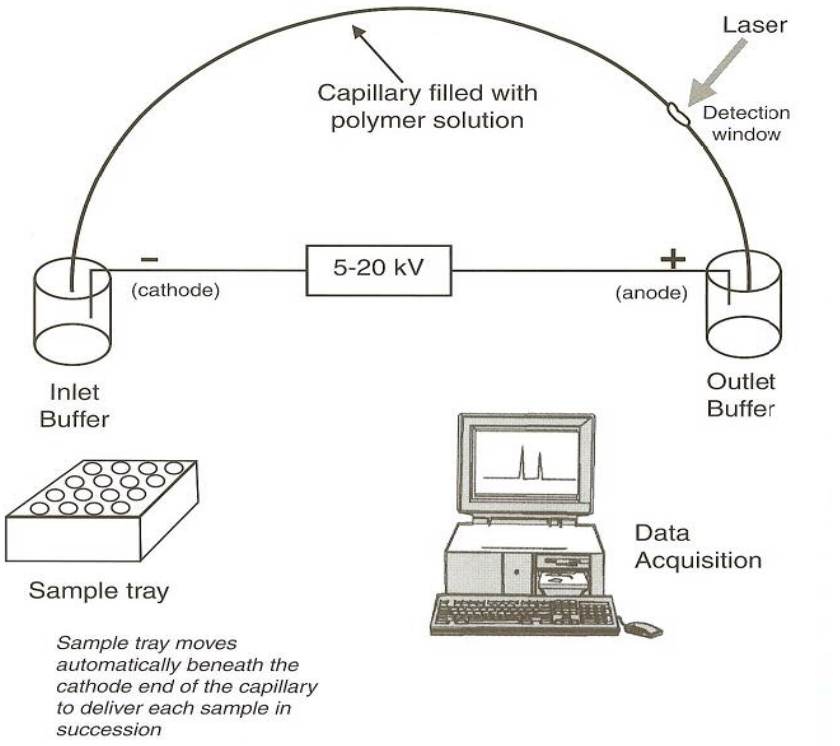

Figure 7. Representation of capillary electrophoresis instrument (Butler, 2005).

2.5. Data Analysis

Data collected from the 310 Genetic Analyzer was analyzed using GeneScan (version 3.1.2) software (Applied Biosystems, Foster City, CA). The data produced are in the form of electropherograms, which reflect changes in fluorescent signal intensity (relative fluorescent units or RFUs) during an electophoretic run as DNA fragments pass the detection window in the capillary. The GeneScan software allows the magnitude of fluorescence and the size of the DNA fragments detected to be determined with resolution down to the single base pair (Butler, 2005). Thus, Amelogenin amplicons can be separated, readily identified and quantitated. Using GeneScan software it was also possible to normalize the quantity of fluorescence in Amelogenin amplicons produced from each input amount of DNA template. Since the amount of internal size standard

added to each sample was constant, the red fluorescence in the 200 bp internal size standard could be normalized over all injections in a run. The degree to which the amount of fluorescence in the 200 bp standard varied from injection to injection was assumed to reflect comparable variations in fluorescence from Amelogenin products, and thus slight variations in their apparent amounts could be normalized across a run as well. Ultimately, the concentration of DNA in a given volume of input template of unknown concentration could be estimated after computing the normalized RFUs in Amelogenin amplification products and plotting those values on the standard curve reflecting fluorescence per nanogram (ng) of amplified reference DNA template.

The results should be informative for forensic laboratories that have a mandate to conserve evidence for confirmatory testing. Knowing how much DNA to expect from fingerprints or different hairs will allow laboratories to extract just as much DNA as necessary to obtain a reliable result.

3. Results

In order to quantitate human DNA recovered from forensic specimens, standard curves of fluorescence (RFUs) versus the quantity of amplified male reference DNA standard were generated (Figure 8). An RFU value greater than 150,000 tends to saturate all pixels in the CCD camera while a value less than 100 increases the chances of allelic dropout. Thus, any quantitative assay should avoid making estimates at these extremes of the standard curve. The ideal RFU value is 1000-4000. The usual threshold range for allele designation established by laboratories is a peak height of 50-150 RFUs (corresponding to 500-1000 of total RFU in an allelic peak). For this study, a minimum peak height threshold was set at 50 RFUs. Therefore, any PCR products containing less than 50 RFUs were not scored or considered to represent a true Amelogenin allele (Figure 9).

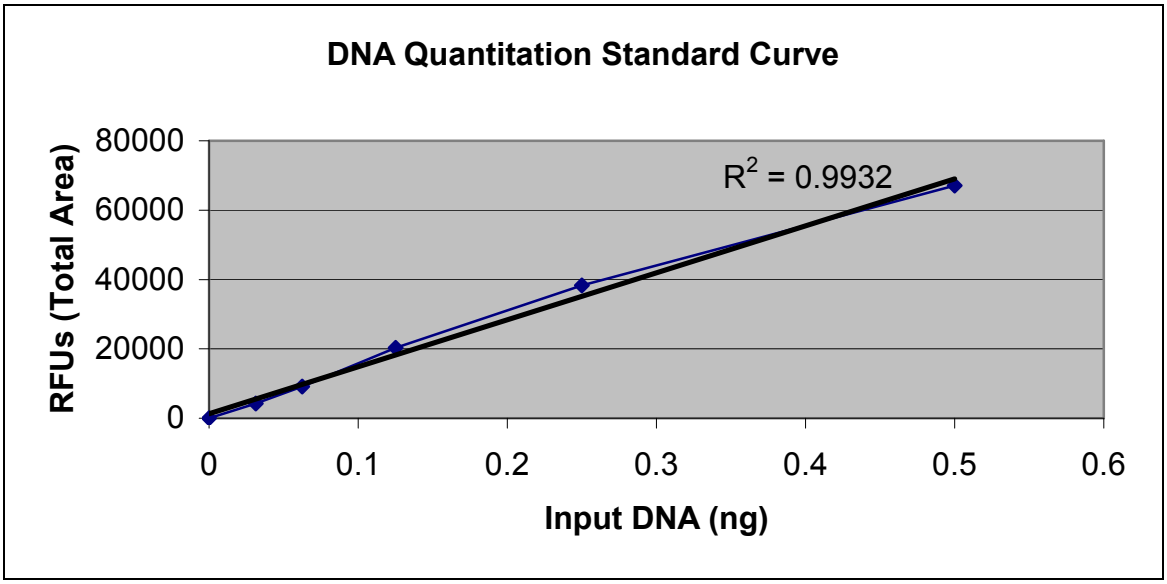

Figure 8. Example of a Standard Curve showing input DNA (ng) versus fluorescence in Amelogenin amplicons (RFUs) created using 2 fold serial dilutions of a 50 µg/mL male reference DNA standard.

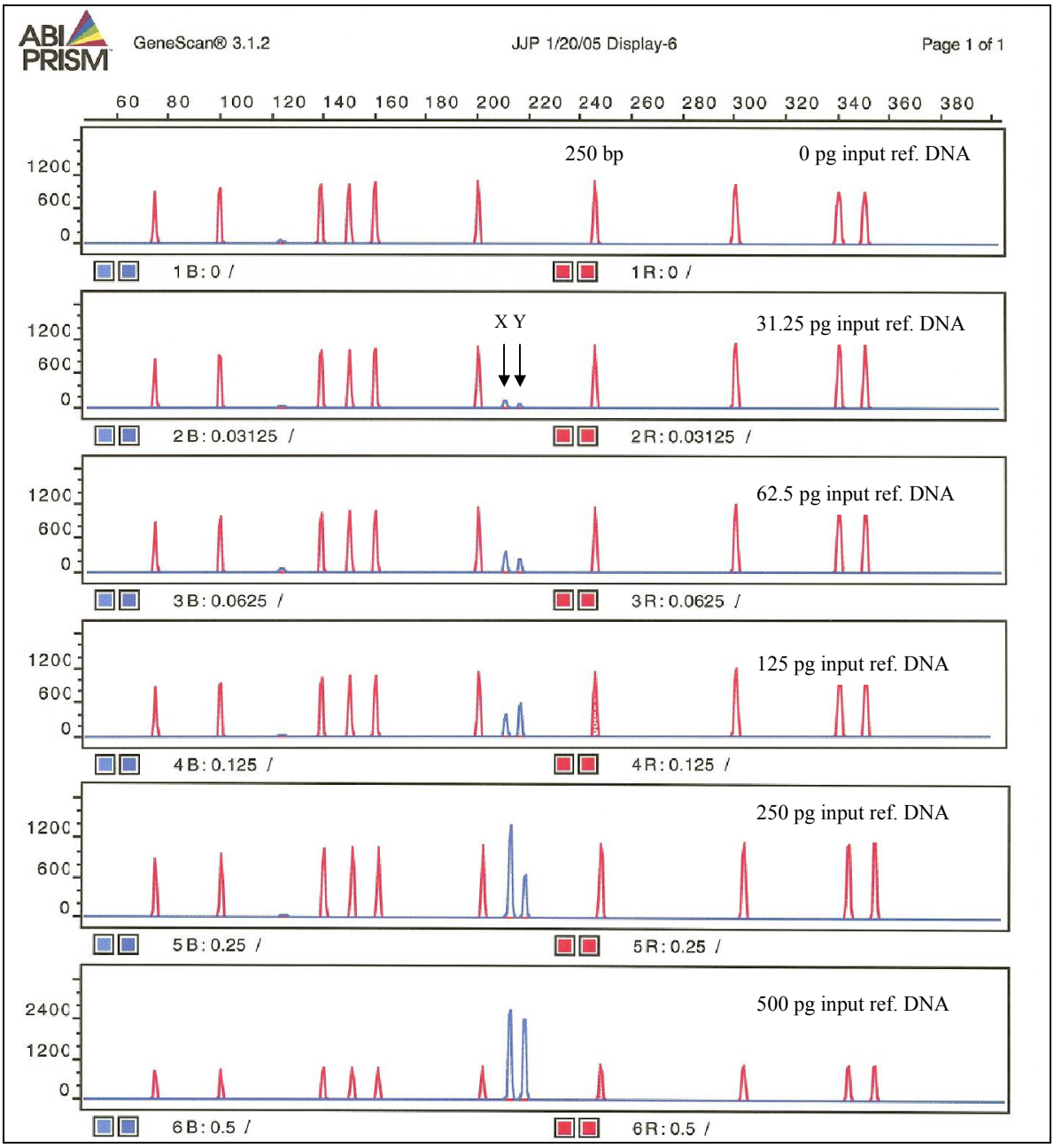

Figure 9. Example of typical profiles produced from different input amounts of the male reference DNA standard. Peaks in red represent the internal size standard, labeled with the ROX fluor, and used to compute the size of any other DNA fragment in the electrophoretic run. The products amplified from the Amelogenin locus are shown in blue and are 210 and 216 bp. The ordinate reflects total RFU contained within a fluorescent peak.

