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Prevalence of Non-self DNA on Three Different Sebaceous Skin Locations

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Abstract

This project serves to provide additional information on the prevalence of non-self DNA on the exposed skin locations specifically the inner upper arm, the nape area of the neck and the neck area just below the ear, where current literature is lacking. Skin tape lifts from these locations were collected from 15 male participants and 13 female participants once a week over a period of three weeks. Only single source profiles were obtained from six volunteers. Eight volunteers had only one mixture DNA profile in nine samples, while mixture DNA profiles for at least two collection occasions were observed in 16 volunteers. The location of skin sample, gender and time of last shower were evaluated for their effect on the detection of non-self alleles. Analysis of variance (ANOVA) showed that none of the variables had a significant effect on the detection of non-self alleles. The mixture of DNA profiles across all datasets were closely examined for the presence of a repeating non-self genotype. Samples collected from the upper arm area presented the highest number of mixtures with non-self alleles being present more than once over the three weeks. A repeating non-self genotype was also observed in the neck area below the ear in one volunteer. For three out of 28 volunteers, these repeating non-self genotypes across all three collection occasions could be attributed to a common contributor. No repeating genotypes were determined for the nape area of the neck. These findings can be meaningful in cases of sexual assault and manual strangulation, where information on the prevalence of non-self DNA on parts of the upper body is important.

Introduction and Literature Review

Forensic relevance of trace DNA

A person or an object can be connected to a crime scene through DNA that is discovered during a forensic investigation. Traditional forensic investigation relied on relatively large amounts of biological materials recovered on the scene of the crime such as visible blood or semen stains to generate a DNA profile. However, the amount of DNA recovered from an object without visible body fluid stains can be very limited. This limited amount is known as trace DNA (Van Oorschot, 2019).

Forensic laboratories follow standard operating procedures for DNA analysis. DNA obtained from biological evidence is amplified using polymerase chain reaction (PCR) and detected through capillary electrophoresis (CE). The result is short repeated sequences of DNA or short tandem repeats (STR) profiles. Forensic testing targets these specific genetic markers since the number of repeat units for each STR is highly variable among individuals. In addition, several STR markers can be combined in a single multiplex reaction. This provides a high power of discrimination for identification purposes (Butler, 2012). Evidence DNA profiles are compared to relevant samples within a case and can be entered into the FBI database for comparison with other crime scene evidence and convicted offender DNA profiles (Butler 2012).

For samples that are suspected to come from multiple individuals, multiple peaks can be observed in a single locus. This is called a DNA mixture, where there are two or more contributors to a DNA profile (Bieber et al., 2016). Interpreting a mixture DNA profile remains a challenging task to forensic scientists to this day. One of the reasons is that even after testing, the number or ratio of contributors can be difficult to determine. Separating different genotypes for database entry

is not always possible (Van Oorschot et al., 2010). But even if a DNA mixture cannot be separated into distinct genotypes, comparisons to known reference samples and statistical evaluations using probabilistic genotyping software can still generate probative associations (Coble & Bright 2019).

Despite the known challenges of DNA profile analysis and interpretation, advanced techniques have enabled forensic laboratories to produce accurate data from very limited amounts of DNA. Trace DNA from high volume crimes, such as property crimes and home invasions, now comprises the bulk of evidence submission to forensic laboratories (Mapes, et al., 2016).

DNA Transfer

The increased sensitivity of DNA profiling systems has greatly increased the ability to generate DNA profiles from touched objects. Even with very small amounts of DNA, also called trace DNA, technological advancements mean trace DNA collected from a crime scene or a victim's body can be connected to a person of interest. The first description of using DNA to connect an attacker to a victim's body is from Wiegand and Kleiber (1997). The authors were able to successfully recover DNA foreign to the "victim" from the neck area in a series of simulated strangulations and even a real case (Wiegand and Kleiber, 1997). One question would be how long an attacker's DNA would still be recoverable. A study conducted by Bowman et al. (2018) investigated active transfer and the persistence of offender DNA on the victim's skin over three time-points—0h, 3h, and 24h post-assault. The study involved a series of mock assaults through direct skin-to-skin contact on the victim's wrist and upper arm using medium and heavy pressure. The authors demonstrated a high transfer rate of offender DNA where heavy pressure was applied. Offender DNA was recovered in most samples 0h post-assault. Offender DNA persisted through

the 3h and 24h time following an assault with 25% and 12% percent of the samples, respectively, yielding enough of the offender genotype for probative statistical weight calculations.

