The Interpretation of Various Skin Conditions on the Transfer of Secondary Touch DNA

A research project submitted in partial fulfillment of the requirements for the degree of Master of Science at George Mason University

By

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Dedication

This is dedicated to my mother and grandmother who have always believed in me, supported me, and pushed me to be the very best person I could be. You both have been my rock since day one and one day I hope to take care of you like you have always taken care of me. Thank you for caring and constantly showering me with love. I love you both.

I also would like to dedicate this to my dog Jackson who has been with me since I was 9 years old. Whenever I had a tough day at school you were always there to play and cheer me up. You may be an old man now but you are still my ray of sunshine. I love you buddy.

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List of Abbreviations

AB – Applied Biosystems

DNA – Deoxyribonucleic Acid

PCR – Polymerase Chain Reaction

PPE – Personal Protective Equipment

Terms and Definitions

Allele - an alternative form of a gene or a section of DNA at a particular genetic location (locus); typically multiple alleles are possible for each STR marker (Butler, 2010)

Allelic Dropout - failure to detect an allele within a sample or failure to amplify an allele during PCR; due to primer binding site mutations or stochastic effects when attempting to amplify low amounts of DNA template (Butler, 2010)

Analytical threshold - an acceptable 'relative fluorescence units' (RFU) level determined to be appropriate for use in the PCR/STR DNA typing process; a minimum threshold for data comparison that identified by the specific forensic laboratory doing the testing through independent validation studies (Butler, 2010)

Artifact - any nonallelic product of the amplification process (stutter or minus A) or anomaly of the detection process (pull-up or spike) (Butler, 2010)

Chelex extraction - a method of DNA extraction involving Chelex resin that produces single-stranded DNA (Butler, 2010)

Deoxyribonucleic acid (DNA) - the genetic material of organisms, usually double stranded; a class of nucleic acids identified by the presence of deoxyribose, a sugar, and the four nucleobases, adenine, thymine, cytosine, and guanine (Butler, 2010)

Electrophoresis - a technique in which molecules are separated by their velocity in an electric field (Butler, 2010)

- A method that separates STRs by their size and charge and is able to detect each STR fragment through fluorescent dyes, that were previously attached during PCR, then proceeds to covert the signal from the dyes to electronic signal (Butler, 2010)

Electropherogram -the graphic representation of the separation of molecules by electrophoresis in which the data appear as ' peaks ' along a line (Butler, 2010)

Fluorescence - the emission of light from a molecule following its excitation by light energy; in the context of DNA analysis, different fluorescent dyes permit simultaneous detection of similar size PCR products through fluorescence emission in different colors (Butler, 2010)

Friction ridge - A raised portion of the epidermis on the palmar or plantar skin, consisting of one or more connected ridge units (IAI et al., 2011)

Friction ridge detail - An area comprised of the combination of ridge flow, ridge characteristics, and ridge structure (IAI et al., 2011)

Heterozygous - having different alleles at a particular locus; manifest as two distinct peaks for a locus in an electropherogram (Butler, 2010)

Homozygous - having two identical or indistinguishable alleles at a particular genetic locus; could occur because the alleles are identical by descent or identical by state; manifest as a single peak for a locus in an electropherogram (Butler, 2010)

Latent Print - Transferred impression of friction ridge detail not readily visible (IAI et al., 2011)

Locus - a unique physical location of a gene (or specific sequence of DNA) on a chromosome; the plural of locus is loci

Lysate - A fluid containing the contents of lysed (broken down) cells (Genlantis, n.d.)

Mixture - a sample resulting from the combination of biological material from two or more contributors (Butler, 2010)

Partial profile - failure to obtain a complete set of results for the DNA loci examined; common causes include degraded or low quantities of template DNA (Butler, 2010)

Polymerase Chain Reaction (PCR) - process that yields millions of copies of desired DNA through repeated cycling of a reaction involving the DNA polymerase enzyme (Butler, 2010)

Short tandem repeats (STRs) - multiple copies of an identical (or similar) DNA sequence arranged in direct succession where the repeat sequence unit is 2 to 6 bp in length (Butler, 2010)

Sister allele - term given to the second allele present in a heterozygous sample (Butler, 2010)

Stochastic Threshold – a detection level set where a potential sister allele of a detected peak may fall below the analytical threshold (Butler, 2010)

Abstract

Secondary DNA transfer has been an increasing topic of study throughout the forensic science community recent years. Little information is known about how the condition of the surface of an individual's hand may play a role in the transfer of secondary DNA. This study evaluated whether individuals' hand conditions, whether oily or dry, played a role in the deposition of DNA onto another individuals' hands and then onto a glass slab. Participants were assigned as the primary DNA contributor, meaning they would be the person touching the glass. Participants were then told their hand condition and the researcher and each participant had direct contact with each other for 30 seconds via handshakes after which the primary placed their hand on a glass substrate. Swabbing's of the glass were taken them extracted, quantified, amplified, and then injected into the AB 3500 Genetic Analyzer. DNA typing results showed that under oily conditions, the secondary contributors profile was likely to show up in more abundance than if that contributor had dry skin, having 35.4% of alleles detected when both contributors had oily hands and 20% of alleles detected when only the secondary contributor had oily hands.

Keywords: *secondary DNA, secondary transfer, forensic science, deposition, primary, hand, contact, handshake, oily, dry, contributor, alleles, transfer , touch DNA*

Introduction

In previous decades there have been numerous investigations into primary transfer of Deoxyribonucleic Acid (DNA) but in recent years, investigators and researchers have begun to look into the possibility that not every individual who shows up on a profile was actually at the scene. In forensic science, throughout all the different disciplines there is one common factor that is followed and lived by, Locard's Exchange Principle. Locard's Exchange Principle states that every contact leaves a trace, going from a victim to the suspect to the crime scene or in any order, there will always be a trace of each left on each. Touch DNA is a term used to describe human DNA that is left behind on an object after it has been handled or the transfer of DNA through skin cells when an object is either being touched or handled (Gammon et al., 2019; Alketbi, 2018). At one point it was believed that DNA was deposited through regular and continuing contact rather than a single contact due to the large amounts of DNA recovered. It was also believed that touching an item only once would leave a few cells to collect and that only a very small amount of DNA could be recovered (Meakin and Jamieson, 2013). Forensic DNA technology has now advanced to the point where only a single human cell can successfully generate a DNA profile (Gammon et. al., 2019).

Anatomy of the Skin

Skin is the largest organ on the human body and it has numerous functions including the regulation of body temperature, moisture retention, protection from invasive organisms such as viruses and bacteria, and sensation. The structure of this organ helps to explain how DNA can be transferred from hand to hand or hand to object in cases of direct or secondary DNA transfer by helping to understand the life cycle of cells on the skin. Skin is made up of two primary layers:

the epidermis and the dermis. The bottom layer of the epidermis is called the basal layer and the top layer is called the cornified layer. In the cornified layer, the eleidin protein is located which becomes keratin as it migrates upwards. Keratin is the key structural material in the outer layer of skin. As it moves up to the surface of the skin, old keratin cells are continuously sloughed off the surface of the epidermis which results in a constant need to restore the keratin that has been lost (IAI et al., 2011).

The dermis is made of various connective tissues which include collagen, elastin fibers, and an interfibrillar gel that is made up of glycosamin-proteoglycans, salts, and water. Located in this layer are two major glands that the body needs: the sudoriferous and sebaceous glands. The sudoriferous glands are further broken down into the eccrine and apocrine glands. These three gland classes contribute to the production of sweat. Each of these glands provide a particular mixture of chemical compounds that either secrete from pores onto the friction ridges of the hands and feet or are transferred to the friction ridges through touching an area such as the forehead or underarm. In the sudoriferous gland, the eccrine gland is located mainly on the palms of the hands and soles of the feet. Although this gland is not present on the hands and feet, the oils produced by it are commonly found on touched samples because people have the propensity to touch their face and other areas with this gland (IAI et al., 2011).

This gland secretes organic compounds such as amino acids that are the primary substance in latent prints and the ability to visualize ridge details. Also a part of the sudoriferous gland is the apocrine gland which is associated with course hair of the armpits and the pubic area. This gland is larger and secretes thicker fluid than that of the eccrine gland. The gland's ducts usually empties into a hair follicle located above the sebaceous gland duct before the

secretions reach the skin's surface. The second major gland, the sebaceous gland, are small saclike organs that are found throughout the body associating primarily with body hair. This gland, unlike eccrine glands, are not found on the hands or feet but are abundantly found on the scalp, face, anus, nose, mouth, and external portions of the ears. The purpose of this gland is to help retain body heat by preventing sweat evaporation and to lubricate hair and the surrounding skin (IAI et al., 2011).

