Spring 5-2021

Determination of Human Shedding Propensity Based on STR Results

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A thesis presented in partial fulfillment of the requirements for the degree of

Master of Science in Forensic Science

John Jay College of Criminal Justice

City University of New York

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May 2021

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This thesis has been presented to and accepted by the office of Graduate Studies, John Jay College of Criminal Justice in partial fulfillment of the requirements for the degree of Master of Science in Forensic Science

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Acknowledgements

I would like to thank several people for their support during my studies and for making this thesis possible.

Firstly, I am immensely thankful for my thesis advisor and mentor, Dr. Mechthild Prinz, for her guidance and support throughout my graduate career. She has contributed to my knowledge, confidence, and growth during the past two years.

I would like to thank the rest of my committee members for devoting their time to reviewing and providing feedback on my work.

My fellow laboratory members were vital to the completion of this research, including Xiao, Natalie, Tebah, Niti, and Dinura. Additionally, I am grateful for my classmates and friends who have offered me encouragement, kindness, and advice.

Finally, I would like to extend my appreciation to my family for always supporting my ambitions. Without their assistance and benevolence, I would not be here today.

Abstract

Trace DNA evidence may be discovered at a crime scene after having been deposited by a person of interest via active or passive transfer. Based on previous studies, passive transfer of one's DNA is influenced by their shedding propensity, or probability of depositing a detectable amount of DNA through touch. Determining the shedding propensity of a person of interest can aid in trace DNA interpretation in forensic casework. This study explored STR profile quality and the presence of a DNA mixture for different skin surface locations, including fingertips before and after handwashing. As expected, unwashed fingers showed a higher prevalence of mixtures than washed fingers. Hand dominance showed no significant effect on right versus left finger STR profile quality. However, right-handed participants exhibited a higher mixture prevalence for samples obtained from their dominant hand. Shedding propensity was determined based on STR profile quality and the number of expected alleles detected from washed finger samples only. Three individuals were high shedders (10.7%), 18 individuals were intermediate shedders (64.3%), and seven individuals were low shedders (25.0%). No trend was seen for shedding propensity, profile quality, or mixture status based on biological sex. STR profile quality can be affected by various factors and future research will combine the use of STR results with quantification data to develop an alternative method of predicting shedding propensity.

1. Introduction and Literature Review

Trace DNA evidence and transfer

Forensic DNA typing is routinely used to connect the biological evidence collected at a crime scene or on the victim's body to a person of interest. If a person of interest has yet to be identified, the source of the evidence can be searched through the U.S. National DNA Database. Forensic testing targets specific genetic markers called short tandem repeats (STRs), which are small, highly polymorphic regions. STRs are detected after performing polymerase chain reaction (PCR) amplification with fluorescent primers and capillary electrophoresis. Among individuals, STR alleles vary in the number of short repeat units present and the length of the respective PCR product (Butler, 2012). The ability to differentiate individuals based on STR typing depends on the STR loci included in the PCR multiplex reaction. Testing highly polymorphic STR alleles or multiple STR loci will increase the powers of discrimination, exceeding 1 in 328 quintillion with 22 loci (Butler, 2012). Due to PCR amplification, STR typing is sensitive enough to detect small amounts of DNA left behind through touching an object or surface. This contact, or trace DNA, evidence now comprises the majority of submissions to DNA laboratories (Mapes et al., 2016).

Trace DNA evidence can be deposited at crime scenes due to either active or passive transfer. Active transfer refers to when an individual has contact with an object or surface, thus transferring "touch" DNA. Passive transfer occurs through indirect means, such as secondary transfer or aerosol transfer (Fonneløp et al., 2015). When one individual actively transfers their DNA to an object and then another individual touches the same object, the second person can deposit both their DNA and the first person's DNA on a

subsequent surface. Although the first person never directly touched the subsequent surface, their DNA would be discovered upon swabbing. Various objects and surfaces, such as cloth rags or gloves, can also act as DNA vectors. Additionally, trace DNA can be deposited on surfaces as aerosolized biological fluids, which can be transferred by actions such as coughing or sneezing. STR DNA typing therefore cannot definitively establish whether the trace DNA found at a crime scene belongs to a person who was present at the crime scene. The interpretation of trace DNA collected from objects following transfer events is very relevant in forensic investigations and has been extensively investigated. Previous studies have indicated that the quantity and quality of DNA an individual leaves behind after touching an object can be affected by several factors, including the object material, the manner of contact, one's activities prior to contact, and an individual's shedder status. Shedder status or shedding propensity can be defined as the probability that an individual will deposit a detectable amount of their DNA on an object through touch and will be discussed in the following section (Lowe et al., 2002).

Certain challenges can arise in the interpretation of STR profiles obtained from touch DNA because the DNA is generally low in quantity. Low-template DNA is prone to PCR-stochastic effects, such as allele drop-out, locus drop-out, increased stutter peaks, and additional artifacts (Gill et al., 2015). When small quantities of DNA are present in a sample, the PCR primers may not reliably amplify the DNA, resulting in unequal sampling of the alleles (Butler, 2012). Allele drop-out refers to the loss of a single allele while locus drop-out is the loss of both alleles at a heterozygous locus. Additional peaks may be present in a STR profile due to stutter products, which occur because of strand slippage during PCR amplification (Butler, 2012). Stutter products can be one or two repeats smaller than

the nominal allele (back stutter) or one repeat greater than the nominal allele (forward stutter) (Butler, 2015). Unusually high stutter peaks with heights greater than 15% of the nominal allele often occur with low-template DNA and can be mistaken for a true allele during STR interpretation (Butler, 2015). These effects must be taken in consideration when analyzing STR profiles to appropriately interpret the results.

For touched objects, the presence of a mixed STR profile can indicate a passive transfer event concurrent with a direct transfer event (van Oorschot et al., 2019). When an individual's reference profile is available, foreign alleles can be readily identified and might suggest that secondary transfer has occurred. However, foreign alleles may also be present from previous direct transfer events prior to the crime. Scientists have been unable to agree on the prevalence of this non-self-DNA, particularly during transfer studies. Some studies indicate that foreign DNA is present at minimal levels (less than 10%) for most of the samples analyzed (Goray et al., 2016; van Oorschot et al. 2014). Other research suggests that the level of passive transfer is relatively low, at rates closer to 10-30% (Daly et al., 2012; Lacerenza et al., 2016; Phipps & Petricevic, 2007; Tan et al., 2019). Lacerenza et al. (2016) discovered that over half of the mixtures identified were low-level mixtures, thus the foreign DNA contributed little to the overall DNA quantity. There were significant differences in the number of mixtures between males and females (Lacerenza et al., 2016). These conflicting results may be due to the differences in the experimental design or methods of sampling and identifying mixtures.

In mixed profiles, the primary donor, or highest contributor, may share alleles with additional contributors, which would mask some of the foreign alleles. Additionally, the highest contributor is not always the individual who participated in the direct transfer event.

In a study conducted by Daly et al. (2012) using volunteers who had not washed their hands, in one instance a male profile was obtained from an object touched by a female participant while the female's profile was not detected. Comparable results were obtained from the transfer experiments performed by Buckingham et al. (2016). Following the handling of an object, the participants deposited higher amounts of non-self-DNA than their own DNA approximately 20% of the time (Buckingham et al., 2016). Similar studies observed the same occurrence at lower rates of 2.9%, 2.4%, and 1% of the samples (Goray et al., 2016; Phipps & Petricevic, 2007; Tan et al., 2019). Several variables may affect the quantity of foreign DNA that is indirectly transferred, including the time since contact with the foreign DNA, the manner of handling, and the substrate of the vector (van Oorschot et al., 2019). One's shedding propensity may also affect their ability to pick up and deposit foreign DNA (Gosch & Courts, 2019). According to the findings of Goray et al. (2016), those with high shedding propensity or "good shedders" will transfer lower proportions of foreign DNA as compared to self-DNA.

Shedding propensity criteria

One of the first mentions of individual differences in the amounts of DNA deposited, or shedder status, was in an article published by Lowe et al. (2002), which classified individuals in the study as either "poor shedders" or "good shedders". Determining the shedding propensity of a person of interest can aid in trace DNA interpretation in forensic casework. In past research, shedding propensity has mainly been assessed based on the quantity and quality of deposited DNA on touched objects through transfer experiments. These studies have yielded a wide range of quantification results,

exhibiting high levels of inter- and intra-variation (Burrill et al., 2019). This variation may be attributed to the type of handled items, duration of handling, or other experimental factors. Since a biological sample has little probative value unless an adequate STR profile can be generated, STR success rates have been investigated in conjuncture with the quantification results to define one's shedding propensity. STR profile quality of a collected sample is a qualitative measurement based on the number of expected alleles detected. A profile with all expected alleles detected is considered a full profile while a profile missing any expected alleles is known as a partial profile. The percentage of expected alleles detected can then be calculated to assess the STR success rate (Lacerenza et al., 2016).

Although there is an abundance of research on the topics of trace DNA, passive transfer, and shedding propensity, the high method variability may contribute to inconsistent conclusions within the scientific community. Lowe et al. (2002) first proposed the concept of shedder status based on a study of only 8 volunteers who washed their hands then handled a plastic tube for ten seconds on five different days. Individuals who deposited samples that produced DNA profiles with 80-100% of their alleles were classified as good shedders (Lowe et al., 2002). Using this definition, 60% of the donors were good shedders and the remaining 40% were bad shedders (Lowe et al., 2002). To expand on this data, Phipps & Petricevic (2007) repeated Lowe's experiment with a few modifications and more volunteers. However, they were unable to acquire any consistently high-quality profiles among their participants, suggesting a vital discrepancy in the experimental design (Phipps & Petricevic, 2007).

Other research involving the handling of plastic tubes also had conflicting results despite using similar methodology. Manoli et al. (2016) and Fonneløp et al. (2017) both classified approximately 25% of the participants as good shedders using the criteria established by Lowe et al. (2002), but the remaining participants were grouped into different categories. While Fonneløp et al. (2017) adhered to the distinction between good and bad shedders, Manoli et al. (2016) included an intermediate status for profiles containing 41-80% of the donor's alleles. This percentage was established based on interquartile ranges calculated from the average percentages of alleles in the profiles acquired from both hands (Manoli et al., 2016). Using this additional criterion, 50% of the participants were intermediate shedders and 26% were poor shedders (Manoli et al., 2016). Contrastingly, Tan et al. (2019) found 11% good shedders, 41% intermediate shedders, and 48% poor shedders based on the number of held plastic tube samples that resulted in reportable profiles, or those with at least 16 of the expected alleles present. Donors with four reportable profiles out of the six collected samples were good shedders, donors with one to three reportable profiles were intermediate shedders, and donors with no reportable profiles were poor shedders (Tan et al., 2019).