Trace DNA evidence can be deposited by active or passive transfer. Active transfer is defined as the transfer of DNA from an individual after direct contact. Active transfer of DNA can also occur without any contact by touching but as a result of an activity within the vicinity of an item, such as speaking, coughing or sneezing (Meakin and Jamieson, 2013). Passive transfer results in the transfer of DNA to an item through an intermediary surface. An example is a person touching a knife that has been previously touched by someone else. This same person then touches his shirt. Both of their DNA will be discovered when the shirt is swabbed and analyzed even though the other person never touched the shirt (Meakin and Jamieson, 2013).

In DNA casework, it is a challenging task for forensic investigators to establish whether DNA found at the crime scene belongs to an individual who was actually present at the scene of the crime, or if the DNA could have been preexisting on the item, or was deposited during the incident but through passive transfer. The other two factors to be considered are how long probative DNA remains on a surface (persistence) and how efficiently a forensic laboratory recovers the DNA. Research into all four factors, transfer, persistence, prevalence, and recovery (TPPR) is needed to inform trace DNA interpretation (van Oorschot et al. 2019).

Shedding Propensity

The amount of DNA deposited on an object, or a skin surface is highly dependent on the shedding propensity of an individual. Lowe et al. (2002) define shedding propensity as an individual's ability to leave a detectable amount of DNA on an object or on a skin surface through direct contact. While previous studies support that some individuals shed more than others, the factors

that affect this are still undetermined. Some of the factors that have been investigated by scientists are gender, handedness, and age.

In a study conducted by Kanokwongnuwut et al. (2018), male individuals were determined to be better shedders than female individuals, and time since hand washing played a role in this observation. Four sets of donor thumbprints on glass were collected and tested at different time intervals after handwashing. The key observations were the variability among donors and the increase in the amount of DNA recovered over time. Giacomoni et al. (2009) studied a list of gender-linked differences between males and females. Sweat rate and sebum production are among the things that were examined. The authors hypothesized that men generally deposit more DNA than women because of their increased sebaceous and sweat production where DNA can accumulate.

Otten et al. (2019) investigated shedder status as a possible factor influencing the extent of passive transfer using working gloves as a vector. The shedder status of 40 participants was determined and participants with different shedding propensities were paired and tasked to handle objects using gloves at two time-points in order to determine the extent of passive transfer. An increased transfer of DNA was observed among the good shedders as demonstrated by the amount of DNA recovered from outside and inside the gloves. The results indicate that the shedder status of an individual affects the extent of DNA transfer.

In a study conducted by Fonneløp et al. (2017), the scientists demonstrated an indirect relationship between the shedding propensity of an individual and the presence of non-self alleles. The authors examined the background DNA in samples from T-shirts worn in an area with frequent human traffic and detected multiple contributors. High shedders were observed to have fewer

contributors while low shedders were observed to have a higher count of non-self alleles on their DNA profile. Non-self DNA transferred to a high shedder may be masked by the person's own DNA (Fonneløp et al., 2017). The same observation was made by Lacerenza et al. (2016) in a comparison study in shedding propensities between men and women using palmar samples. The scientists determined that men have a higher shedding propensity than women and that there was an overall low prevalence of non-self alleles found in the palmar samples collected from the male participants.

Poetsch et al. (2013) investigated the influence of the age of an individual on the amount and quality of DNA left at a crime scene. Handprints from 213 individuals ranging from one to 89 years old were analyzed. Full DNA profiles were observed in 75% of all children up to 10 years old, 9% of adolescents from 11 to 20 years old, 25% of adults from 21 to 60 years old and 8% of elderly people who are more than 60 years old. Only partial profiles were observed from individuals who are older than 80 years. The study revealed that the quantity and quality of DNA recovered can be affected by the age of an individual.