Conditions that affect the ability to leave sufficient DNA from the skin are called deposition factors. These factors influence the quality and even the presence of latent prints which come into play with touch DNA. Deposition factors include the conditions around the contact between friction skin and the objects that are touched such as pre-transfer conditions which include the condition or health of the donor's friction skin and the amount and type of residue on the skin; transfer conditions which are the conditions of the substate/surface being touched, including the texture, surface area, surface temperature, and surface residue; and pressure applied, also considered a transfer condition, during contact, otherwise known as deposition pressure.. In this study, the pre-transfer condition, the condition of the donor's friction skin and the amount and type of residue on the skin will mainly be focused on (IAI et al., 2011).

Touch DNA Overview

As stated before, Locard's Exchange Principle plays a big role in the forensic science community. This theory states that when a perpetrator is committing a crime they will leave traces of their presence at the scene and consecutively leave with trace evidence from that scene, both of which can both be used as forensic evidence in an ongoing criminal investigation (Cale et al., 2016). In the case of DNA, transfer occurs from direct contact of the skin with a substrate,

with shedder status of the individual influencing the amount of DNA left behind (Kanokwongnuwut, et al., 2018). The concept of an individual being a 'shedder' or 'non-shedder' became fairly popular through Lowe et al. who in 2002 conducted a study to assess the ability of an individual to deposit their own DNA profile on an item as well as the potential to detect DNA profiles resulting from secondary transfer, which is now known as shedder status. As mentioned by Lowe et al. in this study, several groups of researchers had shown that the amount of DNA deposited by primary transfer when an individual touches an object appeared to be donor dependent (2002). Along with Shedder status, researchers have also look at hand washing as a potential factor in the amount of shed cells on the skin which contain DNA. DNA expert Suzanne Ryan (2012) has stated that "if a person has not washed their hands recently, there tends to be more DNA present on the surface of the hands as washing will remove many of the shed cells on the surface of the hands". Ryan also references in the article that researchers Quinones and Daniel (2011) verified through their study that the presence of sweat helps to increase DNA yields obtained from touch DNA samples from individuals with sweaty hands.

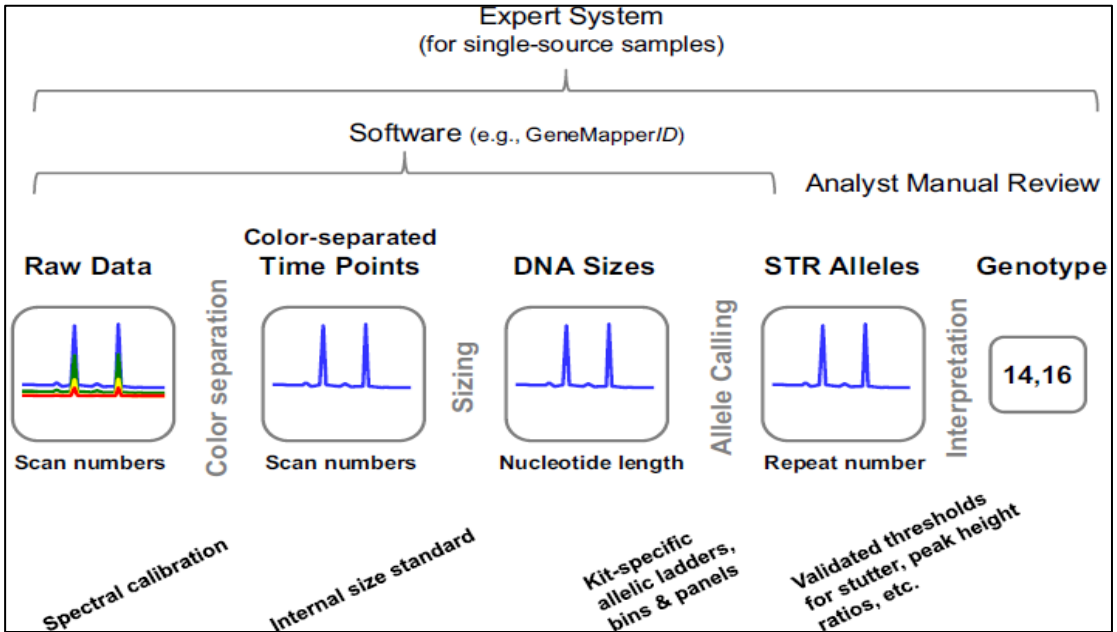
In recent years, studies have concluded that it may be possible to bring traces of another individual into a crime scene and deposit traces of their DNA through secondary DNA transfer (Cale et al., 2016). When looking at the situation of how the DNA got somewhere, the likelihood that it was transferred rather than directly deposited on an item needs to be considered during the analysis of the situation (Szkuta et al., 2017). Secondary transfer takes place when DNA is transferred from one object or individual to another by an intermediate object/person (Cale et al., 2016). As stated by Cale et al. (2016), there are various concerns that forensic DNA analysts should have due to the ability of secondary DNA transfers to potentially (i) falsely link or include

someone in a crime, (ii) introduce extraneous DNA into a forensic sample, and (iii) may lead analysts and other medicolegal professionals to falsely come to the conclusion that DNA left on an object is a result of direct contact.

Analysis Overview

Over time, various collection techniques have been used to obtain touch DNA from substrates. The most common collection techniques include cutting small sections out of a substrate when applicable and placing it in a sterile tube, swabbing the suspected area with a sterile cotton swab and then placing it in a sterile tube, or pressing adhesive tape on substrates such as fabrics and then cutting the tape before also placing it into a sterile tube (Linacre et al., 2009). Once samples have been collected, the next steps involved in the analysis process include: (1) extraction, (2) quantification, (3)Polymerase Chain Reaction (PCR) amplification, and (4) STR typing.

Extraction is the separation and isolation process of DNA from other biological substances contained within the biological sample and the substrate it is deposited on (Butler, 2010). In past years, forensic laboratories have used various extraction methods such as Chelex, solid phase, and organic solvents. But, in more recent years extraction kits have become more



present and have gained popularity due to the reduction of contamination and loss of DNA that has previously been caused through frequent tube changes during the extraction process (Sethi et al., 2013). After extraction has successfully been completed, quantification is done which which helps to determine the amount of DNA that was recovered during the extraction process (Butler, 2010). Various instruments and kits have been created over the years to aid in this process such as the Applied Biosystems (AB) QuantStudio 5 Real-Time PCR System for Human Identification, AB 7500 Fast Real-Time PCR System, AB Quantifiler Trio DNA Quantification Kit, AB Quantifiler Duo Quantification Kit, and the AB Quantifiler Human DNA Quantification Kit. Quantification and amplification can be a crucial step in the analysis process when a sample is considered degraded or have a low copy number (LCN) where there is only a small amount of DNA present in the sample (Sethi et al., 2013). Quantification can help determine how much DNA is in a sample to see if amplifying the sample is needed if there is not enough DNA in the sample. In order to obtain a full DNA profile there needs to be a minimum DNA concentration of 0.5ng/μL to 1ng/μL, anything below this has the potential to yield a partial DNA profile or no DNA profile at all (Sethi et al., 2013). PCR Amplification is a process which is used to copy certain targeted regions of the DNA to create numerous copies of a particular sequence which may vary, known as Short Tandem Repeats (STRs), through cycles of heating and cooling (Butler, 2010).

Figure 1. The transformation process of sample information/data that occurs at one STR locus during data interpretation (Butler, 2015)

This step can be completed by using instruments such as a thermocycler and kits such as the AB Globalfiler PCR Amplification Kit, AB AmpFLSTR Identifiler™ PCR Amplification Kit,

and the AB Identifiler Plus Amplification Kit. STR Typing is the last step in the analysis of a DNA sample, this step is the process of analyzing DNA data previously obtained through capillary electrophoresis and viewing them on a gene analyzing software which takes the raw data and separates it by dye color separated time points, DNA sizes, and STR alleles, as seen above in figure 1.

When analyzing a DNA profile, alleles that have been detected through gene analyzing software are looked at and determined to either be real peaks, contamination, or artifacts such as stutter. Along with stutter, other issues that can arise as the amount of DNA decreases with degraded or low level DNA samples is allele dropout. As seen in figure 2, this is an example showing the general trend of how heterozygote peak balance decreases as DNA template levels also decrease until one of the alleles fails to be amplified (Butler, pg, 92, 2015).