Research has confirmed that the substrate of the handled object will affect the amount and quality of DNA recovered (Daly et al., 2012; Fonneløp et al., 2015). For this reason, scientists have conducted transfer experiments using other materials, such as glass plates (Goray et al., 2016; Oleiwi et al., 2015; Kanokwongnuwut et al., 2018). As with the previous experiments, the determination of shedder status depended on the criteria outlined by the scientists. Daly et al. (2012) working with volunteers that touched either glass, fabric, or wood found 22% good shedders when including donors with profiles containing

six or more alleles, but 13% good shedders when only including those with full profiles. Kanokwongnuwut et al. (2018) determined shedder status based on the amount of stained cellular material in a thumbprint visualized using fluorescence microscopy. This method resulted in 18% high shedders, 36% intermediate shedders, and 45% low shedders (Kanokwongnuwut et al., 2018). The amount of cellular material correlated with the STR profile quality and average RFUs after direct PCR amplification (Kanokwongnuwut et al., 2018). Basing the shedder status determinations on cell counts eliminates the need for swabbing, which can cause a loss of DNA, so the results may be more representative of the population. However, since the criterion for each shedding status fluctuates among these experiments, comparing the conclusions from these studies must be done with caution. Establishing a more standardized definition and methodology for determining shedding propensity would allow scientists to better evaluate this trait among the general population.

Effect of handwashing

Another variable that may contribute to the range of results among scientists is the inclusion of handwashing. The amount of self and non-self-DNA deposited from touch is expected to be lower immediately after handwashing since this process generally removes foreign material from the hands. As the time since handwashing increases, more self-DNA will be present, either from touching oneself or through a natural accumulation on the surface of the skin (Phipps & Petricevic, 2007). The rate of this build-up of detectable DNA is thought to depend on one's shedding propensity (Burrill et al., 2019). However, some studies have concluded that the time since handwashing has insignificant effects on the amount of DNA deposited on a surface (Goray et al., 2016; Fonneløp et al., 2017; Szkuta

et al., 2017). These conflicting results may be due to the actions performed between the time of handwashing and time of sample collection. In the study conducted by Goray et al. (2016), the time since handwashing was reported by the volunteers rather than measured in a controlled setting. Volunteers were not required to wash their hands directly prior to sample collection and activities since the last handwashing varied considerably (Goray et al., 2016). The researchers were thus depending on the volunteer's honesty and ability to recollect past events for their assessment.

Requiring participants to wash their hands in the laboratory provides a better level of experimental control and reduces the potential bias. Furthermore, activities following handwashing should be monitored to eliminate other variables. As an example of a more controlled study, Kanokwongnuwut et al. (2018) required participants to wash their hands with water and provide thumbprints for fluorescent cell counting and direct PCR at controlled intervals. The amount of cellular material detected was directly proportional to the time since handwashing (Kanokwongnuwut et al., 2018). A similar study also using thumbprints and fluorescence demonstrated the accumulation of DNA containing material and a direct correlation between the time since handwashing and STR success rates (Kanokwongnuwut et al., 2020). In another experiment, volunteers were asked to wash their hands and then touch another individual's sebaceous or palmar skin (Zoppis et al., 2014). This research suggested that washing with soap and water removes all detectable self-DNA making the foreign DNA the major component and permitting passive transfer (Zoppis et al., 2014).

Effect of biological sex

Given that males and females have certain biological differences, it would be reasonable to conclude that shedding propensity is dependent on one's gender at birth. Sex hormones will alter how the epidermis metabolizes and responds, leading to variations in the skin between the sexes (Giacomoni et al., 2009). Such differences may affect the amount of DNA deposited following contact since touch DNA is thought to mainly originate from skin cells, sweat, and sebum (Quinones & Daniel, 2012). Although palmar skin does not contain sebaceous glands, sebum is often present on the hands from touching one's sebaceous skin. Fluorescent imaging has shown that cellular material is more often found near the sweat pores (Kanokwongnuwut et al., 2020). Since sweat glands are highly concentrated in the palms and fingers, the presence of sweat can directly contribute to the DNA collected from the hands (Giacomoni et al., 2009). Provided that men generally have higher sebum and sweat production than women, it can be hypothesized that men usually deposit more DNA and thus are classified as high shedders more frequently (Giacomoni et al., 2009).

While some studies have agreed with this hypothesis, there has yet to be a consensus on this topic (Gosch & Courts, 2019). There may have been bias in some of the studies that discovered significant differences between sexes because of unequal numbers of males and females (Fonneløp et al., 2017; Goray et al., 2016). However, Lacerenza et al. (2016) came to the same conclusion based on an experiment with the equal numbers of males and females. This study also determined that women deposited more mixed profiles than men at a rate of 63.3% to 30% (Lacerenza et al., 2016). In contrast, some researchers have found no correlation between biological sex and shedding propensity (Farmen et al.,

2008; Manoli et al., 2016). These discrepancies are likely due to variation in the experimental design or shedding propensity criteria as discussed previously. Additionally, the statistical significance of the differences between sexes was determined using a wide variety of statistical tools.

Effect of hand dominance

An individual's manner of handling objects will generally differ based on their hand dominance. Since activities done prior to sample collection appear to influence the amount of self and non-self-DNA deposited, handedness should be investigated in transfer experiments. Individuals might be more likely to handle items and interact with others using their dominant hand, thus increasing the overall amount of DNA and prevalence of mixtures on the dominant hand. Some studies indicate that the dominant hand will deposit samples of increased STR success rates whereas others have reported the opposite (Phipps & Petricevic, 2007; Manoli et al., 2016). Several contrasting results have been published, concluding that hand dominance has no significant correlation to either profile quality or mixture prevalence (Goray et al., 2016; Lacerenza et al., 2016; Kanokwongnuwut et al., 2018). Since a smaller percentage of the general population is left-handed, it is possible that these results are biased. Left-handed individuals will regularly interact with their non-dominant hand since many objects are designed for right-handed use. Furthermore, the act of shaking hands is conventionally done with the right hand, regardless of one's hand dominance. Therefore, further research is needed to determine the role of handedness in shedding propensity determinations.

Intra-variation for shedding propensity

Although studies have proven that different individuals will vary in their shedding propensity, compounding complexities may result in temporal variation for a given person. Some research has indicated that over 75% of participants change shedder status between replicates spaced a day apart (Manoli et al., 2016). The level of intra-variation may be equivalent to the degree of inter-variation, making it impossible to predict one's shedding propensity (Phipps & Petricevic, 2007). Since a range of potential biological factors have yet to be investigated, the cause of this intra-variation is unknown. Activities conducted prior to sampling may also contribute to the temporal differences. Some studies have found variability in the number of detected alleles from day to day, but no significant changes in the quantity of DNA deposited (Goray et al., 2016). This suggests that the intra-variation seen for STR data may be due to PCR-stochastic effects from the low quantities of DNA deposited. Similarly, there was no significant variation observed in the individual amounts of cellular material deposited on different days in the fluorescent experiment conducted by Kanokwongnuwut et al. (2018).

As mentioned earlier, these studies vary in their experimental designs and how they define shedding propensity, resulting in discrepant conclusions. If the intra-variation is only significant for profile quality, those studies that define shedder status based on the percentage of detected alleles will find more inconsistencies. However, other research has shown that the amount of DNA deposited is also highly variable between replicates (Tan et al., 2019; Oleiwi et al., 2015; Phipps & Petricevic, 2007). It is possible that certain individuals have more of a tendency to deposit reproducible amounts of DNA, particularly those who would be classified as high shedders (Pfeifer & Wiegand, 2017). The number

of replicates and duration of sampling may also contribute to the degree of intra-variation. By examining deposited DNA over an extended period, certain individuals clearly shed more DNA on average than others (Taylor et al., 2016). Establishing long-term trends could provide better insight into whether shedding propensity is a transient trait. Since several biological and behavioral factors appear to influence the level of intra-variation in both DNA quantity and quality, some believe that it is improbable that individuals can be consistently classified into a shedder status (Quinones & Daniel, 2012).

Research Goals

As demonstrated, there has been a wide variety of experimental designs and definitions for shedding propensity. These studies have shown that there is a range of shedding propensities among individuals, which may be affected by several biological and environmental factors. Since the results have been conflicting, further research into the topic is necessary. Most methods have involved the transfer of DNA from hands to objects or glass plates/slides, which inevitably causes a loss of DNA. To counter this dilemma, this project involves samples acquired directly from the skin using tape lifts. The primary goals of this project are to develop definitions for high, intermediate, and low shedders and to determine the shedding propensities of individuals in a general community using STR profiles from skin surface samples. It is hypothesized that shedding propensity can be determined equivalent to previous studies using this novel approach. The secondary purpose is to evaluate individuals' mixture status, or the prevalence of non-self-DNA, for various sample types. In addition, the effect of handwashing, biological sex, handedness, and sampling location were investigated to determine if these factors affect an individual's

STR profile quality and mixture status. It is anticipated that unwashed finger samples will have a higher prevalence of non-self-DNA and thus not be suitable for the determinations of shedding propensity. Lastly, samples were collected in triplicate, spaced one week apart, to evaluate intra-variation in profile quality and mixture status.

2. Materials and Methods

2.1. Sample Collection

Prior to beginning this research, human subject research approval (project #2018-0099) was obtained to collect samples from volunteers. Minimal risk was established since all samples were collected by noninvasive means. All volunteers signed an informed consent form, which indicated the nature of the research and ensured anonymity. Samples were acquired from 15 male and 13 female volunteers from the John Jay College community. Ages ranged from 19 to 75 with an average age of 28 due to the demographic of the community. Volunteers were randomly assigned numbers from 31 to 60 to maintain anonymity and reduce bias. All participants completed a brief questionnaire to indicate biological sex, age, hand dominance, time since last shower, and time since last handwash. Three collections (spaced one week apart) of skin tape lifts using D-Squame (Clinical & Derm, Dallas, TX) adhesive disks were acquired from various areas of the body. There were eight types of samples collected: unwashed left middle and index fingers (LU), washed left thumb (LW), unwashed right middle and index fingers (RU), washed right thumb (RW), left big toe, nape, upper arm, and the area just below the ear. For the washed finger samples, the volunteers were instructed to wash their hands with water only, dry

their hands with paper towel, and wait for 30 minutes without touching any surfaces prior to sample collection. Buccal swabs were then acquired for each volunteer to serve as references.