Phipps and Petricevic (2007) looked at the influence of hand dominance on shedder status. The authors discovered that there is a higher STR yield from the dominant hand than the non-dominant hand. However, research by Goray et al. (2016) and Lacerenza et al. (2016) suggest that hand dominance has no effect on the DNA profile quality. Because we don't yet fully understand the role handedness plays in shedding propensity, it warrants further study.

Prevalence of non-self DNA

It is safe to assume that personal and shared items will have some DNA accumulated on them over time from regular use. This accumulated DNA is called background DNA and its ubiquity or

prevalence is a factor in trace DNA interpretation. If items that contain background DNA are used by someone else, mixed profiles of self and non-self DNA can be expected. Determining which DNA was already deposited on an object and which DNA was added later, possibly during a criminal act, remains a challenge to forensic scientists.

Non-self DNA is prevalent on hands. In a study conducted by Samie et al. (2016). The authors found foreign alleles in 97% of samples directly deposited by hands on DNA-free plastic knife handles. It was observed that the donor was the major contributor in 83% of samples. Additionally, 23% of the profiles were identified as mixtures where the major and minor contributors could not be determined, and 5% were not of sufficient quality for analysis. Goray et al. (2016) investigated the presence of self and non-self DNA in multiple handprints deposited by the same individuals over time. Handprints from ten individuals were studied in order to determine the consistency of DNA deposited and the degree of variability among individuals. A total of 240 handprints were collected three times during the day on four different days spaced over several weeks. Generally, non-self DNA was observed along with self DNA and was found to be the minor component to most mixture samples. Very rare instances where non-self DNA was found to be the major component were from individuals who were deemed as poor shedders.

A study conducted by Graham and Rutty (2008) investigated the presence of background DNA on adult necks as a result of adventitious transfer. The neck surface of 24 adult volunteers was swabbed and the authors showed that 23% of the skin swabs contained at least a few non-donor alleles with 5% of the samples showing six or more non-donor alleles.

Moreover, a study by Bowman et al. (2018) included controls from adjacent skin areas that had not been touched. The number of non-self alleles in these control swabs ranged from 0-63.

Out of the 108 samples analyzed, non-self alleles were present in 104 samples. In addition, at least ten non-self alleles were detected in 44% of the samples (Bowman et al., 2018). This occurrence of foreign DNA without active contact can impact the probative value of DNA results in a strangulation case.

Additionally, in a time-course study conducted by Fantinato et al. (2022) on the detection of non-self alleles on the neck, the authors investigated 20 adult volunteers who co-habited with other individuals such as a partner or children. Samples were taken immediately after showering, 7-8 hours post-shower, and 24 hours post-shower. Overall, more than half (62%) of the samples were determined to be DNA mixtures. A significant increase in the number of mixtures and the non-self allele count was detected over time. In addition to the non-self alleles from known partners, 9% of the samples showed some alleles from unknown contributors.

These findings are relevant for cases of sexual assault and manual strangulation, where information on the prevalence of non-self DNA on parts of the body other than the hands is important, especially if the person of interest is known to the victim. These studies show that the presence of non-self alleles can be due to innocent everyday interactions.

Research Goals

This study aimed to determine the prevalence of non-self DNA on three locations of sebaceous skin, particularly on the upper arm, the nape of the neck, and the neck area below the ear. Fifteen male participants and 13 female participants were invited to donate skin samples on three different occasions, one week apart. On each occasion, time since last shower was recorded.

The first goal was to determine the effect of the skin area, gender, and time since last shower on the presence of non-self DNA. Moreover, if such non-self DNA is detected, the second

goal was to determine whether a pattern could be detected over the three different collection occasions and whether the non-self DNA could be attributed to a common donor.