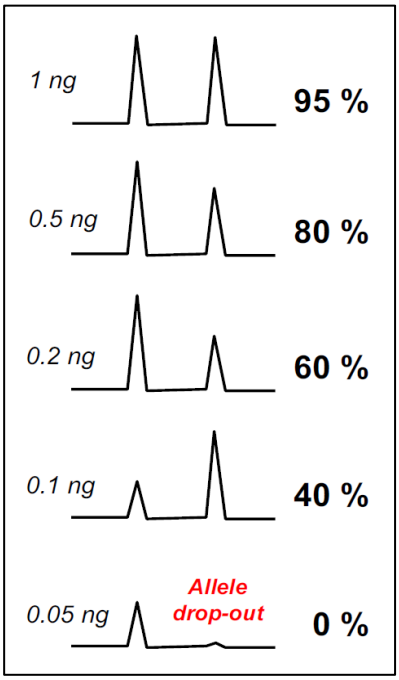


Figure 2. *Trend of allelic dropout as levels of DNA template decrease (Butler, 2015)*

When this occurs it can lead to belief that there is a homozygous allele instead (Butler, 2015). When there is potential allele dropout, this can lead to uncertainty when analyzing the profile and can cause issues with an analyst coming to a conclusion. If an analyst comes to a decision of inconclusive, this means that there is an inability to clearly include or exclude a suspect's DNA profile from a profile obtained from a piece of evidence (Butler, 2015). Other factors that can lead to a decision of "inconclusive" include: (1) no or limited data was collected from the evidence sample due to DNA degradation, (2) results from the evidence sample were too complex to accurately interpret,

or (3) a reference sample was not available to do a known versus unknown profile comparison (Butler, 2015). As stated by John Butler in his book published in 2015, *Advanced Topics in Forensic DNA Typing: Interpretation*, when there is a mixture which contains DNA from two contributors, each separate STR locus can exhibit anywhere from one to four different alleles as seen in figure 3. In figure 3, Butler (2015) is showing hypothetical results from person A, in red, and person B, in blue, showing how there can be peak height changes that occur when there is allele stacking caused by the sharing of alleles and a 1:1 mixture ration.

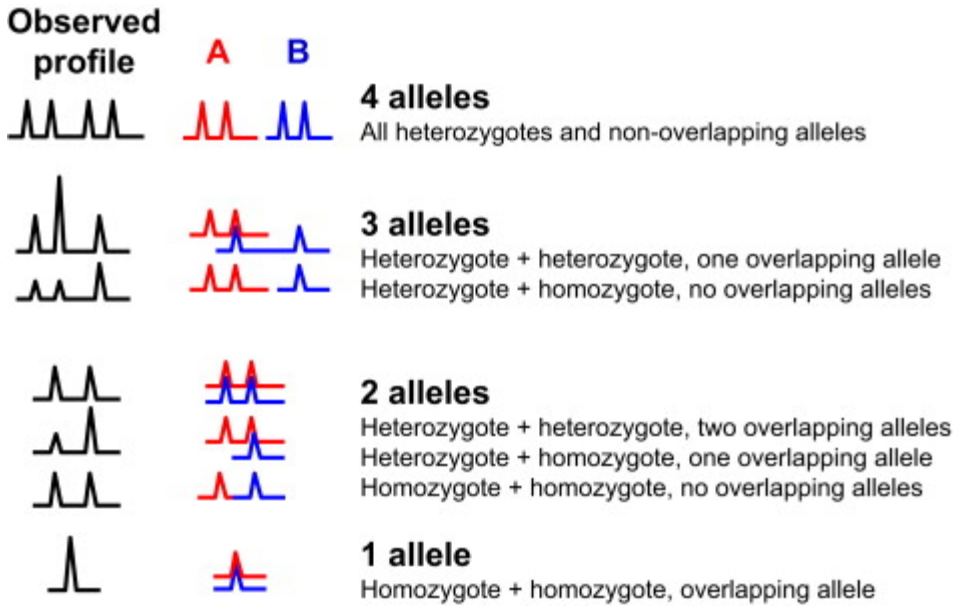


Figure 3. *The various combinations that contributors alleles can appear in profiles (Butler, 2015)*

Purpose of this Study

In recent years there have been more and more studies on secondary DNA and the possibilities of how it may have come about on a scene. While there have been such studies, the question of "Under what circumstances could the chances of finding secondary DNA increase?" and "What may be the reason behind secondary DNA besides the obvious scenario of one person

touching an object and then another person touching that same object?" has yet to be fully answered. The purpose of this study was to determine if certain conditions on an individual's hand such as if it is oily, as in normal nature oils on the skin, or dry, as in washed previously with no nature oils on the skin yet, play a role in how much touch DNA is transferred. Secondary goals were to determine if the state of two individuals hands plays a role in the concentration of touch DNA obtained from a glass slab. By looking into situations of an individual's hand conditions, whether oily or dry, the objective of this study was to determine if in fact hand conditions have an effect on the frequency of secondary DNA being transferred and seen in DNA profiles and if the primary individuals hands play a role in the frequency as well. This study is preliminary step to further research on how the condition of an individual's skin may impact the amount of DNA deposited onto someone or something. In addition, it can be the first step in evaluating how these factors impact DNA profile analysis and be a possible explanation as to why an individual who was actually at a crime scene may not be detected in a profile or why an individual who was not at a scene is detected in a profile.

Literature Review

In 2002, Lowe et al. showed that there is a difference between individuals and how much DNA they tend to deposit on an item after contact. The authors reported the potential to detect secondary transfer DNA profiles under specific laboratory conditions using Low Copy Number analysis protocols and the standard Second Generation Multiplex Plus DNA profiling technique. Through this study, the transfer of DNA from one individual to a second individual through direct contact of the hands and afterwards to an a sterile tube was seen to be possible under distinct laboratory conditions using the AMPLSTR SGM Plus Multiplex at 28 and 34 PCR

cycles following a Low Copy Number analysis protocol. The sterile tubes as well as the two individuals hands were swabbed to investigate the ability of an individual to deposit his or her own DNA profile on an object (Lowe et al., 2002). Overall, this study demonstrated that the quantity and quality of DNA profiles recovered was dependent on each individual involved in the transfer process.

In the years following Lowe et al.'s 2002 study, Cale et al. (2016) evaluated the transfer of DNA through an intermediate looking at whether newer more sensitive technology could lead to the detection of interpretable secondary DNA transfer profiles. Through this study the authors also demonstrated the risks of assuming that DNA recovered from an object resulted from direct contact. In this study was to examine whether having secondary DNA transfer present could potentially complicate forensic DNA typing result interpretations and overall the final conclusion that is drawn from those results. It also looked at whether the texture of the substrate being used, rough or smooth, may aid in the occurrence of secondary DNA transfer (Cale et al., 2016). During the investigation, twelve individuals were paired together and had hand to hand contact for two minutes and immediately handled assigned knives. DNA profiles from the knives were obtained and amplified using Identifiler Plus Amplification Kit and injected into a AB 3130xl Genetic Analyzer. Results indicated that secondary DNA transfer was detected in 85% of the samples, with five samples having the secondary contributor as the only contributor or the major contributor (Cale et al., 2016).

Also in 2016, Georgina Meakin and fellow researchers investigated the deposition and persistence of both directly- and indirectly- transferred DNA on knives. Researchers first prepared knives by instructing each knife be handled in a specific way by a single participant

over two days to simulate regular use. Meakin (2016) stated that having surfaces that are free of DNA before they come into contact with DNA from the offender or victim during a criminal act is unlikely in a real world situation. A background layer of DNA is likely to already be present on surfaces, especially on items that are regularly used, such as clothing and some types of opportunistic weapons. Once the two days were over, each pair of participants shook hands for 10 seconds and immediately stabbed their knife into a foam block repeatedly for 60 seconds. DNA was recovered by mini-tape lifting from triplicate sets of knife handles which came from four participant pairs after regular use, and at one hour, one day and one week after the handshaking and stabbing had taken place. During this study, samples were extracted using the QIAmp DNA Investigator Kit, quantified using the Quantifiler Human DNA Quantification Kit with the ABI PRISM 7900HT Sequence Detection System, and profiles were obtained using the AMPFLSTR NGM Select PCR Amplification Kit on the DNA Analyzer 3730xl in conjunction with the analyzing software GeneMapper 4.0 (Meakin et al, 2016). The researchers found that from the regularly used knives, one volunteer consistently deposited significantly greater amounts than other volunteers while another volunteer was seen to not always leave complete profiles. When looking at the persistence of DNA found on regularly used knives, it was seen that DNA persisted for at least a week but declined with increasing time between DNA deposition and recovery. DNA from a non-donor was co-deposited at <5% of the profiles recovered but for one volunteer there was DNA consistently left from their romantic partner on their knives at about 25% and 11% of the profiles both before and after the handshaking and stabbing events (Meakin et al., 2016). In about 10% of profiles recovered, alleles from one of the contributors were observed as a partial minor profile and were found in three pairings of

volunteers after the handshaking and stabbing events. When looking at the fourth pairing of volunteers, it was observed that only complete single-source DNA profiles matching the regular user's profile were recovered. When looking at the persistence for when indirectly-transferred handshaker DNA was detected, it was found to decline with increasing time between DNA deposition and recovery. Meakin et al. (2016) stated that the results obtained from this study suggest that the sooner an item is sampled after a crime has happened, the greater the chances of recovering indirectly-transferred DNA.