3.1. Quantitation of DNA in Fingerprints (N =50)

Initial studies were designed to confirm and complement the results of Melisa Gulick (2005) and Janice Joslin (2004). Seven volunteers were asked to deposit fingerprints on clean glass microscope slides, using their thumbs, forefingers, and middle fingers on both hands. However, results from preliminary studies suggested that fingerprints from a volunteer's non-dominant hand tended to contain higher and more consistent levels of DNA than fingerprints from the dominant hand. In addition, there appeared to be less secondary transfer of DNA from other donors (i.e. contamination) when fingerprints from the non-dominant hand were used (not shown).

DNA was extracted from fingerprints and the Amelogenin locus was amplified as described in Materials and Methods. Depending on the amount of DNA recovered from the fingerprints, individuals were classified as light shedders, intermediate shedders, or heavy shedders. An individual arbitrarily classified as a light shedder deposited less than 50 pg of DNA in a fingerprint based on quantitative amplification of the Amelogenin locus. One classified as an intermediate shedder deposited between 50 and 300 pg of DNA in a fingerprint and a heavy shedder regularly deposited more than 300 pg of DNA in a fingerprint. The electropherograms shown in Figures 10 through 15 show the relative peak heights of Amelogenin amplicons from volunteers identified as light shedders, intermediate shedders, and heavy shedders.

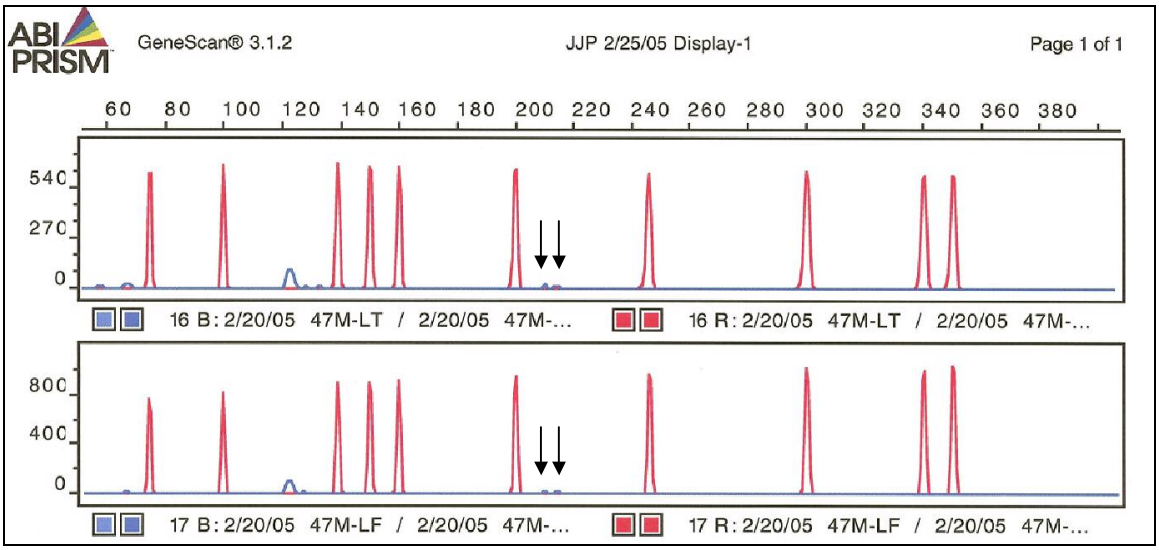

Figure 10. Example of a profile from a male volunteer considered a light shedder by the standards of this study. Allelic peaks from fingerprint DNA samples (47M) <50 RFU. On average, no DNA was recovered from these fingerprints.

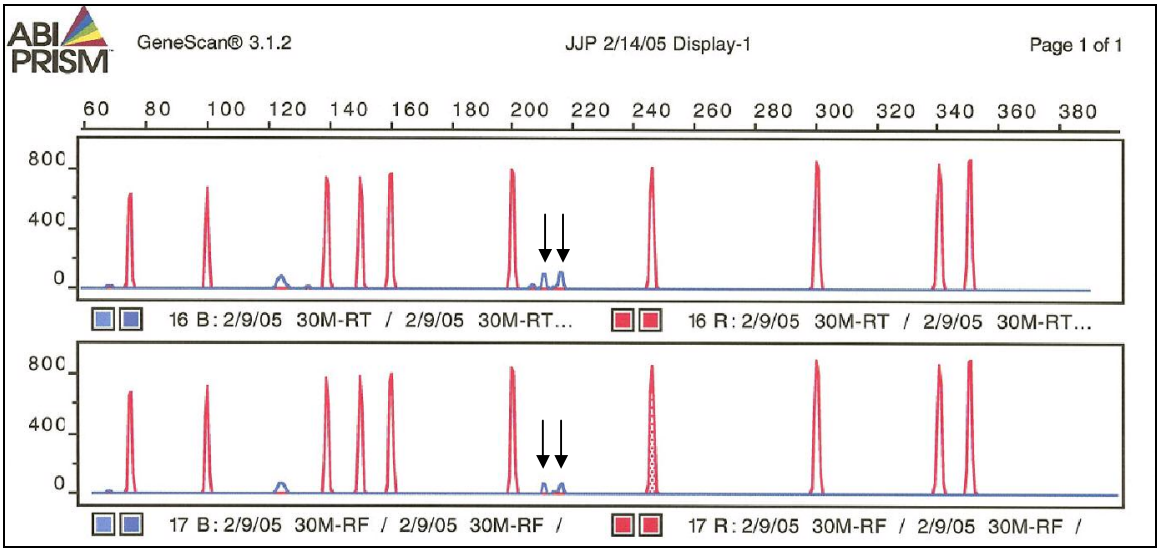

Figure 11. Example of a profile from a male volunteer considered an intermediate shedder by the standards of this study. Allelic peaks from fingerprint DNA samples (30M) >50 RFU. An average of 66 pg of DNA was recovered from these fingerprints.

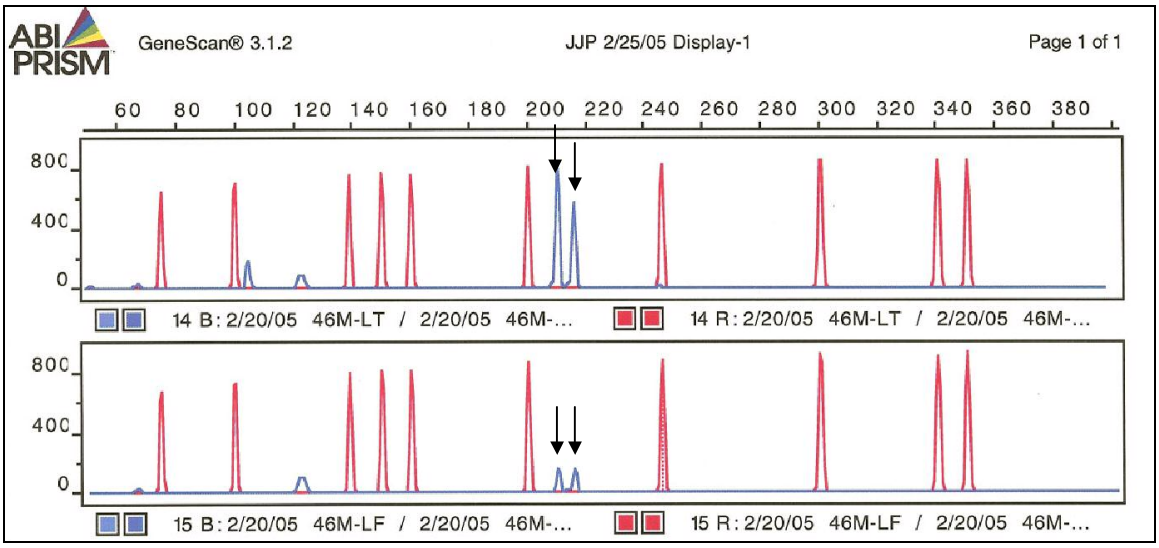

Figure 12. Example of a profile from a male volunteer considered a heavy shedder by the standards of this study. Allelic peaks from fingerprint DNA samples (46M) >50 RFU. An average of 452 pg of DNA was recovered from the fingerprints.

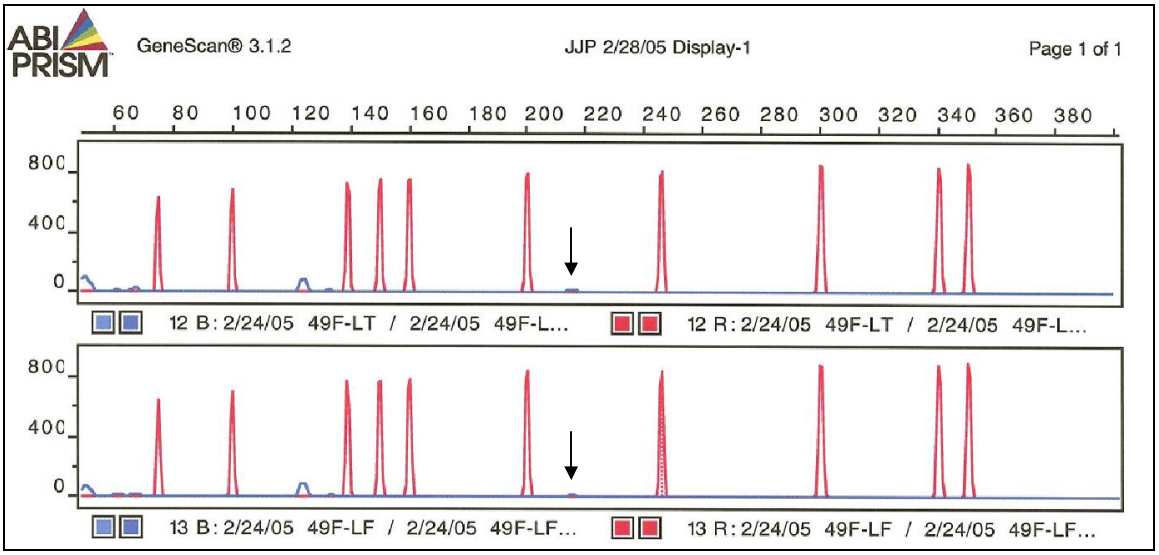

Figure 13. Example of a profile from a female volunteer considered a light shedder by the standards of this study. Allelic peaks from fingerprint DNA samples (49F) <50 RFU. On average, no DNA was recovered from these fingerprints.