Materials and Methods

Sample Collection

Human subject research approval included informed consent from each volunteer and was obtained prior to beginning this research (CUNY IRB project #2018-0099). The donors ranged from 19 to 75 years old (the average was 28 years) and the samples were collected from 15 male donors and 13 female donors. A questionnaire was completed by each participant that indicated the donor's gender, age, handedness, and time since last handwash and shower (see Appendix 1). Samples were collected from various areas of the body, including the nape, the upper arm, and the area just below the ear (see figure 1) on three different occasions, one week apart, using D-Square adhesive tape disks (Cuderm, Dallas, TX). The D-Square adhesive tape disks and all other consumables, which were not certified forensic grade, were UV irradiated prior to sample collection to make sure that they were DNA-free. Tape disk samples were stored in 2 mL reaction tubes and kept in the freezer at -20^{o}C prior to extraction. Buccal swabs were acquired from each volunteer to serve as a reference sample. See Trapani (2021) and Chen (2021) for other samples collected and analyzed from the same set of donors.

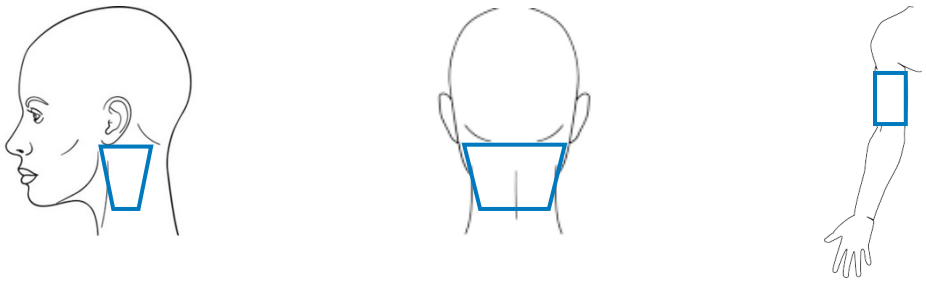

Figure 1. Sampling areas (from left to right): neck area below the ear, nape area of the neck and upper arm area

DNA Extraction and STR Profiling

The QIAamp DNA investigator kit on the QiaCube extraction robot was used for sample DNA extraction (both Qiagen, Germantown, MD) and the QuantiFiler Trio kit on the QuantStudio 5 real time PCR instrument (both Thermo Fisher Scientific, Framingham, MA) was used for DNA quantification. The robotic extraction required an additional step to remove the tape adhesive. After the QIAamp DNA investigator lysis step, the tape was removed, the samples frozen and then defrosted. After defrosting, while still cool the samples were spun in a centrifuge to pellet the adhesive and the supernatant was moved to a new tube (Chen 2021). All samples were amplified using the GlobalFiler STR Human Identification kit with a target of 500 pg of DNA, or for samples with less than 34 pg/µL the maximum allowed volume of 15 µL on a Veriti Thermal Cycler and genotyped on a 3500 Genetic Analyzer (all Thermo Fisher Scientific). 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. For more detail on DNA extraction and genotyping see Trapani (2021).

STR Profile Analysis

Before the data were analyzed, positive and negative controls as well as allelic ladders were checked for each batch of samples. Additionally, peaks that appeared to be artifacts like spikes or pull-ups were identified and unlabeled in all electropherograms. Next, a comparison between the sample STR profiles and the donor reference samples was performed to evaluate the quality of the profile and to identify mixture status. Based on the expected number of alleles present, five categories were established to classify the quality of the DNA profile: full profile (F), high partial profile (HP), low partial profile (LP), negative (NEG), and not suitable for analysis (NS). A “full

profile" classification was assigned to profiles when all expected alleles were detected. DNA profiles containing 13 to 21 complete loci were classified as "high partial profiles", while those that only contained one to 12 complete loci were classified as "low partial profiles." A "negative profile" classification was assigned when no complete heterozygote locus was detected. Finally, profiles "not suitable for analysis" was assigned to negative profiles with at least one non-self heterozygote locus present. Table 1 shows a summary of the DNA profile quality classifications.

Table 1. A summary of DNA Profile Classifications, Codes and Definitions

Classification	**Code**	**Definition**
Full profile	**F**	All expected donor alleles present.
High profile	**HP**	13 to 21 complete donor loci are present.
Low profile	**LP**	One to 12 complete donor loci are present
Negative profile	**NEG**	No complete heterozygote locus from known or non-self donor is present
Not suitable	**NS**	No complete heterozygote locus from known donor, but at least one non-self heterozygote locus is present.