In 2010, Daly et al. carried out a study on *The transfer of touch DNA from hands to glass, fabric and wood* to examine variation in the amount DNA transferred from the hands to the three different substrates. In this study, researchers had 300 volunteers split evenly between each substrate (100 glass, 100 fabric, and 100 wood) with 50% of the volunteers being male and the other 50% female (Daly et al., 2010). Researchers had each volunteer handle their respective substrate for 60 seconds before the DNA was collected using a minitape lift followed quantification using the Quantifiler kit assay, extraction using a Qiagen QIAmp DNA mini kit, and amplification using the AmpFLSTR SGM Plus Amplification Kit at 28 cycles. After analysis, it was found that wood gave the best results followed by fabric and then glass. Results also showed that glass samples had a 9% likelihood of successfully obtaining a profile suggestive of the holder while fabric had a 23% likelihood and wood had a 36% likelihood (Daly et al., 2010). Researchers also considered different variables including "the phenomenon of secondary transfer". This variable was observed when mixed profiles were obtained during the study but results shows that the occurrence was low at about 10% of the total number of samples (Daly et al., 2010). DNA profiles showing more than one individual were found on substrates that had

only been touched by one volunteer. This study demonstrated that the nature of the object could in fact affect the amount of DNA transferred during contact. It also demonstrated that while secondary DNA is possible, profiles obtained from the touched samples were more likely to be from the result of primary rather than secondary transfer (Daly et al., 2010).

Materials and Methods

Materials and Volunteers

Brand new glass slabs were cleaned of any DNA using a mixture of 10% bleach-water followed by a mixture of 70% ethanol-water. A glass slab was swabbed before any handling by volunteers and was designated as the negative control. This slab was used to evaluate the quality control measures of sanitizing areas and wearing proper lab attire put into place prior to the glass slabs being touched. The negative control sample was extracted and quantified along with all other samples collected. Lack of DNA in this sample would mean that the quality control measures put into place were effective. Further procedures were put into place to minimize the introduction of extraneous DNA including wearing personal protective equipment (PPE) during sample collection and using 10% bleach to clean work areas and equipment. The appropriate PPE including of gloves, masks and lab coat were utilized through the process of set-up, DNA collection, and DNA analysis. Gloves were changed between every pair of volunteers and between the various hand conditions to ensure no extraneous DNA would be passed to surfaces being used.

Experimental set-up

To begin, benches were sanitized and wiped down with a 10% bleach solution followed by a 70% ethanol solution. Gloves were then changed and the glass slabs were opened up and went through the same cleaning process as the bench surfaces, between each glass slab gloves were changed to ensure there was no contamination between glass slabs. After each slab was cleaned, it was then labeled with the participants code and the sample type abbreviation code, having the secondary person's (researcher) condition first then the primary person's (participant) condition, as follows:

A# Negative (-) control
A# Positive (+) control
A# Dry/Oily-Primary (D/OP)
A# Oily/Dry-Primary (O/DP)
A# Dry/Dry-Primary (D/DP),
A# Oily/Oily-Primary (O/OP)

Seven (7) individual participants came in one at a time and were paired with the student researcher and labeled as the primary donor over the span of four weeks. Before beginning, each participant was assigned a code and asked to fill out a survey with their name and email and answer questions on the state of their skin as well as read and sign a consent form. Each participant was asked to wash their hands with soap and water and dry their hands immediately following the consent and asked to put on gloves. They were then read instructions of the project process before being asked to provide a buccal swab for later references during analysis. The reference profiles generated from the buccal swabs were used during analysis to determine the relative contributions of the hand shakers' to the profiles obtained from the glass slabs. After a

buccal swab was obtained, a positive control was done by having the participant and the student researcher rub their hands together while their gloves were still on for about five to ten minutes, to help with the production of oils on the skin, and then removed so they could place their hand with medium pressure down on the positive control glass slab in separate areas. Hand washing was then done again after each sample. Following hand washing, if the individual was given the dry hands condition they were instructed to make sure they dried their hands extremely well, keep drying them over the five to ten minute wait time, refrain from touching their face to minimize the amount of oil present on the surface of their hands, and refrain from touching their phones or anything that could introduce foreign DNA to their hands. Contrary, if the individual was given the oily hands condition, after hand washing they were asked to dry their hands but not an extreme amount and to rub their hands across their foreheads or cheeks to collect oils from their skin onto their hands and follow by putting on gloves to help increase oil and sweat production. After removing the gloves, both participants were asked to vigorously shake hands while maintaining constant contact for 30 seconds. After shaking hands, the primary person went on to placing their hand on the glass slab and applying moderate pressure to transfer the DNA from their hand to the slab for five seconds. Both individuals then washed their hands and waited about ten to fifteen minutes and started from the beginning, following the order of conditions as stated previously. Once each volunteer trial was completed, each trials glass slab was swabbed using a double swab wet/dry method using deionized (DI) water to wet the first swab and followed with a dry swab to obtain any DNA that may not have been picked up during the first swipe. Swabs were then placed backwards in their original packaging and allowed to dry overnight in a fume hood. After each set of swabs were obtained from each participant, the glass

slabs were cleaned with the bleach and ethanol solutions and repackaged and taped up in brown paper.

After all samples were obtained, swabs were then ready for cutting. Proper protective equipment was put on (gloves, mask, and lab coat) and benches were then cleaned with the same method as previously stated followed by placing brown paper down to ensure there would be no contamination from the bench to samples. 1.5mL microcentrifuge tube lids were closed before labeling to ensure no extraneous DNA would be introduced to the inside of the tubes, before or during cuttings, and then were labeled with each participants code and the sample type code. After each tube was labeled it was placed into a microcentrifuge tube holder. Two 150mL beakers were filled, one with fresh bleach water solution and the other with 96% ethanol. Two scalpels were removed from new packaging and put into the bleach water to clean them along with two pairs of scissors. Each tool was then transferred into ethanol and then taken out and thoroughly dried, each tool was left in both beakers for about five minutes each. Before each double swab set for each sample type was out down, a fresh kimwipe was also put down and wooden swab sticks were thrown away.

Swabs were cut one package at a time, only opening its respective sample tube when that sample was being completed and shut immediately afterwards. When completing the buccal and negative control swabs, only a very small section of each swab was cut due to DNA being so plentiful on the buccal swab and having no DNA on the negative swab. The positive control along with all other samples were split down the middle of the cotton tip and the scalpel was then slid along the wood to skin the cotton off. After each double swab was done, it was placed into its appropriate labeled tube. After each sample was done, both the scalpel and scissors were

placed into the bleach water and transferred into the ethanol as previously done to sterilize the tools for the next sample. Gloves were cleaned with a bleach solution after each sample within a participant but were changed whenever a new participant sample group was about to be done to ensure there was no cross contamination between sample groups. Once cuttings were finished, the tools were cleaned in the beaker solutions once more and the completed sample tools were placed back into the fume hood until it was time for extractions to be done.

Extraction Methods

DNA was manual extracted from swabs using the Qiagen QIAamp DNA Investigator kit as per the manufacturer's instructions for extracting DNA from cotton swabs. Before beginning the extraction process, a lab coat, mask, and gloves were put on to ensure there to be no contamination of the samples. Two ThermoScientific 24x1.5ml thermomixers were plugged in and turned on, one was set to 56°C at a speed of 900rpm for one hour and the other was set to 70°C for ten minutes to be used later on in the extraction process. An AirClean 600 PCR Workstation was then opened and the inside surface as well as the lip of the bench it was on was cleaned with a 10% bleach solution followed by a 70% ethanol solution. After thoroughly wiping down both surfaces, the work station was turned on. A long paper towel was put down inside the station to help make any clean up easier to handle and the sample tube rack containing the previously cut sample tubes that were being extracted were placed on top of it. All buffers needed were taken out of the kit and placed into the work station, all buffers used were at room temperature before and at the time of use.