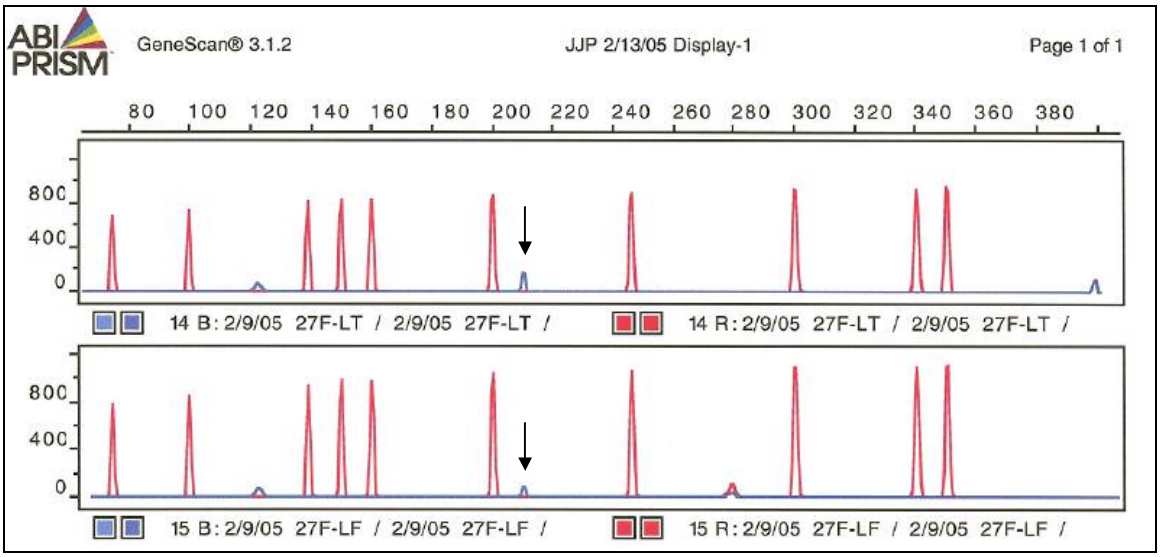

Figure 14. Example of a profile from a female volunteer considered an intermediate shedder by the standards of this study. Allelic peaks from fingerprint DNA samples (27F) >50 RFU. An average of 62 pg of DNA was recovered from these fingerprints.

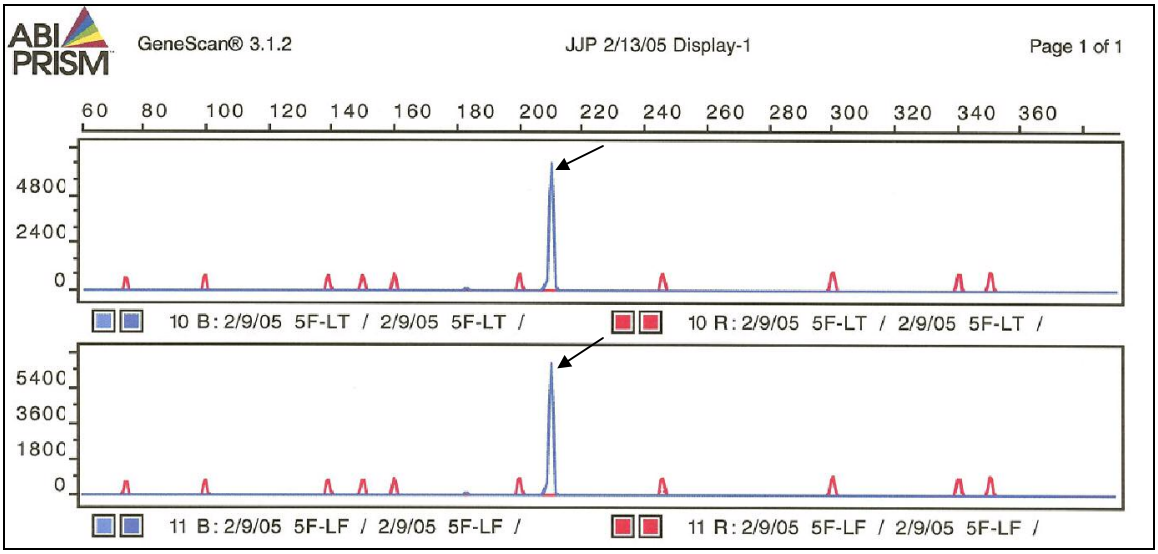

Figure 15. Example of a profile from a female volunteer considered a heavy shedder by the standards of this study. Allelic peaks from fingerprint DNA samples (5F) >50 RFU. An average of 3136 pg of DNA was recovered from these fingerprints.

Some volunteers gave multiple sets of fingerprints, while others gave only one set. The amounts of DNA recovered from each volunteer's set(s) of fingerprints were averaged. All sets of fingerprints could be subdivided into the following groups: dominant hand versus non-dominant hand, male versus female, and processing time (time from when the fingerprint was placed on a slide to when DNA was extracted and quantitated). For each of the groups, a mean, a standard deviation (SD), and coefficient of variation (CV) (gives the standard deviation as a proportion to the mean), was calculated. Using statistical analysis, a p-value was also calculated for each group.

To answer the question, "Is there a significant difference in the amount of DNA recovered from the non-dominant hand and dominant hand?" initial results comparing the average recoveries of DNA from the fingerprints deposited by the thumbs, forefingers, and middle fingers of the non-dominant and dominant hands of seven donors (one male and six female) are summarized in Figure 16. For this initial study, all the fingerprints per volunteer were deposited on the same date and at the same time.

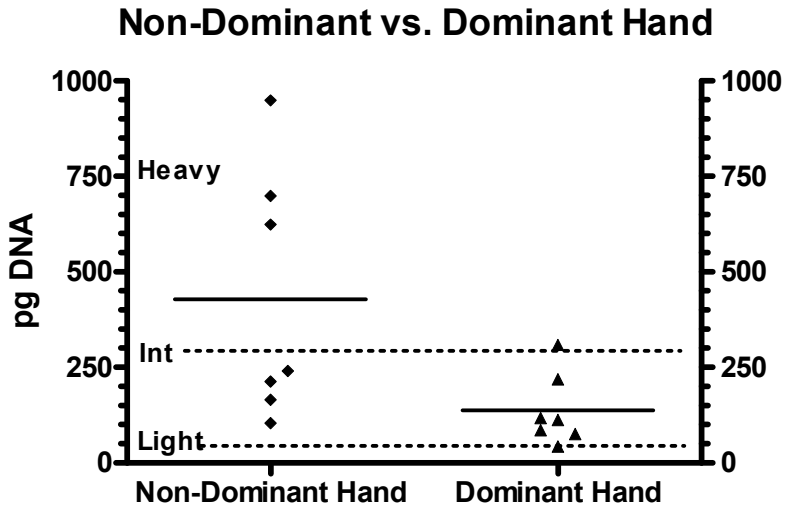

Figure 16. Average recoveries of DNA from fingerprints deposited from the non-dominant hand versus the dominant hand of seven volunteers (one male and six female). Each data point represents the mean of sampling done in triplicate. The dotted lines indicate the shedder status thresholds. The solid lines indicate the total sample means.

In general, fingerprints from a volunteer's non-dominant hand contain higher and more consistent levels of DNA than fingerprints from the dominant hand, as indicated by the greater number of data points within the heavy shedder category for the non-dominant hand. Specifically, the total sample mean for the non-dominant hand (428 pg) was calculated to be approximately three times the total sample mean for the dominant hand (137 pg). Using statistical analysis, non-parametric Wilcoxon signed rank test ($W = 26.00$; $p = 0.0313$), results indicated that the non-dominant hand of volunteers significantly shed more DNA than the dominant hand. For the purposes of this study, assuming individuals use their dominant hands more frequently than their non-dominant hands and thereby deposit less DNA in fingerprints from their non-dominant hands, subsequent studies consisted of only using the non-dominant hand of volunteers.

To answer the question, "Is there a significant difference in the amount of DNA recovered from males and females?" the combined average recoveries of DNA from the male and female donors of each shedder phenotype, as well as the distribution within each shedder phenotype, are summarized in Figure 17 and Table 2.

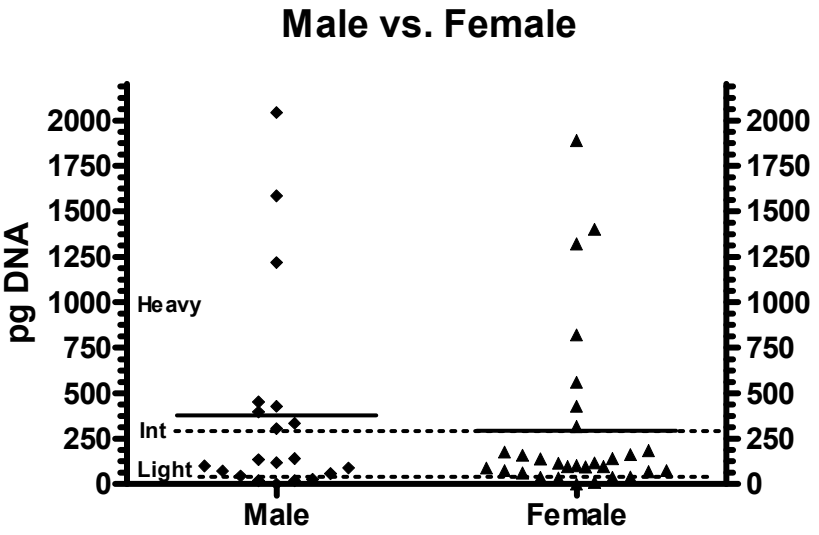

Figure 17. Combined average recoveries of DNA from fingerprints from the non-dominant hand of 20 male and 30 female volunteers. Each data point represents the mean of sampling done in at least duplicate. The dotted lines indicate the shedder status thresholds. The solid lines indicate the total sample means.

	Distribution Within Each Shedder Phenotype		
Sex of Donor	Light	Intermediate	Heavy
Male	25% (5 / 20)	35% (7 / 20)	40% (8 / 20)
Female	20% (6 / 30)	57% (17 / 30)	23% (7 / 30)

Table 2. Distribution of male and female volunteers within each shedder phenotype. The total number of males is 20 and the total number of females is 30.

From Figure 17, it appeared that there are more female heavy shedders than males. However, the data was slightly misleading, since there were 10 more females than males. It is likely that, depending on where the shedder thresholds were set, if more males had been sampled, our results may have supported the conclusion that male shedders deposit more DNA in their fingerprints than do female shedders. The total sample mean for males (379 pg) was slightly greater than the total sample mean for females (295 pg). However, using statistical analysis, non-parametric Mann Whitney test ($U = 289.5$; $p \approx 0.8430$), results indicated that there is no significant difference in the total means for males and females. From Table 2, it appeared that there were more female intermediate shedders than males, and more male heavy shedders than females. One possible reason for this is that the range of recovered DNA for both light and heavy shedders is much wider than for intermediate shedders. Because the range in recovered DNA for intermediate shedders is defined by both lower and upper boundaries, it is likely that fingerprints from intermediate shedders exhibit the greatest reproducibility of the three shedder phenotypes, and that the most accurate part of the assay deals with recovered amounts of DNA between 50 and 300 pg. Likewise, light and heavy shedders are often classified as such from quantitation using Amelogenin products whose fluorescence is more likely to be at the extremes of the assay (i.e. either very low fluorescence or very high fluorescence) also contributing to the magnitude of the CV values.