2.2. STR Profiling

DNA was extracted from all samples using QIAamp® DNA investigator kit on the QiaCube® extraction robot (both Qiagen, Germantown, MD) and quantified using QuantiFiler™ Trio kits on the QuantStudio 5 real time PCR instrument (both Thermo Fisher Scientific, Framingham, MA). Samples were amplified with a target amount of 1ng, or for lower concentrations with the maximum allowed input (15uL) using GlobalFiler™ STR human identification kit and run on a 3500 Genetic Analyzer (both Thermo Fisher Scientific, Framingham, MA). STR profiles were analyzed using GeneMarker® HID (SoftGenetics, Collegetown, PA). The detection threshold was set to 50 RFU. Pull-up detection and stutter correction were applied to all profiles.

2.3. STR Profile Analysis

All controls and allelic ladders were checked for each batch of samples prior to data analysis. Off-base, off-ladder, and pull-up peaks were identified and unlabeled in all STR profiles. Sample STR profiles were then compared to the profiles acquired from the donors' reference samples to determine mixture status and profile quality. Five categories were established for profile quality based on the number of expected alleles present: full profile (F), high partial profile (HP), low partial profile (LP), negative (NEG), and not suitable for analysis (NS). Table 1 summarizes each profile quality classification and the respective

codes utilized for data analysis. When all expected alleles were detected, the profile was classified as a full profile. Profiles containing 13 to 21 complete loci were considered high partial profiles while those with one to 12 complete loci were considered low partial profiles. A negative profile was one with no complete heterozygote locus detected. Lastly, profiles were deemed not suitable for analysis when the profile was negative with at least one foreign heterozygote locus present.

Table 1. STR profile quality classifications, codes, and definitions.

Classification	Code	Definition
Full profile	F	All donor alleles present.
High partial	HP	13 to 21 complete donor loci present.
Low partial	LP	One to 12 complete donor loci present.
Negative profile	NEG	No complete heterozygote locus from known or foreign donor.
Not Suitable	NS	No complete heterozygote locus from known donor, but at least one foreign heterozygote locus present.

Mixture status was determined based on the presence, number, and relative peak height of foreign alleles. Table 2 outlines the following five categories: single source (N), background mixture (MXB), mixture with donor as the major component (MX), mixture with donor as the minor component (MXDM), and not suitable for analysis (NS). Single source profiles were those with two or less foreign alleles present, not including stutter peaks, to account for random drop-in alleles. For high-quality profiles (full or high partial with high RFUs), a background mixture was defined as any profile with at least three foreign alleles present with heights less than 40% that of the donor peaks. Low-quality profiles (partial profiles with low RFUs) were classified as background mixtures when three to five foreign alleles were present with heights equal to or less than that of the donor peaks. Full and high partial profiles with three or more foreign alleles with heights greater

than the corresponding stutter peaks were deemed mixtures with the donor as the major component. However, when both donor heterozygote alleles had lower peak heights than the foreign alleles at two loci or more, the profile was classified as a mixture with the donor as the minor component. Low partial profiles were classified as mixtures with the donor as the major component when six or more foreign alleles were present with heights equal to or less than that of the donor alleles. When at least three loci contained foreign alleles with heights equal to that of the donor alleles and one or more missing donor alleles, the profile was classified as a mixture with the donor as the minor component. All negative profiles were deemed not suitable for analysis (NS).

Table 2. STR profile mixture status classifications, codes, and definitions. Refer to the Appendix for representative profiles for MXB, MX, and MXDM.

Classification	Code	Definition
Single source	N	Two or less foreign alleles present.
Background Mixture	MXB	At least three foreign alleles present, and most loci only show donor alleles. Full and high partial profiles with high RFUs: two or less foreign peaks higher than the stutter peaks and all foreign peak heights less than 40% that of the donor alleles. Low and high partial profiles with RFUs less than 1000: three to five foreign peaks present with heights equal to or lower than that of the donor alleles.
Mixture; Donor as Major Contributor	MX	Full and high partial profiles: three or more foreign alleles present with heights greater than that of the stutter peaks at the same locus. Low partial profiles: six or more foreign alleles present with heights equal to or lower than that of the donor alleles.
Mixture; Donor as Minor Contributor	MXDM	Full and high partial profiles: at least two loci present where both donor heterozygote alleles have lower heights than the foreign alleles. Low partial profiles: six or more foreign alleles present with at least three loci where foreign alleles are equal height to the donor alleles and one or more known donor alleles are missing.
Not Suitable	NS	Negative profile

Profile quality and mixture status results were then sorted by type of sample to assess differences based on sampling location. For both profile quality and mixture status, the number of samples in each category for each sample type was tabulated and graphed. The results for the washed and unwashed finger samples were compared to assess the effect

of handwashing. Differences in the data for the unwashed finger samples based on time since handwashing was also investigated. The effect of biological sex on washed and unwashed finger samples was analyzed by calculating percentages of each profile quality and mixture status for males and females. The effect of hand dominance was analyzed by sorting the data based on handedness and calculating percentages of each profile quality and mixture status for left- and right-handed participants. The Kruskal-Wallis test was utilized for the sampling location, biological sex, hand dominance, and time since handwashing results to establish significance (Kruskal-Wallis Test Calculator, 2020). The Z score test for two population proportions was used to compare the number of full profiles and single source profiles for the unwashed and washed finger samples (Z Score Calculator for 2 Population Proportions, 2020).

A consistency check was performed for both profile quality and mixture status. For profile quality, the washed and unwashed finger sample sets were classified into five categories for consistency across the three collections, as summarized in Table 3. Sample sets were classified as consistently high-quality or consistently low-quality when all three collections were high-quality or low-quality, respectively. Mostly high-quality or mostly low-quality sample sets were those with two collections of high-quality or low-quality profiles, respectively. Sample sets with different profile qualities for all three collections were classified as inconsistent. For mixture status, all sample sets were classified into four categories for consistency across the three collections (see Table 4). When all three collections were single source profiles or two collections were single source profiles and one collection was a background mixture, the sample set was classified as consistently clean. Sample sets with all three collections being one of the types of mixtures were

classified as consistently mixed. Sample sets containing a combination of single source profiles and mixtures with the donor as either the major or minor component were considered inconsistent. An inconclusive sample set was one consisting of two or three negative profiles. The Z score test for two population proportions was used to analyze if mixture status consistency significantly differs between washed and unwashed fingers (Z Score Calculator for 2 Population Proportions, 2020).

Table 3. Consistency determination for profile quality of the washed and unwashed finger sample sets based on three collections per set.

Category	Definition
Consistently high-quality	All three collections are full or high partial profiles.
Mostly high-quality	Two collections are full or high partial profiles.
Consistently low-quality	All three collections are low partial or negative profiles.
Mostly low-quality	Two collections are low partial or negative profiles.
Inconsistent	All three collections are different profile qualities.

Table 4. Consistency determination for mixture status of the washed and unwashed finger sample sets based on three collections per set.

Category	Definition
Consistently clean	All three collections are single source profiles, or two collections are single source profiles, and one collection is a background mixture.
Consistently mixed	All three collections are background mixtures, mixtures with the donor as the major component, mixtures with the donor as the minor component or a combination.
Inconsistent	One to two collections are single source profiles, and one to two collections are mixtures with the donor as the major or minor component.
Inconclusive	Two or more collections are negative profiles.

Shedding propensity was determined by calculating the percentage of expected alleles present in profiles obtained from washed finger samples and evaluating data for all

three collections, for a total of six results per donor. Upper and lower quartiles were calculated from this data to establish the shedding propensity definitions shown in Table 5. Donors who deposited three or more full profiles and had an average expected allele percentage above 94.9% were classified as high shedders. Intermediate shedders were donors who deposited less than three full profiles and less than two profiles with expected allele percentages lower than the lower quartile of 47.7%. Donors who deposited three or more profiles with expected allele percentages lower than 47.7% and had an average expected allele percentage below 47.7% were classified as low shedders. This data was compared to the quantification data on DNA concentrations for each donor, as analyzed by other laboratory members.

Table 5. Shedding propensity classifications and definitions based on unwashed and washed finger samples.

Shedding Propensity	Definition
High	Three or more washed finger samples with full profiles present and donor has an overall average expected allele percentage above 94.9%.
Intermediate	Less than three washed finger samples with full profiles and less than two washed finger samples with expected allele percentages less than 47.7% present.
Low	Three or more washed finger samples with expected allele percentages less than 47.7% present and donor has an overall average expected allele percentage below 47.7%.

3. Results

3.1. Sampling location

3.1.1. Profile quality

Each collected sample was categorized by profile quality based on the number of expected alleles present in the acquired DNA profile. Samples were then sorted based on sample type to assess the effect of anatomical location. Out of the 670 samples, there were 257 full profiles (F), 200 high partial profiles (HP), 169 low partial profiles (LP), 38 negatives (NEG), and 6 profiles that were not suitable for analysis (NS) (see Table 6). The largest number of full profiles was obtained from the ear samples with 71 full profiles, followed by the nape, left unwashed fingers, and right unwashed fingers with 44, 43, and 41 full profiles, respectively (see Table 6 & Figure 1). The right washed fingers generated the most high partial profiles at 39 profiles. The greatest number of low partial profiles were obtained from the toe and arm samples with 37 low partials each. Toe samples generated the most negative samples at 18 profiles. Insufficient profiles were only obtained from three toe samples and three arm samples. Since the finger samples were the primary focus of this research, statistical testing was limited to these samples. The differences in profile quality for washed and unwashed fingers were found to be insignificant at $p < .05$ [$H = 0$, $p = 1$]. However, the number of full profiles was significantly higher for unwashed fingers according to a Z test for two population proportions [$z = 5.6345$, $p < .00001$, two-tailed].

Table 6. Profile quality categories organized by sample type[1].

Sample Type	Profile Quality				
	F	HP	LP	NEG	NS
Left Unwashed	43 (51.8%)	27 (32.5%)	10 (12.0%)	3 (3.6%)	0 (0%)
Left Washed	25 (29.8%)	28 (33.3%)	26 (31.0%)	5 (6.0%)	0 (0%)
Right Unwashed	41 (48.8%)	30 (35.7%)	11 (13.1%)	2 (2.4%)	0 (0%)
Right Washed	10 (11.9%)	39 (46.4%)	30 (35.7%)	5 (6.0%)	0 (0%)
Toe	11 (13.1%)	15 (17.9%)	37 (44.0%)	18 (21.4%)	3 (3.6%)
Ear	71 (84.5%)	9 (10.7%)	4 (4.8%)	0 (0%)	0 (0%)
Nape	44 (53.0%)	22 (26.5%)	14 (16.9%)	3 (3.6%)	0 (0%)
Arm	12 (14.3%)	30 (35.7%)	37 (44.0%)	2 (2.4%)	3 (3.6%)
Total	257 (38.4%)	200 (29.9%)	169 (25.2%)	38 (5.7%)	6 (0.9%)

[1]Percentages out of each row total are shown in parentheses.