To begin the extraction process, after setting a ThermoScientific Finnpipette F1 20-200 microliter (µl) pipette to 20µl, each 2ml microcentrifuge tube was opened up one by one and 20

microliters (µl) of room temperature proteinase K was pipetted into each sample, after each sample the pipette tube used was ejected into the trash and a new tip was put on for this step as well as all further steps. Following the addition of proteinase K, 400µl of Buffer ATL tissue lysis buffer was added to each microcentrifuge tube using a ThermoScientific Finnpipette F1 100-1000µl pipette that was previously set. Once the ATL buffer was added, all lids on the tubes were checked to ensure they were closed tightly and each tube was vortexed for ten seconds each. Following the vortexing, the sample tubes were then placed into the thermomixer set at 56°C and started for the one hour period. After the hour was up, samples were removed from the thermomixer and centrifuged for about ten seconds to remove droplets from the inside of the lids. Sample tubes were then placed back inside their rack and into the work station to add 400µl of Buffer AL lysis buffer to each tube one at a time. The sample tubes were checked to ensure the lids were closed tightly and following this were vortexed for 15 seconds to thoroughly mix the mixture to ensure efficient lysis. After samples had been vortexed, it was observed that a white precipitate had formed. Once this was seen, the QIAamp DNA Investigator Handbook was referenced and it stated that when Buffer AL is added to Buffer ATL, a white precipitate may form but the precipitate would not interfere with the procedure and would dissolve during incubation in the next step, which it did. Once samples were vortexed, they were then placed in the thermomixer set to 70°C and started for ten minutes. After ten minutes, samples were removed and centrifuged once again for about ten seconds to remove drops from inside the lids. Once centrifuged, 200µl of 96% ethanol was then added to each tube and vortexed for 15 seconds followed by centrifuged for about 10 seconds to remove drops from the lids. In the next step of the process, 600µl of the lysate was transferred from each of the microcentrifuge tubes to

a QIAamp MinElute Column in a 2ml collection tube which had previously been removed from the refrigerator directly before this step. During this step, the lysate was transferred without wetting the rim then centrifuged at 8000rpm for one minute. After centrifuging, each column was unattached from their collection tubes, which help flow-through from the previous step, one by one and put into a clean 2ml collection tube discarding the old collection tube. Subsequently, 500µl of the first wash buffer, Buffer AW1, was added to the column without wetting the rim and again centrifuged at 8000rpm for one minute, having the collection tube once again replaced after this step. 700µl of the second wash buffer, AW2, was then added without wetting the rim and the same centrifuging and collection tube replacement was done as previously stated. Following this, 700µl of 96% ethanol was added to each column and again centrifuged at the same setting with the collection tube being replaced. After this final wash, sample tubes were centrifuged at a full speed of 13,000rpm for three minutes to dry the membrane completely since, as stated by the QIAamp Investigator handbook, ethanol carryover into the eluate may interfere with some downstream applications.

Quantification Methods

DNA samples from the swabs were then quantified using the Quantifiler Trio kit in conjunction with the QuantStudio 5 Real-Time PCR System to determine the concentration of DNA present in each sample, as per the manufacturer's instructions. To begin this step of the process, the PCR Workstation was sanitized using previously stated methods and proper PPE was put on. The Quantification Trio Kit was taken out of the refrigerator which is kept at 2-8°C. Five previously autoclaved microcentrifuge tubes were taken out and labeled with standard (Std) one through five and put into a rack. To prepare Std 1, the Quantifiler THP DNA Standard was

vortexed for three to five seconds before pipetting the appropriate volume of standard and Quantifiler THP DNA Dilution Buffer for the dilution series, as shown in Table 1, into the microcentrifuge tube labeled Std 1 and then mixed thoroughly by vortex. To prepare Std 2, the appropriate amount of Std 1 and Quantifiler THP DNA Dilution Buffer was pipetted into its tube, as shown in table 1, then mixed thoroughly. To prepare standards 3-5, the same protocols for Std 2 were followed.

Table 1. *Standard Concentrations (ng/µL) and volumes*

Standard #	**Concentration (ng/µL)**	**Volume of stock solution or standard + dilution buffer**
Standard (Std) 1	50.000	10µL [100ng/µL stock] + 10µL Quantifiler THP DNA Dilution Buffer
Standard (Std) 2	5.000	10µL Std 1 + 90µL Quantifiler THP DNA Dilution Buffer
Standard (Std) 3	0.500	10µL Std 2 + 90µL Quantifiler THP DNA Dilution Buffer
Standard (Std) 4	0.050	10µL Std 3 + 90µL Quantifiler THP DNA Dilution Buffer
Standard (Std) 5	0.005	10µL Std 4 + 90µL Quantifiler THP DNA Dilution Buffer

Once the standards were prepared it was then time to manually setup the reaction plate. To start off, both the Quantifiler THP PCR Reaction Mix and the Quantifiler Trio Primer Mix were vortexed for three to five seconds then centrifuged to ensure it was mixed thoroughly. Following this, 10µL of the PCR Reaction Mix and 8µL of the Primer mix were added to each of the wells on the reaction plate. The plate setup was then completed, as shown in Figure 4, by

adding 2µL of either sample, standard, or control to their appropriate well. The plate was then sealed with the Optical Adhesive Cover and bubbles that had formed in the wells were removed by tapping the plate on the bench then centrifuged at 3,000rpm for about 20 seconds in a Mini PCR Plate Spinner.

	1	2	3	4	5	6	7	8	9	10
A	Std 1	Std 1	Master Mix/NTC	A1/8 O/OP	A2/8 O/OP	A3/8 O/OP	A4/8 O/OP	A5/8 O/OP	A6/8 O/OP	A7/8 O/OP
B	Std 2	Std 2	A1/1 Reference Sample	A2/1 Reference Sample	A3/1 Reference Sample	A4/1 Reference Sample	A5/1 Reference Sample	A6/1 Reference Sample	A7/1 Reference Sample	JK Reference Sample
C	Std 3	Std 3	A1/2 Negative Control	A2/2 Negative Control	A3/2 Negative Control	A4/2 Negative Control	A5/2 Negative Control	A6/2 Negative Control	A7/2 Negative Control	
D	Std 4	Std 4	A1/3 Secondary Positive Control	A2/3 Secondary Positive Control	A3/3 Secondary Positive Control	A4/3 Secondary Positive Control	A5/3 Secondary Positive Control	A6/3 Secondary Positive Control	A7/3 Secondary Positive Control	
E	Std 5	Std 5	A1/4 Primary Positive Control	A2/4 Primary Positive Control	A3/4 Primary Positive Control	A4/4 Primary Positive Control	A5/4 Primary Positive Control	A6/4 Primary Positive Control	A7/4 Primary Positive Control	
F			A1/5 D/OP	A2/5 D/OP	A3/5 D/OP	A4/5 D/OP	A5/5 D/OP	A6/5 D/OP	A7/5 D/OP	
G			A1/6 O/DP	A2/6 O/DP	A3/6 O/DP	A4/6 O/DP	A5/6 O/DP	A6/6 O/DP	A7/6 O/DP	
H			A1/7 D/DP	A2/7 D/DP	A3/7 D/DP	A4/7 D/DP	A5/7 D/DP	A6/7 D/DP	A7/7 D/DP	

This step in removing bubbles was critical in order to avoid noise in the fluorescence signal that bubbles can cause. The reaction plate was ran according to the GMU reference manual under the parameters of 40 cycles, initial cycling stage and holding stage at 95°C, and the final cycling stage at 60°C. This step was performed twice due to the result of low R^2 values during the first Quantification.

Figure 4. *Plate Map Setup of Quantification Reaction Plate*

Amplification Methods

	1	2	3	4	5	6	7	8
A	Positive Control	A1/7 D/DP	A2/7 D/DP	A3/7 D/DP	A4/7 D/DP	A5/7 D/DP	A6/7 D/DP	A7/7 D/DP
B	Negative Control	A1/8 O/OP	A2/8 O/OP	A3/8 O/OP	A4/8 O/OP	A5/8 O/OP	A6/8 O/OP	A7/8 O/OP
C	A1/1 Reference Sample	A2/1 Reference Sample	A3/1 Reference Sample	A4/1 Reference Sample	A5/1 Reference Sample	A6/1 Reference Sample	A7/1 Reference Sample	JK Reference Sample
D	A1/2 Negative Control	A2/2 Negative Control	A3/2 Negative Control	A4/2 Negative Control	A5/2 Negative Control	A6/2 Negative Control	A7/2 Negative Control	
E	A1/3 Secondary Positive Control	A2/3 Secondary Positive Control	A3/3 Secondary Positive Control	A4/3 Secondary Positive Control	A5/3 Secondary Positive Control	A6/3 Secondary Positive Control	A7/3 Secondary Positive Control	
F	A1/4 Primary Positive Control	A2/4 Primary Positive Control	A3/4 Primary Positive Control	A4/4 Primary Positive Control	A5/4 Primary Positive Control	A6/4 Primary Positive Control	A7/4 Primary Positive Control	
G	A1/5 D/OP	A2/5 D/OP	A3/5 D/OP	A4/5 D/OP	A5/5 D/OP	A6/5 D/OP	A7/5 D/OP	
H	A1/6 O/DP	A2/6 O/DP	A3/6 O/DP	A4/6 O/DP	A5/6 O/DP	A6/6 O/DP	A7/6 O/DP	

In the next stage of the experiment, samples were prepared for Amplification. Both the Master mix and Primer set were taken out of the refrigerator to allow them to come to room temperature, they were then vortexed for about three seconds then centrifuged to remove any droplets from the caps of the tubes. The Primer set was kept inside of the box until it was time for use due to the light sensitivity of the fluorescent dyes attached to the primers. To begin, a plate map, shown in figure 5, was constructed to help identify how many wells would be in use and what would go into each of them. Following this, 8µL of Master mix and 3µL of Primer mix were pipetted into a microcentrifuge tube and vortexed for three seconds to mix thoroughly then centrifuged briefly. Once the mixture was ready, 10µL of the mixture was pipetted into each well on the reaction plate. For samples which had a small autosomal concentration greater than 1ng, dilutions of these

samples were done in order to ensure that they met the required input volume by figuring out how much DNA sample to add to distilled water for a mixture total of 15µl.