In order to answer the question, "Does the amount of DNA contained within a fingerprint decompose over time?" studies were performed to compare fingerprint

samples that were processed immediately versus those processed at a later time. Samples processed immediately were characterized as those from which DNA was extracted and quantitated within two days of being collected. Samples processed at a later time were characterized as those from which DNA was extracted and quantitated more than 2 days after being collected. For male versus female fingerprint samples processed immediately, the average recoveries of DNA, as well as the distribution of males and females within each shedder phenotype, are summarized in Figure 18 and Table 3.

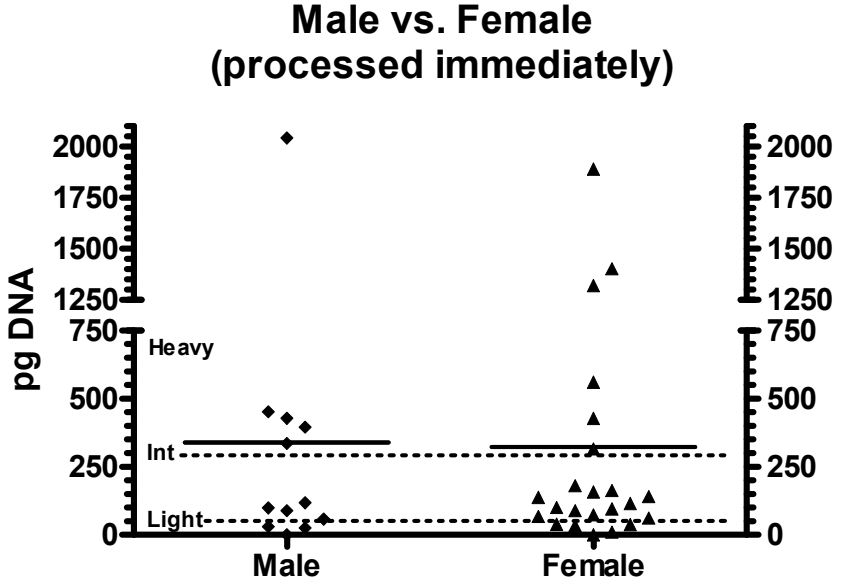

Figure 18. Average recoveries of DNA from fingerprint samples recovered from the non-dominant hand of 12 male and 23 female volunteers that were processed immediately. Each data point represents the mean of sampling done in at least duplicate. The dotted lines indicate the shedder status thresholds. The solid lines indicate the total sample means.

	Distribution Within Each Shedder Phenotype		
Sex of Donor	Light	Intermediate	Heavy
Male	25% (3 / 12)	33% (4 / 12)	42% (5 / 12)
Female	22% (5 / 23)	52% (12 / 23)	26% (6 / 23)

Table 3. Distribution of male and female volunteers within each shedder phenotype. The total number of males is 12 and the total number of females is 23.

Once again, the total sample mean for males (340 pg) was slightly greater than the total sample mean for females (323 pg). However, from Figure 18, these results do not support the conclusion that males deposit more DNA in their fingerprints than females. Individual values for female heavy shedders were larger than for male heavy shedders. However, these results could be misleading, since there are more females than males. Using statistical analysis, non-parametric Mann Whitney test ($U = 136.5$; $p \approx 0.9723$), results indicated that there is no significant difference between the amount of DNA deposited in the fingerprints of males and females when samples are processed immediately. Although the distributions of male and female light shedders are approximately the same, there are more female intermediate shedders than male intermediate shedders, and more male heavy shedders than female heavy shedders. This corresponds to the hypothesis that if males typically deposit more DNA in their fingerprints than females, there would be a higher number of male heavy shedders than female heavy shedders in the general population, and because of how the divisions between shedder groups were set for this study, more females would fall into the intermediate group. Results also indicate that the CVs for light and heavy shedders on

the non-dominant hand are larger than for intermediate shedders (not shown). This corresponds to the hypothesis that because the range of values for intermediate shedders is narrower, the variability within that shedder phenotype would be smaller.

For male versus female fingerprint samples processed later, the average recoveries of DNA, as well as the distribution of males and females within each shedder phenotype, are shown in Figure 19 and Table 4.

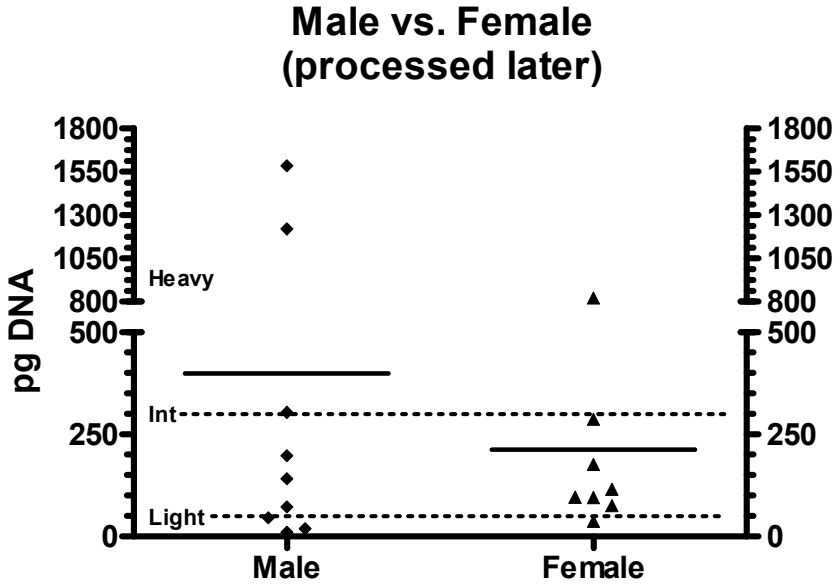

Figure 19. Average recoveries of DNA from fingerprint samples recovered from the non-dominant hand of 9 male and 8 female volunteers that were processed later. Each data point represents the mean of sampling done in at least duplicate. The dotted lines indicate the shedder status thresholds. The solid lines indicate the total sample means.

	Distribution Within Each Shedder Phenotype		
Sex of Donor	Light	Intermediate	Heavy
Male	33.33% (3 / 9)	33.33% (3 / 9)	33.33% (3 / 9)
Female	12.5% (1 / 8)	75% (6 / 8)	12.5% (1 / 8)

Table 4. Distribution of male and female volunteers within each shedder phenotype. The total number of males is 9 and the total number of females is 8.

From Figure 19, the total sample mean for males (399 pg) was approximately two times the total sample mean for females (213 pg). Using statistical analysis, non-parametric Mann Whitney test ($U = 36.00$; $p = 1.00$), results indicated that there is no significant difference between the mean amount of DNA deposited in the fingerprints of males and females when samples are processed later. From Table 4, although there are still more male heavy shedders than female heavy shedders, the magnitude of difference has increased. Although there are still more female intermediate shedders than male intermediate shedders, the magnitude of difference has increased. Also, the magnitude of difference between male and female light shedders has increased substantially in comparison to samples that were processed immediately. Because the amounts of DNA contained within fingerprints are small to begin with, it is possible that some of the differences noticed between samples processed immediately and those processed later are the result of DNA degradation. If degradation has occurred, the results from the samples processed later would be less reproducible.

The fingerprint samples that were processed later were further broken down into samples processed 3 days to 1 month after collection and samples processed after 1 month of collection. For male versus female fingerprint samples processed 3 days to 1 month after collection, the average recoveries of DNA, as well as the distribution of males and females within each shedder phenotype, are shown in Figure 20 and Table 5.

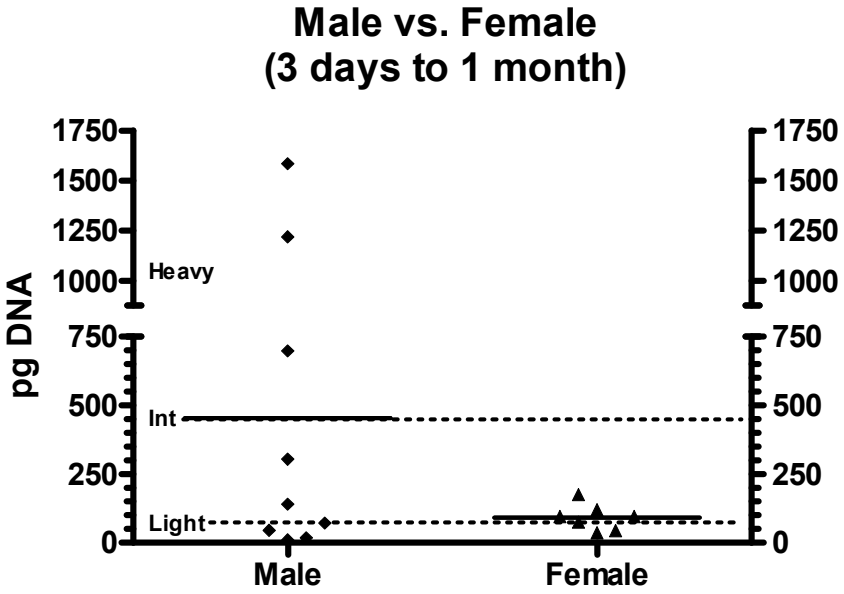

Figure 20. Average recoveries of DNA from fingerprint samples recovered from the non-dominant hand of 9 male and 7 female volunteers that were processed 3 days to 1 month after collection. Each data point represents the mean of sampling done in at least duplicate. The dotted lines indicate the shedder status thresholds. The solid lines indicate the total sample means.

	Distribution Within Each Shedder Category		
Sex of Donor	Light	Intermediate	Heavy
Male	33.3% (3 / 9)	22.3% (2 / 9)	44.4% (4 / 9)
Female	28.6% (2 / 7)	71.4% (5 / 7)	0% (0 / 7)

Table 5. Distribution of male and female volunteers within each shedder phenotype. The total number of males is 9 and the total number of females is 7.

From Figure 20, the total sample mean for males (455 pg) was approximately five times the total sample mean for females (92 pg). Using statistical analysis, non-parametric Mann Whitney test ($U = 36.00$; $p = 1.0000$), results indicated that there is no significant difference between the mean amount of DNA deposited in the fingerprints of males and females for samples processed 3 days to 1 month after sampling. Various data points, as well as Table 5, indicated that, for this experiment, males are distributed within all three shedder phenotypes, but there are no females classified as heavy shedders. The distribution of male and female light shedders on the non-dominant hand are once again approximately the same and there are more female intermediate shedders than male intermediate shedders. It is known that DNA degradation occurs. It is possible that because these samples were not processed immediately, DNA contained within the fingerprints degraded, making the results less reproducible. Another explanation is that certain hygiene habits, such as frequent washing of the hands or wearing lotion, cause less DNA to be deposited in fingerprints.

For male versus female fingerprint samples processed after 1 month of sampling, the average recoveries of DNA, as well as the distribution of males and females within each shedder phenotype, are shown in Figure 21 and Table 6.