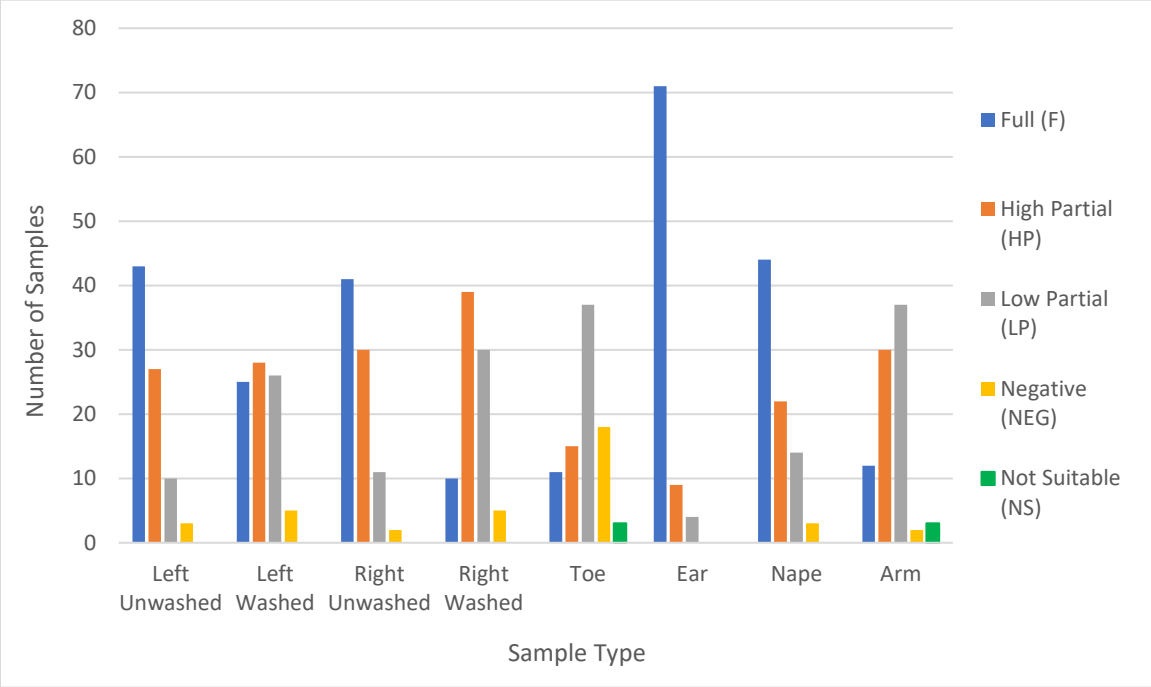

Figure 1. Profile quality by sample type. Ear and nape samples had the highest number of full profiles, unwashed fingers had more full profiles than washed fingers, n=670.

3.1.2. Mixture status

Each sample was then analyzed for the presence and number of foreign alleles, which indicate the sample's mixture status. Approximately half of the collected samples were classified as single source samples (N), 18.4% were background mixtures (MXB) and

15.5% were mixtures with the donor as the major component (MX) (see Table 7). Approximately 5.5% of the samples were classified as a mixture with the donor as a minor component (MXDM) and 5.7% of the samples were negative, thus no mixture status could be determined (NS). The sample type with the highest number of samples classified as MXDM and NA were the toe samples with 18 profiles for each classification. The latter value corresponds to the number of negative profiles acquired from the toe samples. The fewest mixtures were obtained from the ear samples, which were 88.1% single source (see Figure 2). As expected, STR results for unwashed fingers showed the highest prevalence of DNA mixtures (63.5%). The next highest prevalence of mixtures was among the toe samples (48.8%) followed by the washed finger samples (36.3%). Of the washed finger sample mixtures, most had foreign alleles only at background level. Upon comparing the washed and unwashed finger samples, there was a significant difference in the number of single source profiles at $p < .05$ [$z = 4.4469$, $p < .00001$, two-tailed].

Table 7. Mixture status organized by sample type[1].

Sample Type	Mixture Status				
	N	MXB	MX	MXDM	NS
Left Unwashed	36 (43.4%)	22 (26.5%)	20 (24.1%)	2 (2.4%)	3 (3.6%)
Left Washed	49 (58.3%)	21 (25.0%)	9 (10.7%)	0 (0%)	5 (6.0%)
Right Unwashed	20 (23.8%)	28 (33.3%)	31 (36.9%)	3 (3.6%)	2 (2.4%)
Right Washed	48 (57.1%)	20 (23.8%)	10 (11.9%)	1 (1.2%)	5 (6.0%)
Toe	25 (29.8%)	9 (10.7%)	14 (16.7%)	18 (21.4%)	18 (21.4%)
Ear	74 (88.1%)	3 (3.6%)	5 (6.0%)	2 (2.4%)	0 (0%)
Nape	67 (80.7%)	8 (9.6%)	3 (3.6%)	2 (2.4%)	3 (3.6%)
Arm	49 (58.3%)	12 (14.3%)	12 (14%)	9 (10.7%)	2 (2.4%)
Total	368 (54.9%)	123 (18.4%)	104 (15.5%)	37 (5.5%)	38 (5.7%)

[1]Percentages out of each row total are shown in parentheses.

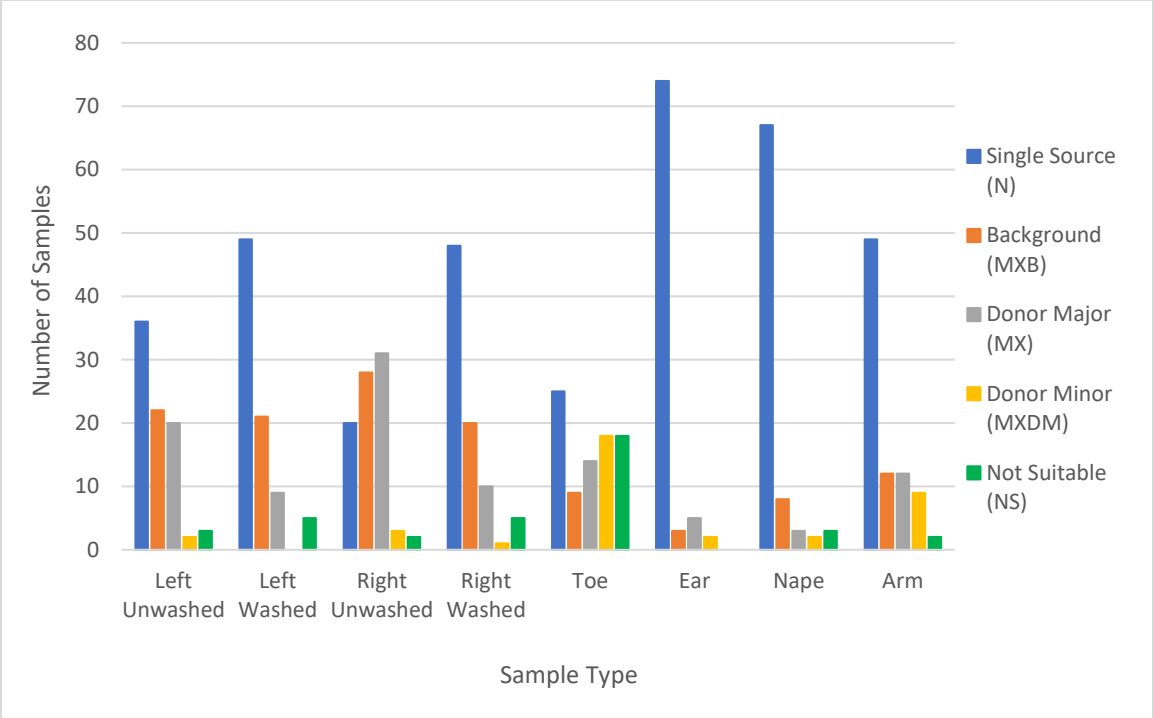

Figure 2. Mixture status by sample type. Ear and nape samples had the highest number of single source profiles, washed fingers had more single source profiles than unwashed fingers, n-670.

3.2. Washed and unwashed finger samples

3.2.1. Time since handwashing

Profile quality and mixture status data for the unwashed finger samples was then sorted according to the time since handwashing, which had been self-reported by each donor (Tables 8 & 9). One donor did not report the time since handwashing for the second sample collection. There was a higher percentage of samples in the <1 hour category (43.6%). The number of full profiles decreased as the time since handwashing increased. However, the differences in profile quality between the three time categories were statistically insignificant at $p < .05$ [$H = 0.305$, $p = .859$]. Similarly, there were no significant differences in mixture status based on time since handwashing [$H = 2.16$, $p = .3396$].

Table 8. Profile quality for unwashed fingers for time intervals since the last handwash[1].

Profile Quality	Reported time since handwashing			
	<1 Hour	1-3 Hours	>3 Hours	Unreported
Full	39 (54.2%)	29 (38.6%)	16 (44.4%)	0 (0%)
High Partial	21 (29.2%)	22 (38.6%)	12 (33.3%)	2 (100%)
Low Partial	11 (15.3%)	3 (5.3%)	7 (19.4%)	0 (0%)
Negative	1 (1.4%)	3 (5.3%)	1 (2.8%)	0 (0%)

[1]Percentages out of each column total are shown in parentheses.

Table 9. Mixture status for unwashed fingers for time intervals since the last handwash[1].

Mixture Status	Reported time since handwashing			
	<1 Hour	1-3 Hours	>3 Hours	Unreported
N	26 (36.1%)	17 (29.8%)	12 (33.3%)	1 (50%)
MXB	16 (22.2%)	19 (33.3%)	15 (41.7%)	0 (0%)
MX	27 (37.5%)	16 (28.1%)	7 (19.4%)	1 (50%)
MXDM	2 (2.8%)	2 (3.5%)	1 (2.8%)	0 (0%)
NS	1 (1.4%)	3 (5.3%)	1 (2.8%)	0 (0%)

[1]Percentages out of each column total are shown in parentheses.

3.2.2. Profile consistency across three collections

To assess the level of intra-variation of these results, the samples sets were grouped into different categories for profile quality (see Table 10) and mixture status (see Table 11). For analyzing profile quality consistency, full and high partial profiles were considered high-quality while low partial and negative profiles were considered low-quality. Consistently high-quality and low-quality sample sets were those with all three collections of high- or low-quality profiles, respectively. Mostly high- and low-quality sample sets were those with two out of three collections being high- or low-quality profiles, respectively. Sample sets with all three collections having different profile qualities were considered inconsistent. Out of the 112 washed and unwashed finger sample sets analyzed for profile quality consistency, 59 were consistently high-quality, 17 were mostly high-quality, 12 were consistently low-quality, 8 were mostly low-quality, and 16 were inconsistent. The largest numbers of consistently high-quality profiles were obtained from

the unwashed finger sample sets while the largest numbers of consistently low-quality profiles were obtained from the washed finger sample sets (see Table 10 & Figure 3). The prevalence of profile quality inconsistency did not seem to be affected by wash status.