The mixture status of a DNA profile was further classified into five categories based on the presence, number, and relative peak height of non-self alleles: 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). A "single source" classification was assigned when there were only one or two non-self alleles present, excluding stutter peaks. A "background mixture" classification was assigned to high and full partial profiles with high RFUs when there were at least three non-self alleles present where peak heights were less than 40% that

of the donor peaks. A “background mixture” classification was assigned to partial profiles with low RFUs when there were three to five non-self alleles present where peak heights were equal to or less than that of the donor peaks. A “mixture with donor as the major component” was assigned to full and high partial profiles when at least three non-self alleles were detected where peak heights were greater than the corresponding stutter peaks. A “mixture with donor as the major component” was assigned to low partial profiles when there are at least six non-self alleles detected where peak heights were equal to or less than that of the donor alleles. A “mixture with donor as the minor component” classification was assigned to full and high partial profiles when both donor heterozygote alleles had lower peak heights than the non-self alleles at two loci or more. A “mixture with donor as the minor component” classification was assigned to low partial profiles when non-self alleles were present in at least three loci where peak heights were equal to that of the donor alleles and there was at least one donor allele missing. Samples previously deemed not suitable for analysis because of lack on donor alleles were all characterized as MXDM. All negative profiles were deemed not suitable for analysis (NS). Table 2 summarizes the STR profile mixture status classifications, codes, and definitions.

Table 2. An outline of STR Profile Mixture Status Classifications, Codes, and Definitions

Classification	Code	Definition
Single source	**N**	2 or less non-self alleles are present
Background Mixture	**MXB**	At least 3 non-self alleles are present where most loci only show donor alleles
		Full and high partial profiles with high RFUs: 1 or 2 non-self peaks are higher than the stutter peaks where all non-self peak heights are less than 40% that of the donor alleles
		Low and high partial profiles with RFUs less than 1000: 3 to 5 non-self peaks are present where peak heights are equal to or lower than that of the donor alleles
Mixture; Donor as Major Contributor	**MX**	Full and high partial profiles: 3 or more non-self alleles are present where peak heights are greater than that of the stutter peaks at the same locus
		Low partial profiles: 6 or more non-self alleles are present where peak heights are equal to or lower than that of the donor alleles
Mixture; Donor as Minor Contributor	**MXDM**	Full and high partial profiles: at least 2 loci are present where both donor heterozygote alleles have lower heights than the non-self alleles.
		Low partial profiles: 6 or more non-self alleles are present with at least 3 loci where non-self alleles are equal height to the donor alleles and 1 or more known donor alleles are missing
Not Applicable	**NA**	Negative profile

For each donor, the samples characterized as mixtures were analyzed for self and non-self alleles. Self and non-self alleles were counted and tabulated for each skin location. Peaks in the -4bp stutter position were excluded from evaluation. Furthermore, samples showing non-self alleles at more

than one collection occasions were screened for repeating non-self alleles and repeating non-self genotypes. Differences in the presence of non-self alleles were evaluated for the effect of time since last shower. Differences in the data was also evaluated to determine whether gender had an effect on the detection of non-self alleles, if present.

Analysis of Variance

Analysis of Variance (ANOVA) using Microsoft Excel was performed in order to determine the effect of gender, skin location and time since last shower in the detection of non-self DNA.

Results

Participants and samples overview

Thirty volunteers that ranged from 19 to 75 years old participated in this study. Samples were collected from three locations, namely the nape area of the neck, the upper arm and the area just below the ear, on three different occasions for three weeks. Time since last shower was recorded for each collection. A total of 270 samples were collected and analyzed. Samples from two volunteers were later on excluded from the dataset since no reference sample DNA profile could be obtained. Overall, 252 samples were analyzed for the study.