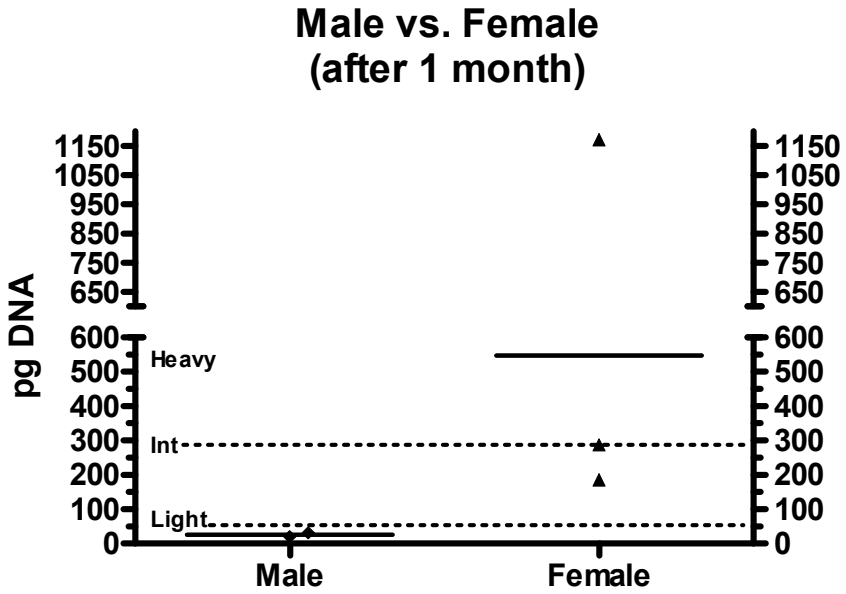

Figure 21. Average recoveries of DNA from fingerprint samples recovered from the non-dominant hand of 2 male and 3 female volunteers that were processed after 1 month of sampling. Each data point represents the mean of sampling done in at least duplicate. The dotted lines indicate the shedder status thresholds. The solid lines indicate the total sample means.

	Distribution Within Each Shedder Category		
Sex of Donor	Light	Intermediate	Heavy
Male	100% (2 / 2)	0% (0 / 2)	0% (0 / 2)
Female	0% (0 / 3)	67% (2 / 3)	33% (1 / 3)

Table 6. Distribution of male and female volunteers within each shedder phenotype. The total number of males is 2 and the total number of females is 3.

It can be seen that there are the fewest number of samples in this portion of the study. Even though the samples were processed after 1 month of being collected, DNA was still recovered from some of the fingerprints. The total sample mean for females (548 pg) was approximately twenty times the total sample mean for males (26 pg). The minimum number of values needed to be able to perform statistical analysis was three; therefore statistical analysis was unable to be performed on these fingerprint samples. For those fingerprints that did not yield detectable DNA, it is likely degradation of the template was responsible.

From the experiments comparing fingerprint samples that were processed immediately versus those processed later, it was found that no conclusive statements could be made with regard to the question, "Does the amount of DNA contained within a fingerprint decompose over time?" However, knowing what we know about DNA degradation, it seems likely that DNA degradation occurs, especially in LCN DNA samples such as fingerprints. Further studies need to be performed to confirm this statement.

In a further extension of the study of reproducibility of depositing DNA in fingerprints from different shedder phenotypes and processed at different times, individual donors were analyzed repeatedly (Table 7, Figure 22).

Processed Earlier (Mean ± SD; CV)	Processed Later (Mean ± SD; CV)
181 pg ± 157; CV = 87%	287 pg ± 396; CV = 138%
698 pg ± 840; CV = 120%	31 pg ± 39; CV = 129%
120 pg ± 85; CV = 71%	669 pg ± 1417; CV = 212%
44 pg ± 39; CV = 90%	185 pg ± 202; CV = 109%
18 pg ± 31; CV = 173%	20 pg ± 35; CV = 173%
30 pg ± 42; CV = 141%	10 pg ± 14; CV = 141%

Table 7. Differing amounts of DNA recovered from the non-dominant hand of 6 volunteers (3 male and 3 female) depending on the time of processing. Each cell shows the sample mean, standard deviation (SD), and coefficient of variation (CV).

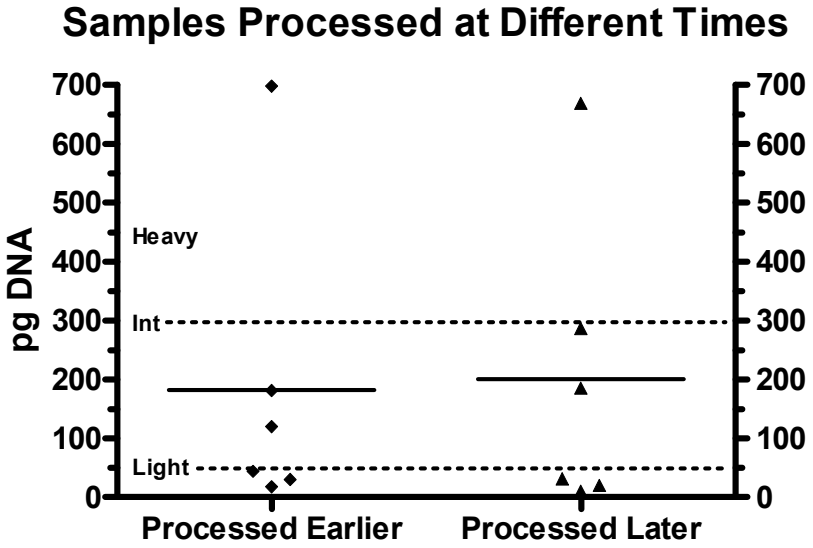

Figure 22. Average recoveries of DNA from fingerprint samples recovered from the non-dominant hand of 3 male and 3 female volunteers depending on the time of processing. Each data point represents the mean of sampling done in at least duplicate. The dotted lines indicate the shedder status thresholds. The solid lines indicate the total sample means.

For two volunteers, the amount of DNA recovered from a fingerprint that was processed at an earlier time was greater than the amount of DNA recovered at a later time. For one volunteer, the amount of DNA recovered from a fingerprint that was

processed at an earlier time was approximately the same as the amount of DNA recovered at a later time. For three volunteers, the amount of DNA recovered from a fingerprint that was processed at an earlier time was less than the amount of DNA recovered at a later time. Based on these results, it is difficult to make any conclusive statements with regard to the reproducibility of depositing DNA in fingerprints from different shedder phenotypes and processing the samples at different times. Using statistical analysis, non-parametric Wilcoxon signed rank test ($W = -5.000$; $p = 0.6875$), results indicated that there is no significant difference in the average recoveries when comparing processing times. Further, the results suggest that, for these volunteers, the amount of DNA deposited in a fingerprint is highly variable when comparing different processing times. It is very possible that external factors such as hand washing, wearing lotion, and other hygiene factors, may underlie the high degree of variability seen in these experiments. Also, some individuals seem to be highly variable due to the biological chemistry of their skin. Because of these factors, there will always be a certain amount of inherent variability when performing this type of study.

3.2. Secondary Transfer

DNA can be transferred from one individual to another through items that are handled by both individuals, a process known as secondary transfer. Thus, two individuals who are shedders and who handle a common object, or even shake hands, may exchange DNA and subsequently deposit their DNA and that of the other person on items subsequently touched. Secondary transfer can also occur from different areas of the same individual's body. For example, on some occasions, male DNA was recovered

from a female's fingerprint based upon the recovery of DNA from the female's fingerprint that directed the amplification of Y-chromosomal Amelogenin amplicons in detectable quantities. In the profiles produced from female fingerprints shown below in Figure 23, products from both the X and Y chromosome are visible.

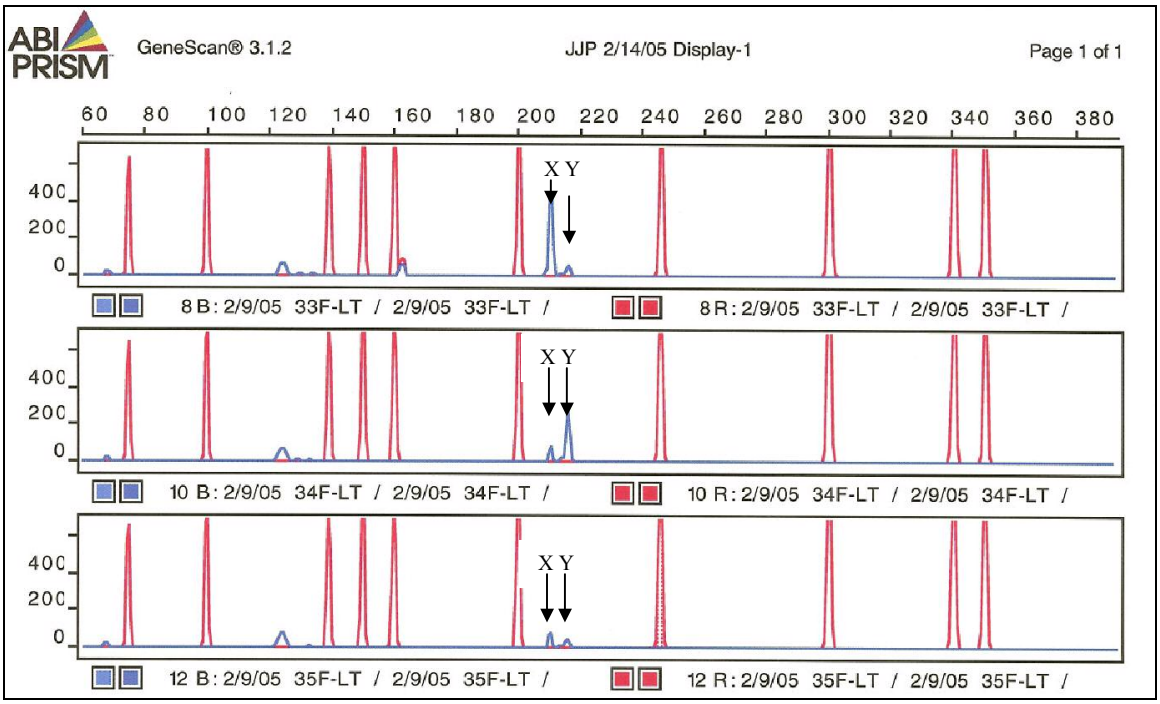

Figure 23. Profiles from female volunteers (33F, 34F, 35F) exhibiting secondary transfer of DNA from another individual (a male, evident from X,Y present in all panes).

One possible explanation for this result is that volunteers 33F, 34F, and 35F picked up male DNA through secondary transfer from an object they had come in contact with prior to depositing their fingerprints on the glass microscope slides. This object could have been anything from a door handle to the steering wheel of a shared

automobile. It should also be noted that amplification of DNA extracted from buccal swabs from these donors did not exhibit Y-chromosomal sequences (not shown).

3.3. DNA Quantitation from Hair Root Samples (N = 20)

In addition to fingerprints, isolated hairs are a common type of forensic evidence recovered from a crime scene or from a victim of sexual assault. While the hair shaft does not contain nuclear DNA, the hair root is useful as a source of nuclear DNA for analysis. Therefore, DNA from hair roots obtained from three areas of the body was quantitated. Three head, pubic, and limb hairs were obtained from each of 20 volunteers. An empirical size rating (scored microscopically) between 1 and 3 was assigned to each hair root (Figures 24 through 26), and DNA extracted from the roots was amplified and analyzed on a 310 Genetic Analyzer using Amelogenin primers.