Table 10. Profile quality consistency for washed and unwashed finger samples across three collections[1].

Sample Type	Number of Sample Sets				
	All High	Mostly High	All Low	Mostly Low	Inconsistent
Left Unwashed	19 (67.9%)	2 (7.1%)	1 (3.6%)	1 (3.6%)	5 (17.9%)
Left Washed	12 (42.9%)	6 (21.4%)	7 (25.0%)	1 (3.6%)	2 (7.1%)
Right Unwashed	20 (71.4%)	1 (3.6%)	0 (0%)	3 (10.7%)	4 (14.3%)
Right Washed	8 (28.6%)	8 (28.6%)	4 (14.3%)	3 (10.7%)	5 (17.9%)
Total	59 (52.7%)	17 (15.2%)	12 (10.7%)	8 (7.1%)	16 (14.3%)

[1]Data organized by sample type and grouped into five main categories: all high-quality, mostly high-quality, mostly low-quality, and inconsistent. Percentages out of each row total are shown in parentheses.

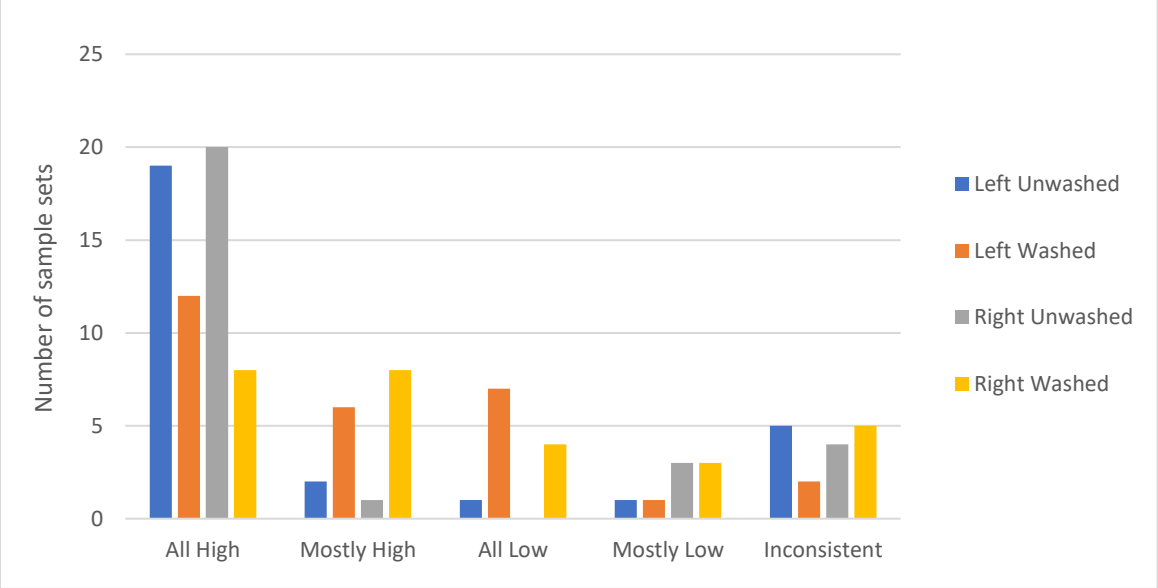

Figure 3. Profile quality consistency across three collections. Compared to unwashed fingers, washed fingers had more sample sets that were consistently low-quality and fewer that were consistently high-quality across all three collections, n=112.

3.2.3. Mixture status consistency across three collections

When analyzing the finger sample sets for mixture status consistency, sample sets were classified into four different categories. Sample sets with all single source profiles or two single source profiles and one background mixture were considered consistently clean. Consistently mixed sample sets were those with all three collections demonstrating some level of foreign DNA. When a sample set included a combination of single source samples and mixed samples, it was classified as inconsistent. Lastly, inconclusive sample sets were those consisting of two or more negative profiles. Out of the 112 sample sets, 52 were consistently clean, 29 were consistently mixed, 29 were inconsistent, and 2 were inconclusive. The highest number of inconsistencies were acquired from the right unwashed finger samples, with 10 inconsistent sample sets (see Table 11 & Figure 4). However, the difference in inconsistent sample sets for unwashed and washed fingers was insignificant [$z = 0.5971$, $p = .5485$, two-tailed]. Combined, the right and left unwashed finger samples produced the greatest amount of consistently mixed sample sets. The difference in the number of consistently mixed sample sets for unwashed and washed fingers was statistically significant at $p < .05$ [$z = 2.985$, $p = .00278$, two-tailed]. There was also a significant difference in the number of consistently clean sample sets between unwashed and washed fingers [$z = -2.849$, $p = .00438$, two-tailed].

Table 11. Mixture status consistency for washed and unwashed fingers across three collections[1].

Sample Type	Number of Sample Sets			
	Clean	Mixed	Inconsistent	Inconclusive
Left Unwashed	12 (42.9%)	9 (32.1%)	6 (21.4%)	1 (3.6%)
Left Washed	19 (67.9%)	3 (10.7%)	5 (17.9%)	1 (3.6%)
Right Unwashed	5 (17.9%)	13 (46.4%)	10 (35.7%)	0 (0%)
Right Washed	16 (57.1%)	4 (14.3%)	8 (28.6%)	0 (0%)
Total	52 (46.4%)	29 (25.9%)	29 (25.9%)	2 (1.8%)

[1]Data organized by sample type and grouped into four main categories: consistently clean, consistently mixed, inconsistent, and inconclusive. Percentages out of each row total are shown in parentheses.

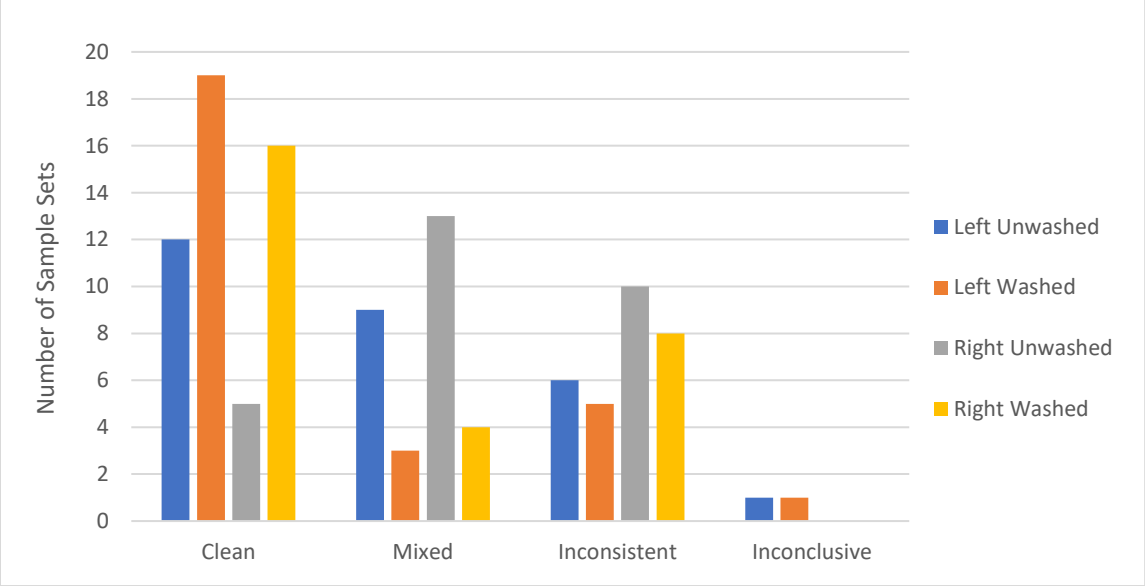

Figure 4. Mixture status consistency across three collections. Compared to unwashed fingers, washed fingers had more consistently clean sample sets across all three collections, n-112.

3.2.4. Biological sex and hand dominance

The results for unwashed and washed fingers were organized by biological sex and handedness to determine if these factors affect the mixture status and profile quality of the STR profiles. Table 12 shows percentage of samples for each profile quality and mixture status for each of the sexes. Among both male and female volunteers, most samples were full and high partial profiles. Male participants deposited about 10% fewer full profiles

than the females. Nearly half of the finger samples were classified as single source samples for both sexes. Neither profile quality nor mixture status were statistically different among sexes at 95% confidence [H = 0.0436, p = .835; H = 0.0982, p = .754].

Table 12. Comparison of finger samples profile quality and mixture status for female versus male volunteers.

Sex	Profile Quality Percentages					Mixture Status Percentages				
	F	HP	LP	NEG	NS	N	MXB	MX	MXDM	NS
Female	40.7	37.4	18.7	3.2	0	49.0	20.6	25.2	0.0	3.2
Male	31.1	36.7	26.7	5.5	0	42.8	30.6	17.2	3.3	5.5

Percentages of profile quality for each sample type separated by handedness are shown in table 13. Only 5 participants were left-handed while the remaining 23 participants were right-handed. As shown in the table, the results appear to be correlated to the wash status rather than hand dominance. Regardless of handedness, the profile quality and mixture status of the dominant hands were not significantly different from the non-dominant hands at $p < .05$ [H = 0.0263, p = .871; H = 0.0359, p = .850]. For the right-handed volunteers, the unwashed finger samples were on average 56.2% full profiles, 30.7% high partial profiles, 10.2% low partial profiles, and 2.93% negative profiles. For the left-handed volunteers, the unwashed finger samples were on average 23.3% full profiles, 50.0% high partial profiles, 23.3% low partial profiles, and 3.34% negative profiles. There were more negative samples among the washed fingers of the left-handed volunteers, with an average of 16.7% negative profiles versus 3.63% negative profiles among the washed fingers of the right-handed volunteers. No unsuitable profiles (NS) were acquired from any of the washed or unwashed finger samples. Differences in profile quality based on handedness were insignificant at $p < .05$ [H = 0, p = 1].

Table 13. Comparison of profile quality determinations for right-handed donors versus left-handed donors.