Profile Quality

DNA profile quality was evaluated for each sample based on the number of expected alleles detected in the profile. Samples were then sorted based on the location: the nape area, the upper arm area, and the neck area just below the ear. DNA samples collected from the neck area below the ear generated the highest percentages of full DNA profiles and none of the samples were deemed either negative or not suitable for analysis. Over 50% of the samples collected from the nape area were full profiles and approximately 3% were deemed negative. The majority of samples collected from the upper arm area were low partial profiles which are consistent with the lower DNA concentrations obtained for this location (Chen 2021). Overall, approximately 51% of the samples resulted in a full DNA profile, 24% of the samples resulted in a high DNA profile, and approximately 22% of the samples resulted in a low DNA profile; while 2% of the samples were classified as negative DNA profile, and 1% was deemed not suitable for analysis. Table 3 summarizes the profile quality scores based on sample type.

Table 3. A summary of profile qualities based on sample type

Sample Type	Profile Quality				
	Full	**High Partial**	**Low Partial**	**Negative**	**Not Suitable**
Arm	12 (14.3%)	30 (35.7%)	37 (44.0%)	2 (2.4%)	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%)
Total	127 (50.6%)	61 (24.3%)	55 (21.9%)	5 (2.0%)	3 (1.2%)

Mixture Status

Mixture status was evaluated for sample type based on the presence and number of non-self alleles. Non-self alleles are defined as alleles that did not come from the sample donor. Out of the 28 volunteers, at least one of the nine samples showed a mixture DNA profile for 22 volunteers. A single mixture DNA profile from only one collection occasion was obtained from eight volunteers and mixture DNA profiles for at least two samples were observed in 14 volunteers. Overall, 22% of the 252 samples were classified as mixtures profiles while approximately 76% came from a single source and the remaining 2% had been negative and did not have a mixture designation (not applicable; NA). Out of the 52 mixture DNA profiles, the donor was the major component for the majority of mixtures, in 23 or 41% of the mixtures the non-self alleles were minor background peaks (MXB), while 20 or 36% were classified as a mixture (MX) with several high non-self allele peaks. Mixtures, where the donor is the minor contributor (MXDM), constituted 23% of the 52 mixture DNA profiles and 5.2% of the overall samples. Table 4 shows a complete summary of the different mixture profile classifications based on the three different skin locations.

Table 4. A summary of mixture status based on sample type

Sample Type	Mixture Status				
	N	**MXB**	**MX**	**MXDM**	**NA**
Arm	49 (58.3%)	12 (14.3%)	12 (14.0%)	9 (10.7%)	2 (2.4%)
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%)
Total	190 (75.7%)	23 (9.2%)	20 (8.0%)	13 (5.2%)	5 (2.0%)