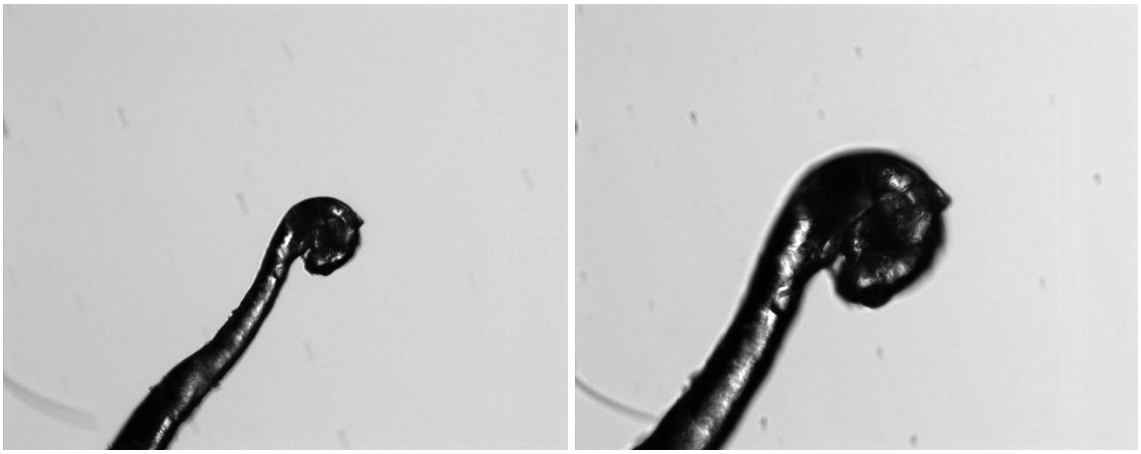

Figure 24. Example of a limb hair root with a visual size rating of 1 (left pane 10 X magnification; right pane 20 X magnification).

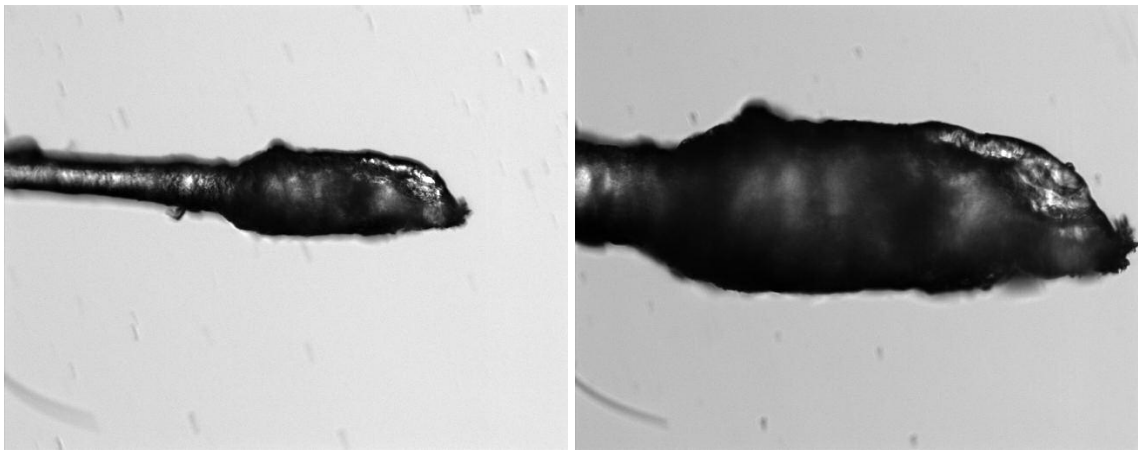

Figure 25. Example of a head hair root with a visual size rating of 2 (left pane 10 X magnification; right pane 20 X magnification).

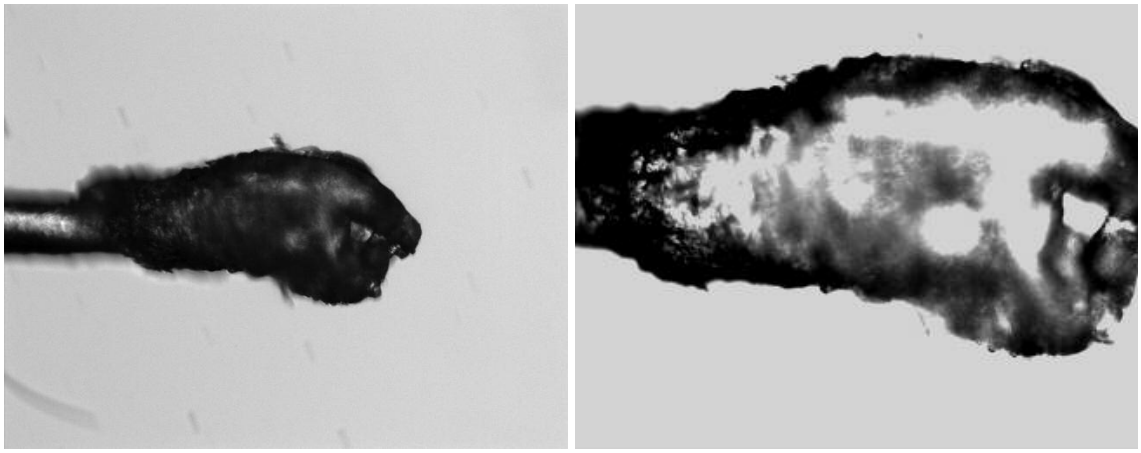

Figure 26. Example of a pubic hair root with a visual size rating of 3 (left pane 10 X magnification; right pane 20 X magnification).

A hair root given a visual size rating of 2 is larger than a hair root given a visual size rating of 1. In the right panes of Figures 24 and 25, the hair root given a visual size rating of 2 takes up more space in the pane than does the hair root given a visual size rating of 1. A hair root with a visual size rating of 3 is larger than a hair root with a

visual size rating of 2. In the right panes of Figures 25 and 26, the hair root given a visual size rating of 3 takes up more space in the pane than does the hair root given a visual size rating of 2.

Butler (2005) stated that a typical forensic hair root sample from a pulled hair contains 1-750 ng of DNA. In this study, the average amount of DNA recovered from the head, pubic, and limb hairs of all 20 individuals were 27.5 ± 41.2 ng. Therefore this study corroborates Butler's statement.

To answer the question, "Is there a significant relationship between DNA recovery and region of the body from where the hair was plucked?" the combined average recoveries of DNA from the head hair roots, pubic hair roots, and limb hair roots of 20 volunteers (9 male and 11 female) are shown in Figure 27.

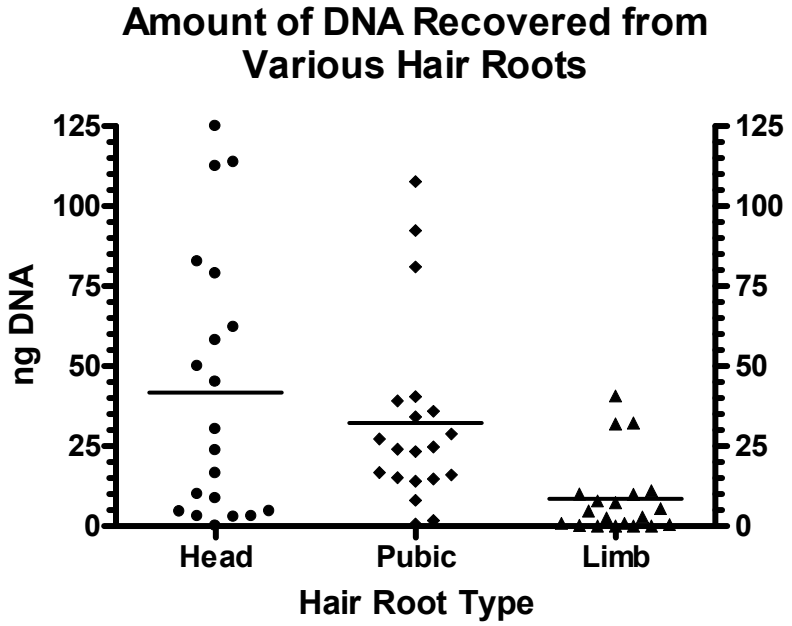

Figure 27. Combined average recoveries of DNA from hair root samples of 20 volunteers (9 male and 11 female). Each data point represents the mean sampling done in triplicate. The solid lines indicate the total sample means.

In general, head hair roots contain more DNA than either pubic or limb hair roots, while pubic hair roots also contain more DNA than limb hair roots. Therefore, a visual decrease in DNA recovery can be seen from head hair roots to pubic hair roots to limb hair roots. Although there does not appear to be a substantial difference in the amount of DNA recovered from head and pubic hair roots, the amount of DNA recovered from limb hair roots is substantially less than either head or pubic hair roots. Specifically, the total sample mean for head hair roots (41.8 ng) was approximately one and a half times the sample mean for pubic hair roots (32.3 ng). The total sample mean for pubic hair roots (32.3 ng) was approximately four times the total sample mean for limb hair roots (8.5 ng). Using statistical analysis, non-parametric Friedman test ($F = 11.20$; $p \approx 0.0037$), results indicated that there is a significant difference in the total sample means. Using

Dunn's Multiple Comparison test, when comparing head and pubic hair roots, there is no significant difference between them ($p > 0.05$). However, there is a significant difference when comparing head and limb hair roots ($p < 0.05$) and when comparing pubic and limb hair roots ($p < 0.01$). Therefore, there does appear to be a significant relationship between DNA recovery and region of the body. One possible explanation for this result is that the size of limb hairs is typically much smaller than head or pubic hairs and morphologically more variable than head or pubic hairs. The smaller the hair, the smaller the hair root, therefore the less DNA that will be recovered from that root.

In order to answer to answer the question, "Is there a significant difference in the amount of DNA recovered between male and female head hairs, male and female pubic hairs, and male and female limb hairs?" the average recoveries of DNA from male and female head, pubic, and limb hair roots are shown in Figure 28.