Profile Quality	Percentage of Right-handed (n=23)				Percentage of Left-handed (n=5)			
	LU	LW	RU	RW	LU	LW	RU	RW
F	57.4	33.3	55.1	11.6	26.7	13.3	20.0	13.3
HP	29.4	34.8	31.9	49.3	46.7	26.7	53.3	33.3
LP	8.82	29.0	11.6	34.8	26.7	40.0	20.0	40.0
NEG	4.41	2.90	1.45	4.35	0.00	20.0	6.67	13.3
NS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Washed fingers showed a higher prevalence of single source samples for both right- and left-handed volunteers. Table 14 shows percentages of each mixture status for the 23 right-handed and five left-handed individuals. Apart from the "left washed" finger sample set, which has the percentages in the MXB and MX categories reversed, mixture status results are similar across handedness. As suggested by the higher prevalence of mixtures and previous studies, unwashed finger samples may be more affected by activities prior to sampling. Since handedness can alter one's pattern of touching objects, it was hypothesized that there would be differences in the mixture status of the unwashed fingers between left- and right-handed volunteers. However, overall mixture status did not significantly differ for either right or left finger samples based on handedness at $p < .05$ (see Table 15). When comparing the number of mixtures for the dominant versus non-dominant hand, right-handed donors deposited significantly different numbers of mixtures and single source samples from the right unwashed fingers [$z = 2.5494$, $p = .0108$, two-tailed; $z = -2.2317$, $p = .02574$, two-tailed]. Left-handed donors did not exhibit the same trend [$z = -1.1366$, $p = .254$, two-tailed].

Table 14. Comparison of mixture status determinations for right-handed donors versus left-handed donors.

Mixture Status	Percentage of Right-handed (n=23)				Percentage of Left-handed (n=5)			
	LU	LW	RU	RW	LU	LW	RU	RW
N	42.7	59.4	24.6	56.5	46.7	53.3	20.0	60.0
MXB	26.5	29.0	31.9	24.6	26.7	6.67	40.0	20.0
MX	25.0	8.70	39.1	13.0	20.0	20.0	26.7	6.67
MXDM	1.47	0.00	2.90	1.45	6.67	0.00	6.67	0.00
NS	4.41	2.90	1.45	4.35	0.00	20.0	6.67	13.3

Table 15. Results from the Kruskal-Wallis tests for differences in mixture status of unwashed fingers between left- and right- handed donors at $p < .05$.

Sample Type	H statistic	p-value
Left Unwashed	0.0109	.917
Right Unwashed	0.0982	.754

3.3. Shedding propensity determinations

STR data for all three collections for washed finger samples from both hands, constituting a total of six results per donor, were used to determine individual shedding propensity. The individual percentages of expected alleles and the average over all six washed finger samples were determined for each donor (Table 16). Shedding propensity criteria were established upon calculating the upper and lower quartiles from the results. Donors who deposited three or more full profiles among the six washed finger samples and an average expected allele percentage above 94.9% were classified as high shedders. Intermediate shedders were donors who deposited less than three full profiles and less than two profiles with expected allele percentages below the lower quartile of 47.7%. Donors who deposited three or more profiles with expected allele percentages less than 47.7% and produced an average percentage less than 47.7% were classified as low shedders. Based on this criteria, three donors (10.7%) were high shedders, 18 donors

(64.3%) were intermediate shedders, and seven donors (25.0%) were low shedders (see Table 17).

Table 16. Data used for shedding propensity determinations, including average percentages of expected alleles and standard deviation for washed finger samples[1].

Donor	Average Expected Alleles (%)	Standard Deviation (%)	Number of Washed Samples less than 47.7%	Number of Washed Samples between 47.7% and 100%	Number of Washed Samples at 100%
31	73.2	37.97	2	1	3
32	98.1	2.95	0	2	4
35	86.7	9.17	0	5	1
36	43.0	24.84	3	3	0
37	82.9	18.67	0	4	2
38	72.8	19.53	1	5	0
39	26.6	16.16	5	1	0
40	72.1	23.85	2	4	0
41	80.6	14.59	0	6	0
42	82.9	16.03	0	6	0
43	71.4	29.21	2	3	1
44	87.0	17.18	0	4	2
45	25.5	13.54	6	0	0
46	80.7	20.22	0	5	1
47	43.5	19.48	4	2	0
48	85.6	12.47	0	4	2
49	100.0	0.00	0	0	6
50	92.3	12.35	0	2	4
51	88.0	10.84	0	4	2
52	55.9	30.99	3	3	0
53	34.7	29.23	4	2	0
54	98.4	3.98	0	1	5
55	71.2	19.98	0	6	0
56	46.3	21.61	4	2	0
57	78.6	34.00	1	4	1
58	82.5	10.00	0	6	0
59	77.9	13.82	0	5	1
60	43.9	10.08	5	1	0

[1]High shedders are highlighted in yellow, low shedders are highlighted in blue, and intermediate shedders are unhighlighted.

Table 17. Number and percentage of donors classified as each shedding propensity based on the data in table 15.

Shedder Status	Number of Donors	Percentage of Donors
High	3	10.7
Intermediate	18	64.3
Low	7	25.0

4. Discussion

Variations in the methodology and criteria for defining shedding propensity have led to inconsistent results regarding individual propensity to deposit DNA and the influence of biological sex, handwashing, and hand dominance on the quantity and quality of DNA deposited. This research has demonstrated again that the determination of individual shedding propensities may be more complicated than previously suggested. Several factors were investigated during this project using a novel approach of sampling from the skin using tape lifts. By eliminating the need for swabs, the overall DNA yield was expected to increase, which would result in fewer negative profiles. Out of the 670 samples analyzed, only 5.7% were negative and 0.9% were not suitable for analysis. The majority of the collected samples produced high-quality profiles that were either full or high partial. This suggests that sampling directly from the skin reduces the transfer loss of DNA and results in higher quality STR profiles. Additionally, the high STR success rate can be attributed to the increased sensitivity of the modern multiplexes and instrumentation. Since tape lifts capture cellular material from a defined skin surface area, it is presumed that the inter-variability in the quantity and quality of DNA collected by this method is proportional to that of samples deposited via transfer experiments.

Sampling locations

To test whether the sampling location would alter the STR results, a range of sample types were analyzed. Given previous research on the origin of DNA from skin samples, some variation in the STR profile qualities was expected (Oleiwi et al., 2015; Quinones & Daniel, 2012). Profile quality and mixture status were diverse among the different types of samples; however, no statistically significant trends were observed (data not shown). High-quality profiles were most frequently obtained from the ear and nape samples while low-quality profiles were mostly acquired from the toe samples. These differences could be a result of variations in sebaceous secretions or sweat production at each anatomical location, which are known to affect the transport of DNA to the skin surface (Zoppis et al., 2014). The toe samples may have generated poor quality samples because of skin surface irregularities, such as calluses. In terms of mixture status, the ear and nape samples had the highest occurrence of single source and background mixture profiles (combined more than 90%). These high percentages may be due to decreased contact with foreign objects that would act as vectors for non-self-DNA transfer. Since this project was focused on unwashed and washed fingers, an explanation of the variation among the other sampling locations will require further investigation.

The overall prevalence of mixtures of all three categories combined was 39.4%, which is consistent with some of the literature (Daly et al., 2012; Lacerenza et al., 2016). Of those mixtures, 46.5% were considered background, or low-level mixtures, which is higher than what was reported by Lacerenza et al. (2016) for palmar surfaces. When analyzing the 168 washed finger samples only, the mixture prevalence is nearly equivalent with 36.3% mixtures. However, here a larger percentage of those mixtures (67.2%) were

background mixtures. This is more consistent with studies that have observed a high prevalence of low-level mixtures (Goray et al., 2016; van Oorschot et al. 2014). Since this research classified mixtures based on the presence, number, and relative height of foreign alleles, it is possible that some profiles may have been misclassified. If any secondary donor shares multiple alleles with the primary donor, fewer foreign alleles would be detected. This could lead to the profile being classified as a single source when it should be classified as a mixture. However, this is unlikely when analyzing samples using a multiplex system. Furthermore, since low-template DNA will often generate profiles with PCR stochastic effects, it can be difficult to distinguish between a background mixture and single source profile. For this mixture type, drop-ins and other artifacts, such as increased stutter, could lead to a mixture determination when the profile is truly a single source sample.

Unwashed and washed finger samples

Since most DNA transfer is associated with direct or indirect touch by the hands, the finger samples were analyzed in more depth than the other sample types. The profile quality of unwashed finger samples was not significantly affected by the self-reported time since last handwash. Although this result is consistent with previous studies, other research has shown that certain activities will influence the amount of deposited DNA (Goray et al., 2016; Fonneløp et al., 2017; Szkuta et al., 2017). The project questionnaire did not account for activities performed prior to sampling. Without knowledge of the participants' behaviors, it will be difficult to determine a correlation between time since handwashing and quality or quantity of DNA deposited. Furthermore, the participants only selected one

of three time ranges provided (<1 hour, 1-3 hours, or >3 hours) rather than giving an exact time since last handwash. Additional experimentation would need to be performed to more accurately assess the impact of the time since handwashing on STR results for unwashed hands.

Although the data for time since handwashing indicates no effect of handwashing on STR results, the evaluation of unwashed versus washed finger samples suggests an effect 30 minutes after handwashing with no activity allowed. Several trends were observed when comparing the profile quality and mixture status of the washed versus unwashed finger samples. More low-quality profiles and single source profiles were acquired for the washed finger samples. A greater number of full profiles was generated from the unwashed finger samples, likely due to an increased amount of viable cellular material. The difference in the results from the self-reported time since handwashing and the 30 minutes post-handwashing data is likely due to the controlled manner of handwashing in the laboratory. By washing with water only, the volunteers removed both self and non-self-DNA from the surface of their fingers. Since DNA is thought to accumulate on the skin over time at a rate unique to one's shedding propensity, the washed fingers provided a neutral baseline for all volunteers (Burrill et al., 2019). Additionally, participants were instructed not to touch any surfaces or themselves between the time of handwashing and time of sampling. This restriction of activity was expected to significantly reduce the prevalence of mixtures for the washed finger samples. This hypothesis was confirmed with an observed decrease in the number of mixtures and increase in the number of single source profiles for washed finger samples relative to unwashed finger samples.

Profile quality and mixture status consistency across the three sample collections was then analyzed as a measure of intra-variation. Most finger sample sets were consistently high-quality profiles (52.7%), while a smaller percentage were completely inconsistent (14.3%). For mixture status, 72.3% of the sample sets were considered consistent and 25.9% of the sample sets were inconsistent. It was expected that there would be a higher level of variation for unwashed finger samples due to the increased uncertainty in activities performed prior to sampling. However, the number of inconsistent sample sets for both profile quality and mixture status were nearly the same for washed and unwashed fingers from both hands. This suggests that the level of intra-variation is affected by factors other than handedness and handwashing. There was a significant difference in the number of consistently clean and consistently mixed sample sets between washed and unwashed fingers, which corresponds to the conclusion that handwashing with restricted activity prior to sampling has an influence on mixture status. Given that there was more consistency across the three collections for the washed finger samples and single source profiles are more representative of the propensity to deposit self-DNA, this sample type was deemed the most suitable for the shedding propensity determinations.