Effect of skin location

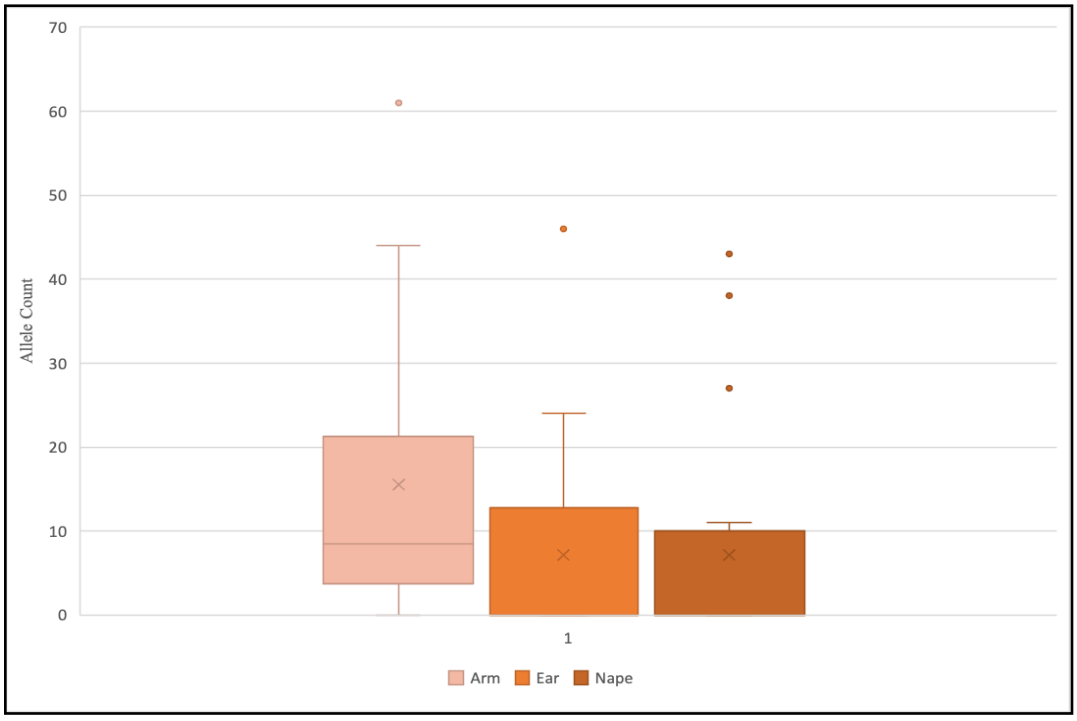

Figure 2. A box and whisker plot showing the non-self alleles for the different skin locations for each donor (n = 22 each). Horizontal lines show the median, x shows the mean, the box size shows the interquartile range, and the whiskers show the top 25% range excluding outliers.

The samples were analyzed for the three different skin locations collected on three different occasions. The non-self allele count ranged from 0 to 61 (mean = 10) and the averages are 16, 7 and 7 for the upper arm area, the neck area below the ear and the nape area of the neck, respectively. The six donors with only single source samples were not included in this analysis. As can be seen in figure 2, the arm samples had the highest average and widest range of number of foreign alleles. Analysis of Variance (ANOVA) was performed in order to determine the effect of skin location on the detection of non-self alleles. The null hypothesis assumes that skin location does not significantly affect the detection of non-self alleles whereas the alternative hypothesis assumes that the detection of non-self alleles is significantly affected by the location of the skin. The calculated f-ratio values for the three collections were as follows: 0.1, 1.7 and 2.5. (p-value = 0.07). This shows that skin location does not significantly affect the detection of non-self alleles.

Effect of gender

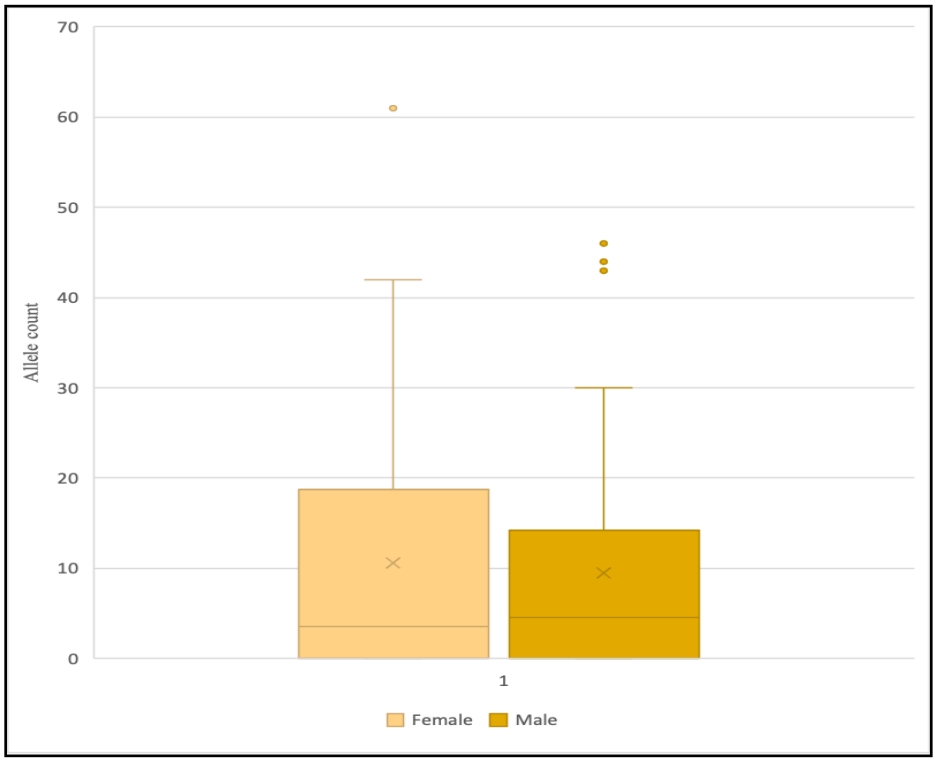

Figure 3. A box and whisker plot showing the non-self alleles for the different skin location based on the participants' gender (female n = 10, male n = 12). Horizontal lines show the median, x shows the mean, the box size shows the interquartile range, and the whiskers show the top 25% range excluding outliers.