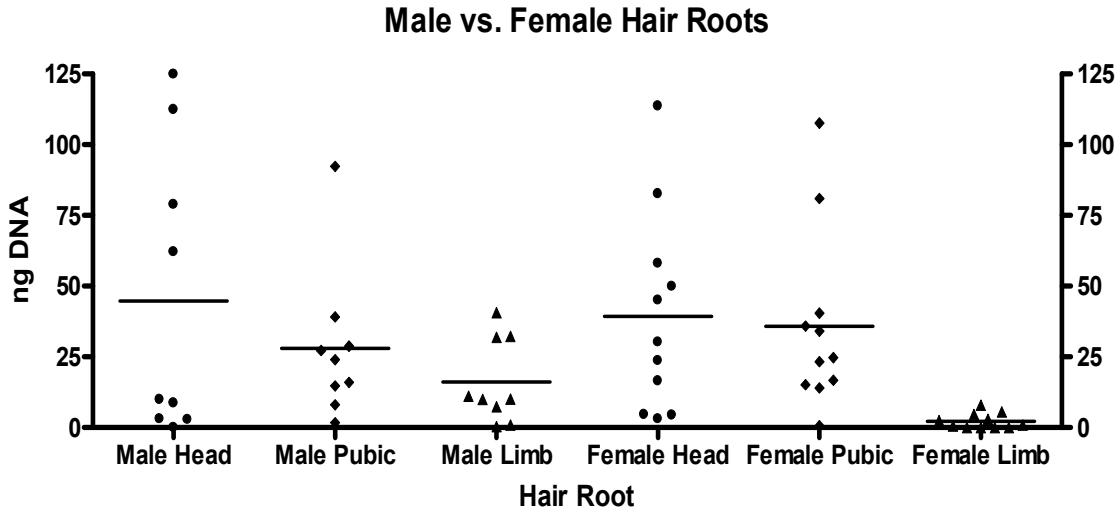

Figure 28. Average recoveries of DNA from head, pubic, and limb hair roots of 9 male and 11 female volunteers. Each data point represents the mean of sampling done in triplicate. The solid lines indicate the total sample means.

From Figure 28, it appeared that the trend of decrease was present for both males and females. For males, results indicated that the total sample mean for head hair roots (44.8 ng) was greater than the total sample mean for pubic hair roots (28.0 ng), which was greater than the total sample mean for limb hair roots (16.0 ng). The same trend can be seen for females. The total sample mean for head hair roots (39.3 ng) was greater than the total sample mean for pubic hair roots (35.8 ng), which was greater than the total sample mean for limb hair roots (2.3 ng). Using statistical analysis, non-parametric Kruskal-Wallis test ($U = 20.19$; $p \approx 0.0012$), results indicated that there is a significant difference in the total sample means. Using Dunn's Multiple Comparison test, there is no significant difference when comparing male head, pubic, and limb hair roots. However, when comparing female head, pubic, and limb hair roots, there is a significant difference between head and limb hair roots ($p < 0.01$) and pubic and limb hair roots ($p < 0.01$). Also, the total sample mean for male limb hair roots (16.0 ng) was approximately seven times the total sample mean for female limb hair roots (2.3 ng), even though there is no significant difference between them ($p > 0.05$). One possible explanation for this result is that males typically have larger / thicker hair than do females, especially limb hairs. Therefore, in general, male hair roots might be expected to contain more DNA than their female counterparts. In sum, there does not appear to be a significant difference in the amount of DNA recovered between male and female hair roots, though there is between hair root types of females. Therefore, there is no gender effect. However, when comparing within gender, there is a significant difference in DNA recovery.

To answer the question, "Is there a significant relationship between DNA recovery and apparent hair root size (visualized microscopically)?" the combined average recoveries of DNA from the visual hair root size ratings 1, 2, and 3 of 20 volunteers (9 male and 11 female) are shown in Figure 29.

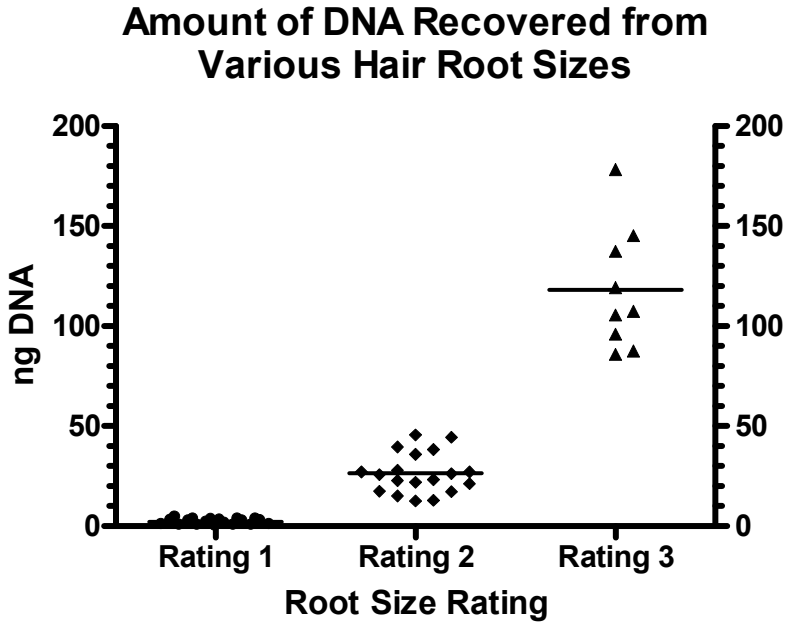

Figure 29. Combined average recoveries of DNA from hair root size ratings 1, 2, and 3 of 20 volunteers (9 male and 11 female). Each data point represents the mean sampling done in triplicate. The solid lines indicate the total sample means.

The apparent visual size rating of hair roots correlates well with the amount of DNA recovered from each class. The total sample mean for size 2 hair roots (26.4 ng) was approximately twelve and a half times the total sample mean for size 1 hair roots (2.1 ng). The total sample mean for size 3 hair roots (118.0 ng) was approximately four and a half times the total sample mean for size 2 hair roots. One possible explanation is that in general, the larger / thicker the hair root, the more DNA will be recovered from that root.

It is simple to determine a size rating for a hair root that either can hardly be seen or that is huge. However, it is harder to determine a size rating for a hair root that is "in between" those sizes. Therefore, there is less variation between the amount of DNA recovered from male and female hair roots with a visual size rating of 2 than for 1 or 3. Using statistical analysis, non-parametric Kruskal-Wallis test ($U = 39.64$; $p < 0.0001$), results indicated that the total samples means are significantly different. Using Dunn's Multiple Comparison test, there is a significant difference between root sizes 1 and 2 ($p < 0.001$), root sizes 1 and 3 ($p < 0.001$), and root sizes 2 and 3 ($p < 0.05$). Therefore, there is a significant relationship between DNA recovery and apparent hair root size (visualized microscopically). As root size increases, DNA recovery increases.

To answer the question, "Is there a significant relationship between DNA recovery and body region within each individual volunteer?" individual DNA recoveries from three body regions of 20 volunteers (9 male and 11 female) are shown in Figure 30.

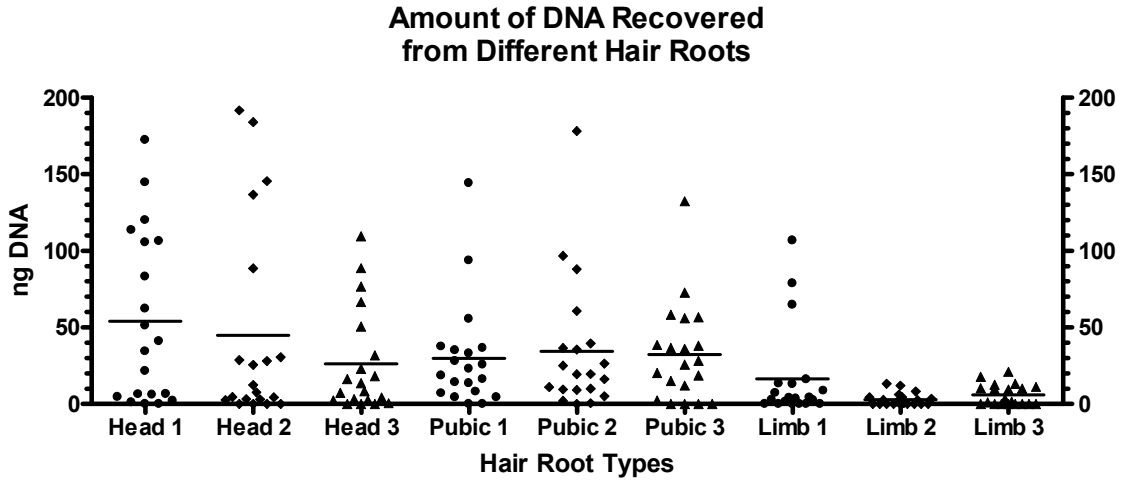

Figure 30. DNA recoveries from three body regions of 20 volunteers. Each data point represents one sample per volunteer. The solid lines indicate the total sample means.

In general, donors appeared to show consistency in DNA recovery among the three hair types in that a donor whose head hair yields more DNA will also yield higher DNA recoveries for the other hair types (individual values not shown). The total sample means for the three head root samples were 54.2 ng, 44.9 ng, and 26.2 ng. The total sample means for the three pubic hair roots were 30.0 ng, 34.5 ng, and 32.4 ng. The total sample means for the three limb hair roots were 16.4 ng, 3.0 ng, and 6.1 ng. Using statistical analysis, non-parametric Friedman test ($F = 38.59$; $p < 0.0001$), results indicated that there is a significant difference in the total sample means. However, using Dunn's Multiple Comparison test, results indicated that there is no significant difference between the three head hair roots ($p > 0.05$), between the three pubic hair roots ($p > 0.05$), and between the three limb hair roots ($p > 0.05$). However, when comparing head or pubic hair roots to limb hair roots, there is a significant difference ($p < 0.05$). Therefore, results have shown that there is a significant relationship between DNA recovery and body region within each individual donor.

4. Discussion

Due to standards requiring all forensic laboratories to quantitate the amount of human DNA found in an evidentiary sample, as well as for PCR amplification purposes, it is essential to be able to accurately quantitate human DNA from evidentiary samples. Forensic laboratories working for the prosecution are mandated to preserve as much evidence as possible to ensure further testing by the defense (if the defense chooses to do so). Being able to estimate the amount of DNA that may be recovered from a forensic sample, such as a fingerprint or hair root, allows the forensic scientist to preserve evidence. For example, if five pubic hairs from an unknown source were collected from a sexual assault victim, and each pubic hair had a visible hair root, it is very possible that a reliable profile will be obtained from only one hair root, preserving the remaining four for further testing.

Several methods currently exist to aid forensic scientists in the quantitation of DNA, including Quantiblot® and real-time PCR. There are advantages and disadvantages to both of these methods. Quantiblot® (Applied Biosystems, Foster City, CA) is a well-established quantitation method and allows for the efficient detection of human specific DNA. However, it does not differentiate between male and female DNA and, because it is based on subjective visual comparison, often sample quantities are misestimated. Real-time PCR (Applied Biosystems, Foster City, CA) is accurate, sensitive, and fast. It can target specific chromosomes and therefore differentiate between male and female DNA allowing the proportions of each template to be estimated within a mixed sample. However, the cost of the equipment is high and space, training,

and quality monitoring, different from that currently in place in a forensic laboratory, is required. The novel human DNA quantitation method used here targets the Amelogenin locus, which distinguishes the X chromosome from the Y chromosome. Advantages to the Amelogenin method (or Q-TAT) are that it is accurate, sensitive, and reasonably inexpensive. More importantly, the method utilizes the same equipment, maintenance, and reagents as typical STR analyses, while meeting the requirements for DNA quantitation published by accrediting agencies.