Effect of biological sex

The effect of biological sex on the STR results was investigated since several studies have observed significant differences in the quantity and quality of DNA deposited by males and females (Fonneløp et al., 2017; Goray et al., 2016; Gosch & Courts, 2019). Differences based on sex can be explained by the biological variations between males and females. It has been shown that males generally produce more sweat and sebum than

females, which may cause a higher amount of DNA to accumulate on the surface of the skin (Giacomoni et al., 2009). Upon touching a surface or object, males would therefore deposit more of their own DNA. Since the quantity of DNA usually corresponds to the quality of the resulting STR profile, it was expected that males would deposit more high-quality profiles. Additionally, males have been shown to deposit less foreign DNA than females, so a lower prevalence of mixtures was expected (Lacerenza et al., 2016). Contrary to the first hypothesis, female finger samples generated a slightly higher percentage of full profiles, at 40.7% versus the male finger samples at 31.1%. Furthermore, the average percentage of expected alleles from the washed finger samples of females was higher with 77.1% versus males with 65.4%. However, this difference was not statistically significant at $p < .05$. There was also no correlation between biological sex and mixture prevalence. While some previous research agrees that there is no difference in deposited DNA based on biological sex, a larger sample size would be required to confirm this conclusion (Farmen et al., 2008; Manoli et al., 2016).

One potential reason for discrepancies among this study and those that discovered significant differences between males and females is the novel use of tape lifts. This method may have reduced the variability among men and women. The tape lifts used for this research had a uniform diameter so the quantity and quality of DNA deposited was independent of sampling area, pressure, and friction. Other studies required participants to handle various items or place palm prints on glass slides (Fonneløp et al., 2017; Goray et al., 2016). Women generally have smaller hands than men and may handle objects with different levels of pressure or friction. Since pressure and friction have been linked to higher DNA deposition, this may cause the perceived differences in shedding propensity

among males and females (Tobias et al., 2017; Goray et al., 2010). Goray et al. (2016) determined that participant hand size had no correlation to the amount of DNA deposited or the number of alleles detected, but they did not investigate potential pressure effects for that study. Additionally, both Goray et al. (2016) and Fonneløp et al. (2017) had small sampling sizes with few participants. It is also possible that the sampling population of this study was too homogenous with all volunteers recruited from the John Jay College of Criminal Justice. However, a university population was also utilized by Lacerenza et al. (2016) and they observed a gender correlation for DNA yield and profile quality.

Effect of hand dominance

Due to the increased activity of the dominant hand, it was expected that an individual would deposit more DNA, and thus higher quality STR profiles, from the unwashed fingers of their dominant hand. Likewise, a higher prevalence of mixtures was expected for the dominant hand samples. However, there were no significant differences in the profile quality results based on hand dominance. Left-handed and right-handed participants generated similar STR data for all finger sample types. The left-handed donors generated fewer full profiles across all finger sample types, but this difference was not significant. This result is consistent with Goray et al. (2016) and Kanokwongnuwut et al. (2018), but conflicts with Manoli et al. (2016). For right-handed donors, the dominant hand (right unwashed fingers) showed a higher prevalence of mixtures than the non-dominant hand (left unwashed fingers), but about half of those were background mixtures. Left-handed donors deposited equal levels of mixtures from both left and right fingers. There may be fewer differences in mixture status among the left-handed participants

because many objects are intended for right-handed use. For this reason, left-handed individuals often contact objects using both hands with equal regularity while right-handed individuals will preferentially use their right hand. However, since there were only five left-handed participants in this study, further research would be required to state the significance of this data.

Shedding propensity determinations

Using the data for the washed fingers, which was a total of six data points per donor, each donor was classified as a high, intermediate, or low shedder. The average percentage of expected alleles ranged from 25.5% for the lowest shedder to 100% for the highest shedder. As with previous studies, there were only a few individuals who more frequently deposited better quality STR profiles than others (Burrill et al., 2019). However, the data exhibited a continuous spectrum rather than clearly delineated ranges for each category. Determining the upper and lower ranges proved to be a difficult task since there has been no consensus throughout the literature. Using quartile calculations alone would cause 25% of donors to be placed into each of the extreme categories, which would not accurately represent the data. Instead, a combination of quartile calculations and previously defined upper limits (full profiles) were utilized (Daly et al., 2012; Manoli et al., 2016).

Since the criteria used for shedding propensity determinations has varied significantly throughout the literature, the percentages of each category have also differed. The categorization used for this research yielded 10.7% high shedders, 64.3% intermediate shedders, and 25.0% low shedders. The percentage of high shedders is most consistent with Daly et al. (2012) and Tan et al. (2019) who reported 13% and 11%, respectively. Several

other studies confirm that the prevalence of high shedders is much lower than was originally determined by Lowe et al. (2002), but state values closer to 20% (Manoli et al., 2016; Fonneløp et al., 2017; Kanokwongnuwut et al., 2018). The percentage of intermediate shedders was higher than any of the previous studies discussed. Several of these studies also incorporated the DNA quantity or amount of cellular material detected (Daly et al., 2012; Kanokwongnuwut et al., 2018). When determining shedding propensity based on cellular deposition, Kanokwongnuwut et al. (2018) discovered that the "light shedders" deposited profiles with an average of 47% of the expected alleles present. This value is equivalent to the lower quartile calculated from the STR results for the washed finger samples. However, Kanokwongnuwut et al. (2018) discovered a higher percentage of low shedders at 45%. These discrepancies are to be expected considering the differences in the extraction efficiency and instrument sensitivity.

There were some inconsistencies when comparing this data to the quantification results from the washed finger samples. Although there were similar trends among the STR and quantification results, the conclusions differed. Based on the quantification data, two participants were classified as low shedders, two participants were high shedders, and the remaining participants were intermediate shedders (Chen et al., 2021, p.154). This discrepancy is due to the different methods of analysis, which were dictated by the nature of the data. While the quantification data is continuous and has no upper limit, the STR data is restricted given that a profile can only contain 0-100% of the expected alleles. It is therefore not possible to directly compare the results from the two sets of data.

Since profile quality was used for the determinations of shedding propensity, a degree of inconsistency was expected to exist for individual donors. As demonstrated by

the consistency check, profile quality can vary across the three collection days. When analyzing the average expected allele percentages calculated for each donor from the six washed finger samples, the standard deviations ranged from 0% to 38%. This result indicates that certain individuals exhibit higher fluctuations in shedding propensity when samples are taken in weekly intervals. The three highest shedders exhibited the lowest standard deviations, which agrees with the conclusions made by Pfeifer & Wiegand (2017). Individuals with intermediate and low shedding propensities may be more influenced by temporal differences in activities, health, or environment.

5. Conclusion

As the submission of touch DNA in forensic investigations increases, there is a heightened need for scientific assessment of the probative value of this type of evidence. Contact traces also pose interpretation difficulties since they generally consist of low template DNA from multiple contributors. In order to better understand the significance of touch DNA collected at a crime scene, several factors must be investigated. It was established that different individuals may have a higher probability of depositing self and non-self-DNA following transfer events. The ability to predict a person of interest's shedding propensity would allow a more informed interpretation of the transfer event leading to the detected touch DNA evidence. Several studies have attempted to develop an adequate method for identifying shedding propensity and the factors that may affect it. Since there has been no agreed upon criteria for each shedder status, these studies have

varied in their conclusions. The goal of this research was to attempt to confirm these results through a novel method of skin tape lifts.

STR profiles were successfully obtained using skin tape lifts from various anatomical locations. There were no significant differences in the STR data based on either biological sex or hand dominance. While right-handed participants generated more mixed profiles from their right unwashed fingers, most of the mixtures were low level. These results contradict several previous findings and may be a consequence of the small sample size. Handwashing had a significant effect on the mixture prevalence among the hand samples. Since there was a higher prevalence of mixtures among the unwashed finger samples, the washed finger samples were deemed the optimal sample type for analyzing shedding propensity.

Across the three collection days, there was some intra-variation which may indicate that shedding propensity is a transient trait. However, the highest shedders demonstrated the lowest level of intra-variation. Criteria for each shedding propensity was established by calculating the interquartile ranges and the average expected allele percentages over the six data points per donor. Based on these criteria, the majority of the participants were classified as intermediate shedders while few were classified as high shedders. There were some discrepancies between these results and those obtained from the quantification analysis conducted by another student (Chen et al., 2021). However, the two highest and lowest shedders were identified using both analyses. In conclusion, this research has provided a foundation for future studies in the prediction of individual shedding propensity.

6. Future Studies

While the results from this research have offered insight into a novel method for determining shedding propensity, further experimentation is required to confirm the findings. The next phase of this research will involve a population study of several ethnic groups with a target of 400 adult participants. Since the various anatomical locations tested did not yield results that were informative for shedding propensity, samples will be collected from washed fingers. The factors of biological sex, handwashing, and hand dominance should be further explored with this larger number of participants. Additional biological and behavioral factors will also be studied: ethnicity, age, body mass index, skin disease, drug and medication use, skin-sun reaction, sebum density, and skin hydration. Given that obtaining replicates may not be feasible in criminal investigations, future studies should attempt to identify and predict shedding propensity from a single collection. However, this may not be possible given the level of temporal variability that individual's exhibit. An exploration of the effects of various skin parameters on shedding propensity may provide a better understanding into the degree of intra-variation exhibited by individuals.