Figure 5. *Plate Map Setup of Amplification Reaction Plate*

To determine what type of dilution to perform, whether 1:10 or 1:100, a calculation of one divided by the small autosomal concentration was done to find the Amp load, as seen in table 2. If the output value had a value greater than zero (0) after the decimal point it would need a 1:10 dilution but if the value was zero (0) then it would need a 1:10 dilution done. Once calculations, as seen in table 3, were completed, the dilution mixtures were completed and 15µL of the dilutions as well as 15µL of samples which did not need to be diluted were pipetted into their respective wells. Samples were then placed into the Proflex Thermocycler, set to run 29 cycles, to copy DNA in the event that there were low levels of DNA in any of the samples obtained.

Table 2. *Reference samples dilution table*

A	B	C	D	E	F	G
Sample Name	**Target conc. of DNA (ng)**	**Small Autosomal concentration**	**Amp load (1/ C2)**	**Volume of DNA (µL)**	**Dilution**	**H_2O (µL)**
A1-1 Ref	1	25.503→25.5	0.0392	4	1:100	11
A2-1 Ref		3.3001→3.30	0.303	3	1:10	12
A3-1 Ref		9.3435→9.34	0.107	1	1:10	14
A4-1 Ref		1.9579→1.96	0.510	5	1:10	10
A5-1 Ref		8.9571→8.96	0.112	1	1:10	14

A6-1 Ref		18.8081→18.81	0.0532	5	1:100	10
A7-1 Ref		2.1286→2.13	0.469	4	1:10	11
JK Ref		19.2167→19.22	0.0520	5	1:100	10

Table 3. *Equations and sample calculations for 1:10 and 1:100 dilutions*

Dilution	**1:10 dilution**	**1:100 dilution**
Equations	$Small\ autosomal\ concentration \times dilution\ factor\left(\frac{10\mu L\ DNA}{90\mu L\ H_2O}\right)$ = X ng/µL	$Small\ autosomal\ concentration \times dilution\ factor\left(\frac{1\mu L\ DNA}{99\mu L\ H_2O}\right)$ = X ng/µL
Equation to find the amount of DNA needed	$\frac{1ng}{\text{Xng/µL}} = Amount\ of\ DNA\ needed\ for\ the\ dilution\ (\mu L)$	
Equation to find amount of H_2O needed	15µL (total volume needed) - Amount of DNA needed for the dilution (µL) = Amount of Distilled water needed to be added to DNA (µL)	
Example	$\frac{3.3\mu L}{1\mu L} \times \frac{10\mu L\ DNA}{90\mu L\ H_2O} = 0.37ng/\mu L \rightarrow \frac{1ng}{0.37ng/\mu L} = 3\mu L\ DNA$ $15\mu L\ (total\ volume) - 3\mu L\ DNA = 12\mu L\ H_2O\ needed$	$\frac{25.5\mu L}{1\mu L} \times \frac{1\mu L\ DNA}{99\mu L\ H_2O} = 0.26ng/\mu L \rightarrow \frac{1ng}{\frac{0.26ng}{\mu L}} = 3.85 \rightarrow 4\mu L\ DNA$ $15\mu L\ (total\ volume) - 4\mu L\ DNA = 11\mu L\ H_2O\ needed$
Example Total Volumes Needed	3µL DNA + 12µL H_2O = 15µL total volume	4µL DNA + 11µL H_2O = 15µL total volume

3500 Capillary Electrophoresis Methods

To prepare for the final step of the process, an additional plate map setup was prepared, as seen in figure 6, to predetermine where all samples and allelic ladders would be placed once put into a reaction plate. Once the plate map was complete, calculations were done to determine

how much Hi-Di Formamide was needed by multiplying 9.6µL, the required volume as stated by the manufacturers manual, by the number of samples including both the positive and negative controls. The same calculation was done to determine how much of the GeneScan 600 LIZ(ILS) was needed but instead 0.4µL was multiplied by the total number of samples. In total 567µL of Hi-Di Formamide and 24µL of GeneScan 600 LIZ(ILS) were added together into a microcentrifuge tube and vortexed to thoroughly mix the two together and this was now known as the master mix.

	1	2	3	4	5	6	7	8
A	Ladder A1	A1/8 O/OP	Ladder A3	A3/7 D/DP	Ladder A5	A5/6 O/DP	Ladder A7	A7/5 D/OP
B	A1/1 Reference Sample	A2/1 Reference Sample	A2/8 O/OP	A3/8 O/OP	A4/7 D/DP	A5/7 D/DP	A6/6 O/DP	A7/6 O/DP
C	A1/2 Negative Control	A2/2 Negative Control	A3/1 Reference Sample	A4/1 Reference Sample	A4/8 O/OP	A5/8 O/OP	A6/7 D/DP	A7/7 D/DP
D	A1/3 Secondary Positive Control	A2/3 Secondary Positive Control	A3/2 Negative Control	A4/2 Negative Control	A5/1 Reference Sample	A6/1 Reference Sample	A6/8 O/OP	A7/8 O/OP
E	A1/4 Primary Positive Control	A2/4 Primary Positive Control	A3/3 Secondary Positive Control	A4/3 Secondary Positive Control	A5/2 Negative Control	A6/2 Negative Control	A7/1 Reference Sample	JK Reference Sample
F	A1/5 D/OP	A2/5 D/OP	A3/4 Primary Positive Control	A4/4 Primary Positive Control	A5/3 Secondary Positive Control	A6/3 Secondary Positive Control	A7/2 Negative Control	JK Positive Control
G	A1/6 O/DP	A2/6 O/DP	A3/5 D/OP	A4/5 D/OP	A5/4 Primary Positive Control	A6/4 Primary Positive Control	A7/3 Secondary Positive Control	JK Negative Control

H	A1/7 D/DP	A2/7 D/DP	A3/6 O/DP	A4/6 O/DP	A5/5 D/OP	A6/5 D/OP	A7/4 Primary Positive Control	Hi-Di Formamide

Figure 6. *Plate Map Setup of CE Reaction Plate*

The master mix was dispensed in 10µL increments in all wells on the reaction plate that would be used and then was followed by 1µL of either sample or allelic ladder pipetted into their perspective wells as noted before in the plate map setup. Formamide was placed into any additional empty wells in a column to prevent dry injections which can introduce air into the system and in turn shorten the lifespan of the capillary (Butler, 2011). Once all wells were filled, a rubber plate septa was placed on the reaction plate and it was centrifuged long enough to thoroughly mix the content and remove any bubbles that remained at the bottom of the wells. After centrifuging, the plate was placed inside the thermocycler and denatured for three (3) minutes at 95°C. The plate was then removed from the thermocycler and immediately snapped cooled for an additional three (3) minutes by placing it in a frozen cooling block. After the three minutes were up, the reaction plate was then placed into the autosampler of the 3500 Genetic Analyzer and electrophoresis was started with an injection time set to 15 seconds. Following electrophoresis, the data obtained from the instrument was downloaded to be analyzed using the GeneMapper ID-X Software.

Results and Discussion

Samples were evaluated for the following: (1) if secondary DNA transfer (alleles resulting from the secondary contributor) was detected, and (2) under which conditions were more alleles found from the secondary contributor than the primary contributor, and (3) under

which conditions were more alleles found from the primary contributor than the secondary contributor.

Using AB GeneMapper ID-X v1.6, samples were analyzed using a stochastic threshold of 1100 RFU to specifically tell if a peak was in fact a homozygous allele or not. Most alleles were seen to come way under the stochastic threshold but this could have been due to touch DNA sample usually having low levels of DNA, the samples may have degraded over time from sitting before being extracted, or due to original samples not being fully extracted and having only a partial amount of the samples volume extracted. Reference sample DNA profiles that were taken from volunteers were first analyzed to determine what peaks were in fact actual alleles and what peaks were artifacts, as seen below in figure 7. Researchers were able to obtain alleles for all loci expect A3 due to the profile not being able to be interpreted by the software. This issue could have been cause by low levels of DNA being in the sample, pipetting issues when placing samples into wells, as well as not having the exact settings applied through the software that were needed for that sample.