Quantitation of DNA using Q-TAT is accomplished by generating standard curves relating X / Y amplicon fluorescence (RFUs) produced from known amounts of input male reference DNA template. The concentration of DNA in unknown forensic samples can then be estimated from the standard curve. Using the methods outlined in Materials and Methods, reproducible standard curves were generated, regardless of whether Amelogenin primers were purchased as part of a sex-typing kit or synthesized separately.

The specific goal of this study was to apply the Q-TAT method to **quantitate the amount of human DNA recovered from typical trace forensic samples such as fingerprints and hair roots**. In doing so, the concentration of DNA from three classes of fingerprint donors, as well as from three different types of hair were determined.

Lowe et al (2002) described individuals as good shedders (individuals who deposit an adequate amount of DNA on the object they touch to determine their DNA

profile) or poor shedders (individuals who deposit DNA on the object in amounts too low to produce a profile using typical DNA typing methods). Prior studies from this laboratory (Joslin, 2004 and Gulick, 2005) enabled classes of fingerprint donors to be subdivided even further. They described individuals as good shedders, moderate shedders, or poor shedders based upon the characteristics of the STR-DNA profile produced from fingerprint DNA.

In the fingerprint portion of this study, 50 volunteers were utilized. The DNA in a fingerprint transferred to a substrate is contained within sloughed skin cells, perspiration, and debris of the ridges. Depending on the amount of DNA recovered from their fingerprints, volunteers were classified as light shedders, intermediate shedders, or heavy shedders. A light shedder regularly deposited less than 50 pg of DNA in a fingerprint. An intermediate shedder regularly deposited between 50 and 300 pg of DNA in a fingerprint. A heavy shedder regularly deposited more than 300 pg of DNA in a fingerprint. The recommended amount of human DNA template for STR typing using currently available multiplex kits is 0.5 – 1.0 ng of chromosomal DNA (Profiler Plus and Identifiler Manuals, Applied Biosystems, Foster City, CA).

It appears that fingerprints deposited on glass microscope slides from the non-dominant hand contain more DNA than fingerprints originating from the dominant hand, probably because of the steady depletion of cells from the dominant hand through constant use. To determine if there is a significant difference between the amount of DNA recovered from the non-dominant and dominant hand, initial studies quantitated

DNA recovery from fingerprints deposited by the thumb, forefinger, and middle finger of both the non-dominant and dominant hands of seven volunteers. Results indicated that, in general, fingerprints from a volunteer's non-dominant hand contain higher and more consistent levels of DNA than from the dominant hand. The total sample mean for the non-dominant hand was found to be approximately three times the total sample mean for the dominant hand. Also, statistical analysis proved that there is a **significant difference in the amount of DNA recovered from the non-dominant hand and dominant hand** ($p = 0.0313$). Because of these results, further studies included using only the non-dominant hand of volunteers.

Gulick (2005) determined that, in general, males deposit more DNA in their fingerprints than females. To explore this observation quantitatively, the average recoveries of DNA from the fingerprints of males and females were compared. Depending on where thresholds for the different shedder phenotypes are set, results appeared to support the conclusion that male shedders do deposit more DNA in their fingerprints than do female shedders. However, statistical analysis proved that there is **no significant difference in the amount of DNA recovered from males and females** ($p \approx 0.8430$). When comparing the distribution of male and female volunteers within each shedder phenotype, it appeared that the number of males and females classified as light shedders are very similar, but in the intermediate class the number of females is greater by two fold. This proportion is reversed among heavy shedders with males outnumbering females two fold. The interpretation of these results is that, based upon our arbitrary classification scheme, more males shed larger amounts of DNA in their fingerprints than

do females. Extended to the general population, males are likely to deposit more DNA in their fingerprints than females and thus a higher percentage of males would be expected to fall in the heavy shedder category, with a higher percentage of females in the intermediate group.

Joslin (2004) determined that after storage at room temperature, the amount of DNA recovered from a fingerprint decreases substantially. To determine if the DNA contained within a fingerprint decomposes over time, studies were performed to compare fingerprint samples that were processed immediately (within two days of collection) versus at a later time (more than 2 days after collection). Results corroborated previous results in that statistical analysis proved that there is no significant difference in the amount of DNA recovered from the fingerprints of males and females, whether samples were processed immediately or later. However, it is likely these results are misleading due to the fact that more females were used than males. No conclusive results were found that could corroborate Joslin's finding (Joslin, 2004) that the DNA contained within a fingerprint decomposes over time. However, it is likely that DNA degradation occurs, especially in LCN DNA samples such as fingerprints.

One of the themes that emerge from our results is that the amount of DNA deposited in the fingerprint of an intermediate or heavy shedder can vary substantially. Factors responsible for the high degree of variability could include external factors such as hand washing, lotion use, and other hygiene issues, as well as the biological chemistry of the skin.

Several studies, including those of Joslin (2004) and Gulick (2005), have indicated that evidence of secondary transfer can be detected in DNA profiles produced from low copy number samples, such as profiles generated from fingerprints. This study corroborates those findings. Occasionally, male DNA was recovered from a female's fingerprint, most likely due to secondary transfer.

Another common type of trace forensic evidence is hair. In the hair root portion of this study, 20 volunteers were utilized. DNA from three types of hair roots (head, pubic, and limb) was quantitated and a visual size rating between 1 and 3 was subjectively assigned to each root. According to Butler (2005), a typical forensic hair root sample from a pulled hair contains 1-750 ng of DNA. This study corroborates Butler's observation because the average amount of DNA recovered from a pulled hair root in our study was 27.5 ng.

To determine if there is a significant relationship between DNA recovery and region of the body from where the hair was plucked, average recoveries from each sample type were compared. In general, a trend of decreasing means was noticed from head hair roots to pubic hair roots to limb hair roots. Statistical analysis proved that there is a **significant relationship between DNA recovery and body region** ($p \approx 0.0037$), specifically when comparing head or pubic hair roots to limb hair roots. These results support the hypothesis that since limb hairs are generally smaller than head or pubic hairs, the roots will be smaller and will contain less and more variable quantities of DNA.

To determine if there is a significant difference in the amount of DNA recovered from the hair roots of male and female volunteers, average recoveries from all male and female samples of each root type were compared. The trend of mean decrease was present in both male and female volunteers for each type of hair root. Also, the mean amount of DNA recovered from male limb hair roots was approximately seven times that of females. Statistical analysis proved that there is a significant difference in the total sample means ($p \approx 0.0012$). However, further analysis proved that there is **no significant difference in the amount of DNA recovered between male and female head hairs, male and female pubic hairs, or male and female limb hairs**. These results corroborate what would likely be seen in the general population, that males appear to have larger or thicker limb hair roots than females and so male limb hair will contain more DNA than female limb hair.

To determine if the size of a hair root estimated microscopically correlates with the amount of DNA recovered, hair roots were assigned a subjective size rating between 1 and 3 (1 being smallest). Results indicated that the average recoveries of DNA from samples of each size category correlated well with the visual size ratings, in that the larger the hair root, the more DNA recovered from the root. Roots with a size rating of 2 contained significantly more DNA than roots with a size rating of 1. Roots with a size rating of 3 yielded the greatest recovery of DNA, greater than roots rated as 2 or 1. Moreover, reproducibility in recovery of DNA from roots in the 2 or 3 size class is much greater than for roots in the size 1 category. Statistical analysis proved that there is a

significant relationship between DNA recovery and root size. As root size increases, DNA recovery increases.

It was suggested before the study began that there might be more variability in the amount of DNA recovered from three different hair roots of the same individual. To determine if there is an overall significant relationship between DNA recovery and body region within each individual donor, results from three different head, pubic, and limb hair roots of the 20 volunteers were compared. Comparison of the amount of DNA recovered from head, pubic, and limb hair roots of the same volunteers are consistent in DNA recovery. Statistical analysis proved that there is no significant difference between the three individual hair roots for each type. However, there is a significant difference in the amount of DNA recovered from head or pubic hair roots when compared to limb hair roots ($p < 0.05$). Therefore there is a **significant relationship between DNA recovery and body region within each individual donor**. This can be explained by the size differences of the different types of hair. Limb hair is significantly smaller in size than either head or pubic hair. The smaller the hair, the smaller the root, the less DNA contained within the root.

Our study underscores the fact that DNA can be recovered and quantitated from trace evidence recovered from crime scenes, such as fingerprints and hair roots. Low copy number (LCN) DNA from fingerprints deposited on an ideal substrate (a clean glass microscope slide) or pulled hair roots from various parts of the human body can be quantitated following extraction, amplification, capillary electrophoresis, and data

analysis methods commonly used in forensic laboratories. Our results also emphasize the importance for forensic laboratory personnel to process samples as soon as possible, in order to prevent degradation. It is also important for laboratory personnel to be aware of secondary transfer on forensic samples bearing fingerprints. Thus, the DNA profile produced from DNA recovered from a fingerprint must be analyzed and matched against reference individuals (suspects and / or victims) with secondary transfer kept in mind.

Especially for limb hair roots, the ability to accurately quantitate the amount of DNA contained within the root is important when dealing with terrorist bombings. Many times, limb hair is recovered from debris from explosive devices, often stuck to the tape used to assemble the bomb. If limb hair containing roots is found, DNA can be extracted, amplified, and quantitated. Perhaps then a profile of a possible bomb suspect can be obtained.

Future studies to be performed could involve:

- In-depth analysis on reasons why males deposit more DNA in their fingerprints than females.
- Quantitating DNA recovery from other "forensic" samples**.**

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Title of Study: QUANTITATION OF HUMAN CHROMOSOMAL DNA IN FINGERPRINTS AND HAIR ROOTS USING THE AMELOGENIN LOCUS

Pages in Study: 66 Candidate for the Degree of Master of Science

Major Field: Forensic Science

Scope and Method of Study: The purpose of this study was to use this method to obtain an estimate of DNA recovery from forensic samples, such as a fingerprint and hair root. Participants for the fingerprint portion of this study were 50 male and female volunteers, from either the OSU-CHS campus or family and friends residing out of state. Participants for the hair root portion of this study were 20 male and female volunteers, again from either the OSU-CHS campus or family and friends residing out of state. DNA was extracted, amplified, and analyzed on a 310 Genetic Analyzer. The amount of fluorescence in the X and/or Y amplification products produced from unknown samples was compared to fluorescence in products amplified from known inputs of human DNA to create a standard curve.

Findings and Conclusions: It was found that in general, males tend to deposit more DNA in their fingerprints than do females, though the difference is not significant. Depending on the set threshold, a higher percentage of males were found to be heavy shedders, and a higher percentage of females were found to be intermediate shedders. One possible explanation is that females are more concerned about personal hygiene than males. Females tend to wash their hands more and use more products such as lotions than do males. It was also found that the amount of DNA recovered from a pulled hair root supports the visual comparisons of root size ratings. The larger / thicker the hair root, the more DNA recovered from the root.

ADVISOR'S APPROVAL: Dr. Robert Allen