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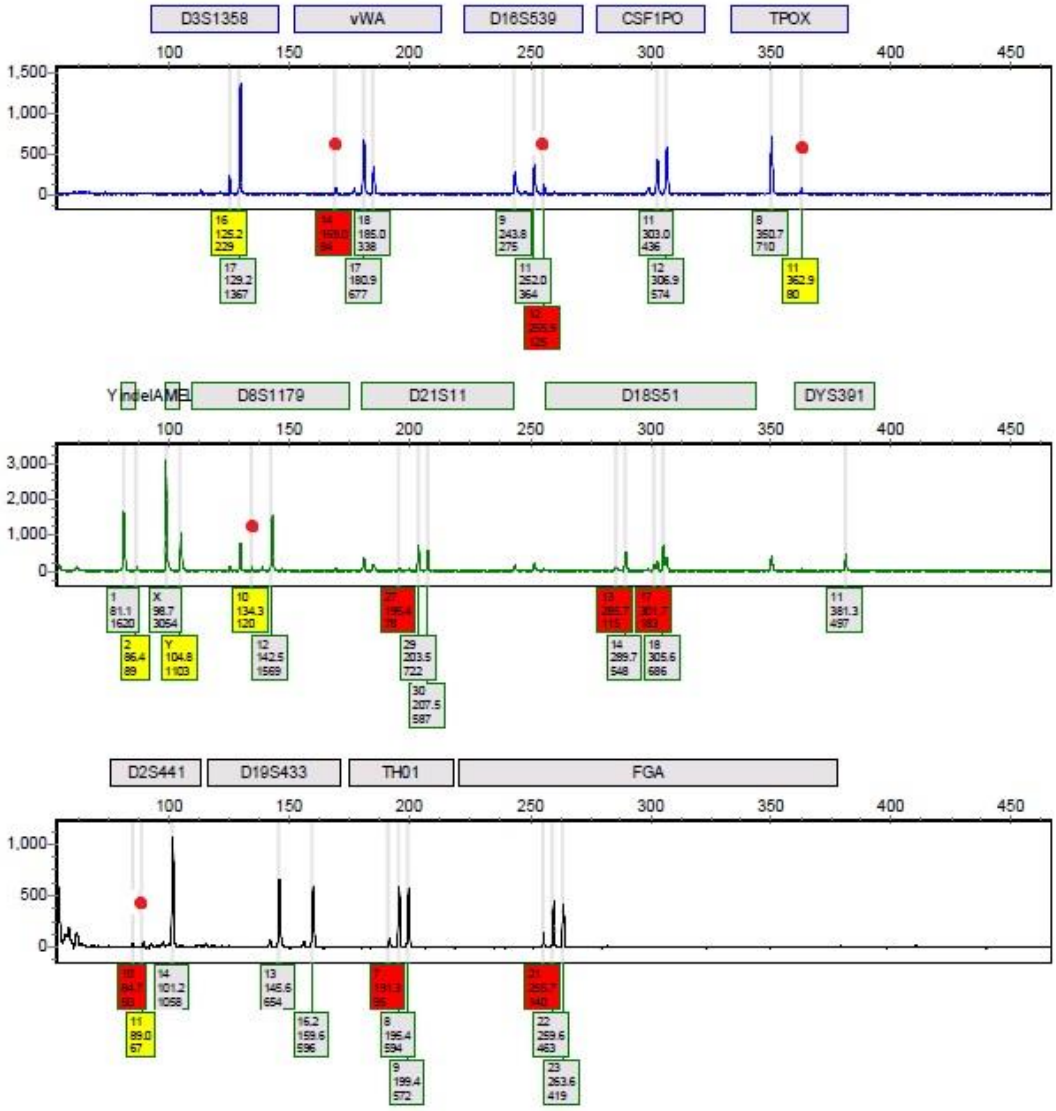
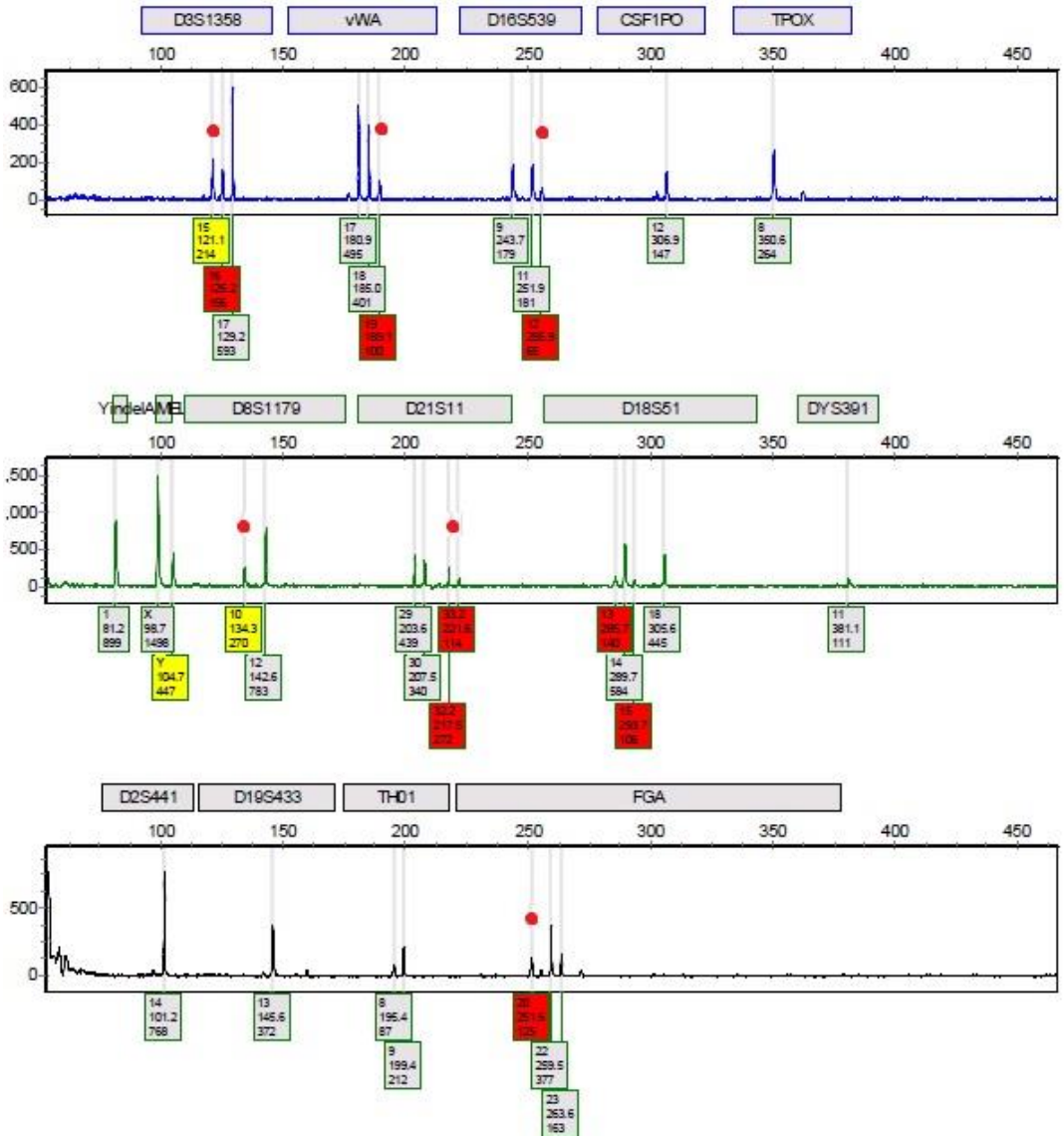
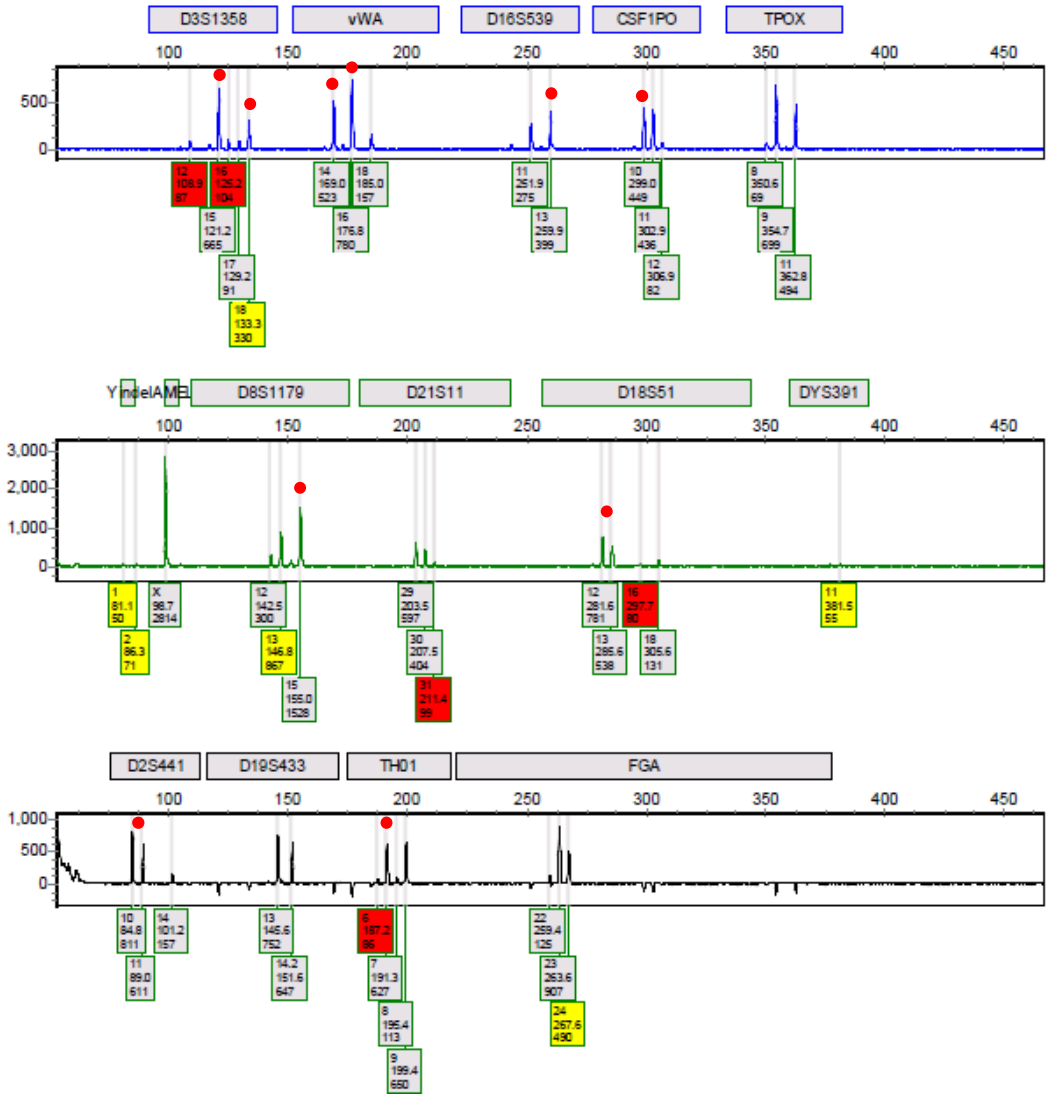
8. Appendix



Supplemental Figure 1. Presented is a partial electropherogram of the full profile acquired from the second collection of the right unwashed hand of donor 40. Red dots indicate some of the foreign alleles used to classify the sample as a background mixture (MXB). Indicated foreign alleles have peak heights less than 40% of the donor alleles' peak heights.



Supplemental Figure 2. Presented is a partial electropherogram of the high partial profile acquired from the first collection of the left unwashed hand of donor 40. Red dots indicate some of the foreign alleles used to classify the sample as a mixture with the donor as the major component (MX). Indicated foreign alleles have peak heights greater than those of the nearby stutter peaks.



Supplemental Figure 3. Presented is a partial electropherogram of the high partial profile acquired from the second collection of the toe of donor 40. Red dots indicate some of the foreign alleles used to classify the sample as mixture with the donor as the minor component (MXDM). The following loci contain foreign alleles with peak heights greater than those of the donor alleles: D3S1358, vWA, D16S539, CSF1PO, D8S1179, D18S51, and D2S441.