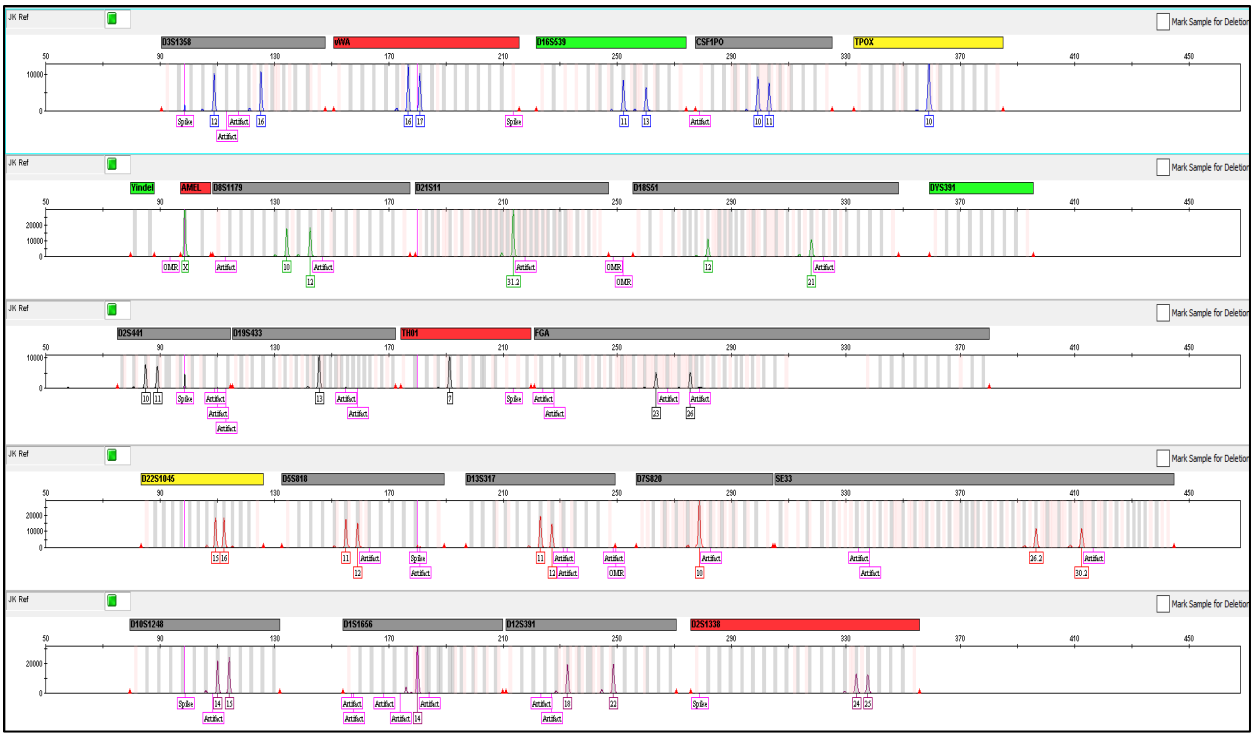


Figure 7. *Electropherogram of the secondary contributors reference DNA profile*

Positive controls were taken with each sample trial with the primary contributors hand print which produced a profile. The alleles from the control for sample A3 was used as a guide to what their profile possibly could be by subtracting out the secondary contributors profile from each condition and comparing what was left to the primary contributors positive control. Once reference profiles were completed and all respective alleles were determined for each loci in all contributors profiles, each of the sample DNA mixture profiles were compared to them, as seen in figure 8. After comparisons were complete, alleles from the secondary contributor were compared to each of the samples alleles to determine in which samples that profile showed up.

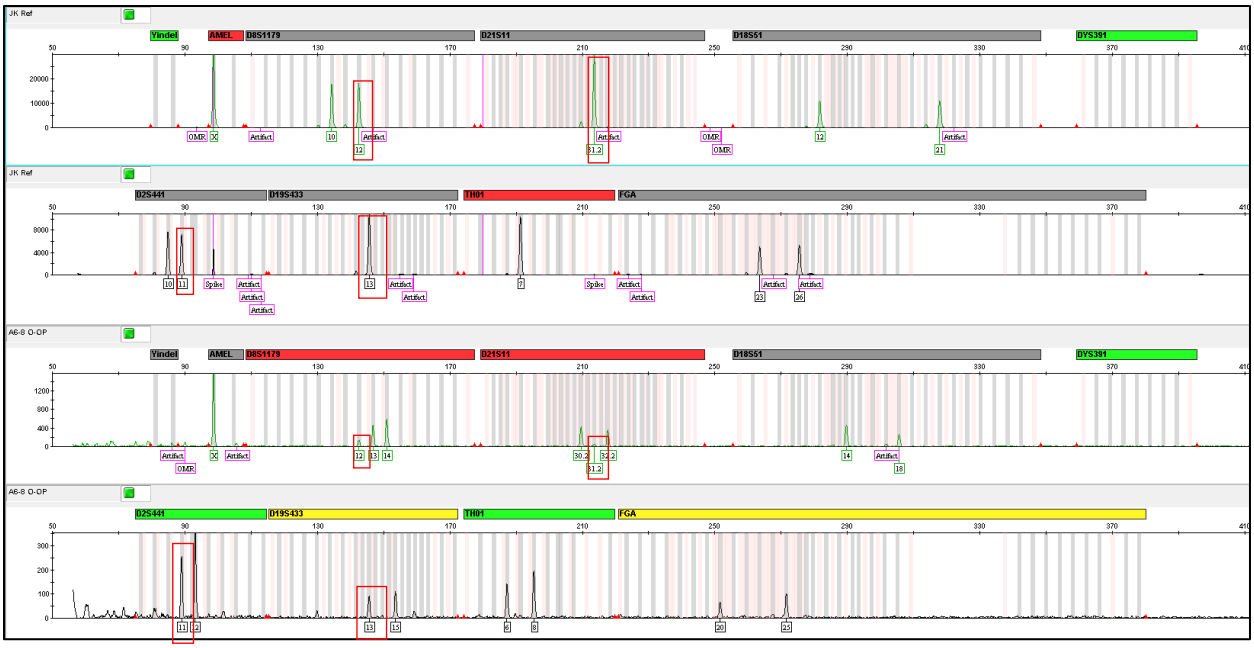


Figure 8. *Electropherogram DNA profile comparison of the secondary contributor and sample A6 O/OP*

After the comparison was done it was found that the profile of the secondary contributor was detected in all samples but not all 22 expected loci were seen to have alleles possibly due to allele dropout from having low level samples.

When analyzing the condition of Dry/Oily Primary, with the secondary contributor having dry hands, it was noticed that about 15.6% of alleles seen were also seen in the secondary contributors profile. When looking at the condition of Oily/Dry Primary, having the secondary contributor with dry hands, it was seen that about 20% of alleles were also seen in the secondary contributors profile. For the condition of both contributors having dry hands, it was seen that about 9.1% of the alleles seen were also a part of the secondary contributors profile. Finally for the last condition of both contributors having oily skin, an overall percentage of 35.4% of alleles were noticed to be from the secondary contributors profile for all samples, having the highest observed allele percentage. Numbers of alleles observed over expected, the percentage of alleles

observed, along wither the number and percentage of loci observed for each condition are noted in table 4.

Sample	Number of Alleles Observed/Expected – Secondary Contributor	Percent of number of Alleles Observed	Number of Loci Observed	Percentage of Loci Observed
D/OP	48/308	15%	72/156	46.2%
O/DP	62/308	20%	62/156	39.7%
D/DP	28/308	9.1%	45/156	28.8%
O/OP	109/308	35.4%	127/156	81.4%

Table 4. *Number and percentage of the secondary contributors alleles observed, and overall loci observed for each condition*

In table 5, the percentage of alleles from the secondary contributor were broken down by each sample and each condition. When comparing individual sample conditions to each other, it was noticed that the percentages of each sample when both contributors had oily hands were, for the most part, the highest percentage of the secondary contributors alleles seen. This result is in agreement with previous research done by Quinones & Daniel (2012) which found that there was an increase in DNA yield from samples originating from individuals with sweaty hands. Under this condition though, there were two outliers that stood out, samples A4 and A5, which both had higher percentages for the condition of Oily (Secondary)/Dry-Primary than for the condition of

both contributors having oily hands. For the Oily (Secondary)/Dry-Primary condition, 50% of the secondary contributors alleles were observed in sample A4 and 45.5% were seen at A5. This deviation could have resulted from having less loci missing for that specific condition but may have been due to chance as well.

Sample	**Percent of Alleles from Secondary Contributor Observed (%)**			
	D/OP	**O/DP**	**D/DP**	**O/OP**
A1 F 23	11.4%	4.5%	2.3%	43.2%
A2 F 25	4.5%	13.6%	4.5%	40.9%
A3 M 26	15.9%	11.4%	18.2%	43.2%
A4 F 28	15.9%	50.0%	27.3%	45.5%
A5 F 18	0.0%	45.5%	4.5%	6.8%
A6 F 19	25.0%	13.6%	0.0%	29.5%
A7 F 46	36.4%	2.3%	6.8%	38.6%

Table 5. *Percent of alleles from secondary contributor observed broken down by sample number and condition*

The lowest percentages were seen under the condition of both contributors having dry hands. Under this condition samples A1, A2, A5, A6, and A7 all had percentages under 10% while samples A3 and A4 were outliers having 18.2% and 27.3% which again could have resulted because of chance or because of more loci having alleles present in the profile for this condition.

Sample	Number of Alleles from the Secondary and Primary Contributors Observed (including shared alleles)							
	D/OP		O/DP		D/DP		O/OP	
	Secondary	Primary	Secondary	Primary	Secondary	Primary	Secondary	Primary
A1 F 23	5	10	3	4	2	3	19	32
A2 F 25	2	1	6	10	2	2	19	9
A3 M 26	7	20*	5	7*	8	8*	20	21*
A4 F 28	7	16	22	13	14	34	20	33
A5 F 18	0	0	20	7	2	3	3	6
A6 F 19	11	11	6	4	0	1	13	41
A7 F 46	17	33	1	1	3	4	17	26

*Since there was no reference sample to compare A3 to and only a positive control which could also be missing alleles, there is some uncertainty of some of the exact alleles A3 had

Table 6. *A comparison of the number of alleles from the secondary and primary contributors observed*

A comparison between alleles observed from the secondary and primary contributors is seen in table 6. When compared side by side, it was observed that while the secondary contributor did have alleles that appeared in each sample profile, the majority of the alleles that contributed to the profile were from the primary contributor. The biggest difference in allele count was noticed whenever the primary contributor had oily hands. This result could imply that more DNA transferred to the glass from that contributor due to them actually touching the glass and more surface area of their skin touching the glass allowing more cells to deposit.

Sample	**Number of Alleles from the Secondary Contributor (Distinct Alleles vs Shared Alleles)**							
	D/OP		**O/DP**		**D/DP**		**O/OP**	
	Distinct	**Shared**	**Distinct**	**Shared**	**Distinct**	**Shared**	**Distinct**	**Shared**
A1 F 23	3	5	2	3	0	2	9	19
A2 F 25	1	2	3	6	2	2	14	19
A3 M 26	7	7	5	5	7	8	17	20
A4 F 28	2	7	14	22	2	14	8	20
A5 F 18	0	0	15	20	0	2	1	3
A6 F 19	4	11	4	6	0	0	3	13
A7 F 46	3	17	0	1	1	3	6	17

Table 7. *A comparison of the number of shared alleles vs the number of distinct alleles of the secondary contributor seen in samples*

Since it was seen that alleles were shared between both contributors, a second table, table 7, showing the number of alleles distinct to the secondary contributor and primary contributors was created to do a comparison of both charts. An additional chart seen below, table 8, was also created to show side by side the difference between the alleles that were distinct to the secondary contributor and the alleles that were shared between both the secondary and primary contributor.

This was done to also show how including shared alleles could potentially falsely inflate the data observed but it must also be considered that the alleles observed could in fact truly be shared alleles between the two contributors. When compared there were no large gaps between observed alleles. Overall there were seven compared allele groups out of the total 28 groups that had a difference of alleles observed that was ten or larger. The results of table 8 could potentially show how an allele could be falsely linked to a contributor by looking at how there was such a difference for certain samples once the shared alleles were not accounted for. When looking between tables 6 and 8, the largest differences seen between the shared alleles and the distinct alleles from the secondary contributor in comparison to the primary contributor were mainly when both contributors had the same condition of either both having dry hands or both having oily hands.

Sample	Number of Alleles from the Secondary and Primary Contributors Observed (only alleles distinct to the secondary contributor)							
	D/OP		O/DP		D/DP		O/OP	
	Secondary	Primary	Secondary	Primary	Secondary	Primary	Secondary	Primary
A1 F 23	3	10	2	4	0	3	9	32
A2 F 25	1	1	3	10	2	2	14	9
A3 M 26	7	20*	5	7*	7	8*	17	21*
A4 F 28	2	16	14	13	2	34	8	33
A5 F 18	0	0	15	7	0	3	1	6
A6 F 19	4	11	4	4	0	1	3	41
A7 F 46	3	33	0	1	1	4	6	26

*Since there was no reference sample to compare A3 to and only a positive control which could also be missing alleles, there is some uncertainty of some of the exact alleles A3 had

Table 8. *A comparison of the number of alleles from the secondary and primary contributors observed (only alleles distinct to the secondary contributor)*

Challenges and limitations

One of the most impactful limitations to this study was perhaps the number of participants that were able to be obtained. While results were able to be obtained from the seven participants, a larger population size could have potentially aided in the visibility of a trend throughout each condition. A second limitation that came about was the amount of time available to carry out analytics. Due to unforeseen circumstances, the amount of time available to perform all necessary instrumentation processes carefully and accurately was shortened causing researchers to move more quickly to finish all analysis before leaving the laboratory. Because of this time shortage, necessary steps such as reanalyzing through the AB 3500 Genetic Analyzer for certain samples could not be done. This was another large limitation because if reanalyzing had of been completed, the potential for more alleles within each sample could have been increased allowing better quality results as well as potential statistics to be done. One last limitation was having a low level DNA that was available to test. Having low level DNA limited the analysis due to the inability for most alleles to reach the stochastic threshold which helps analyst confirm if an allele is a true allele or an artifact such as stutter. Having low level also inhibits the ability to perform vital statistics such as a likelihood ratio to determine the likelihood an allele belonged to one individual over another.

Most challenges faced were due to inexperience conducting DNA laboratory research. While expert and knowledgeable individuals were aiding in this process, small mistakes were made by the student researcher that were not noticed until after a step was completed. One challenge that was also a limitation was due to a reagent blank not being created at the time of extraction. Since reagent blanks are used throughout the entire analytical process, from

extraction to analyzing, this limited the ability to know if there was contamination introduced by reagents used for PCR. This also became a challenge upon profile analysis because it was unclear where unknown alleles appeared from and if they were drop-in alleles or actual contamination within the sample.

Conclusion

Overall, data showed that secondary DNA transfer is most likely to appear under oily conditions when the skin has had time to create its own oils allowing excess skin cells to be sloughed off onto another surface. While the secondary contributor was still a minor contributor in samples, it still helped provide a foundation to evaluate under what conditions it may be more likely to see secondary transfer from someone. Small trends in the amount of alleles present under each condition were noticed but due to some inconsistencies appearing, absolute trends were not able to be identified.

Impact to the field

The findings of this research could potentially help impact the forensic DNA community by furthering the knowledge needed to understand how the condition of an individual's skin, specifically the hands, can impact the amount of secondary DNA that may be transferred. This also could help to increase awareness of the potential for individuals to be included in a profile and potentially as a suspect for a crime they were nowhere near simply due to the fact that they came in contact with someone who actually was.

Future directions

From previous research, it is known that DNA can implicate that an innocent individual was present at a crime scene when they in fact were not. With further research, more has come to light on different factors that may affect secondary touch DNA such as environmental factors, deposition pressure, substrate texture, and shedder status. The forensic community has yet to fully understand what may affect the transfer of DNA from one person to another and in turn an object. While this study went over two conditions that may affect the amount of DNA being transferred, it is only a preliminary step to what further knowledge has yet to be found out.

Further directions to help increase knowledge of this topic would be to look at different substrate types that may affect how much DNA is absorbed; environmental factors such as temperature and humidity to look at the persistence factor of secondary touch DNA; and an age factor to see if different age ranges plays a role in how much DNA is seen in samples. Additional factors that could be investigated could be a time factor to also determine the persistence of the DNA as well as the quality of the DNA after various amounts of time to give researchers and forensic scientist knowledge about how long a sample may have been at a scene; a comparison of alleles seen between direct touch from two contributors and alleles seen through secondary transfer; and concentrations of how much DNA was obtained from each condition during quantification and if it can also be used to help predict shedder status. This investigation could also lead to more research on the understanding of shedder status by being able to predict under what skin conditions an individual may shed more cells and give off more DNA than under another condition.

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