The detection of non-self alleles based on gender across all skin locations was evaluated by sorting the results by gender. Suitable mixture samples for every skin location were obtained from 10 female and 12 male volunteers. As can be seen in figure 3, the average number of non-self alleles detected is similar between females (n = 10) and males (n = 12); 11 and 9, respectively. The calculated f-ratio value using ANOVA was 0.12 (p-value = 0.74). This reveals that gender has no significant effect on the detection of non-self alleles.

The effect of gender on the detection of non-self alleles was also determined separately for each skin location (see figure 4). The average number of non-self alleles was determined for each skin location and the effect of gender was analyzed using ANOVA. The calculated f-ratio values were 0.01, 0.04 and 0.09 for the arm, the ear and the nape area, respectively (p-values were 0.92, 0.83, and 0.76, respectively). All three values show that gender has no significant effect on the detection of non-self alleles on the aforementioned skin locations.

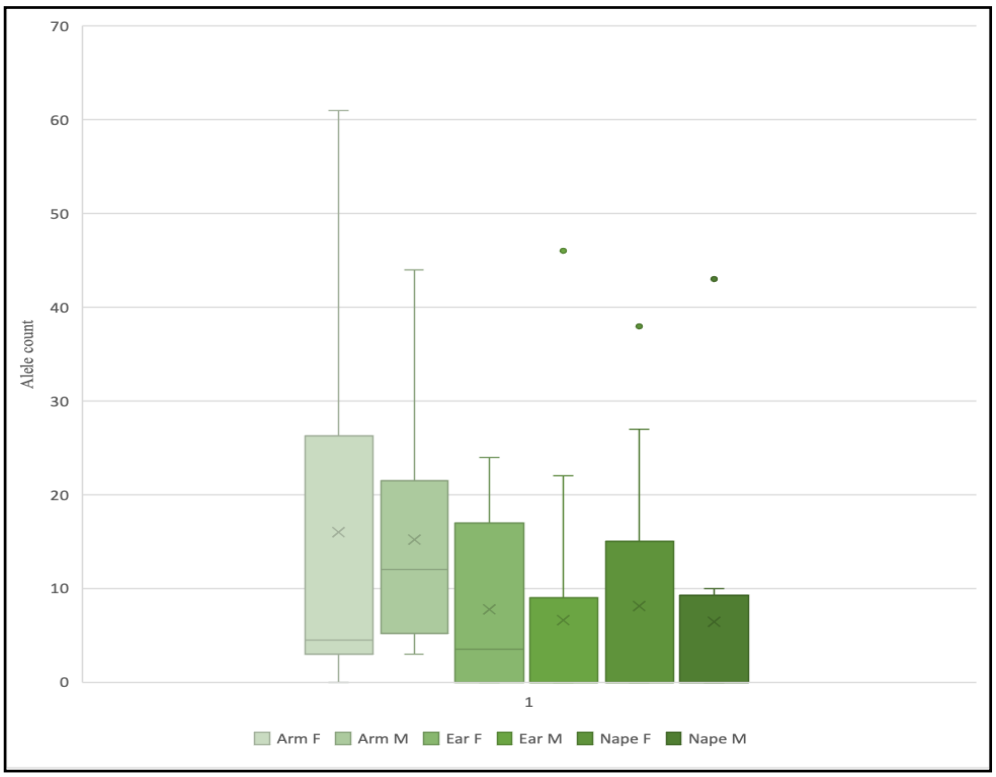

Figure 4. A box and whisker plot showing the non-self alleles based on gender for every skin location (female n = 10, male n = 12). The calculated non-self allele averages are as follows: 16, 15, 8, 7, 8 and 6. Horizontal lines show the median, x shows the mean, the box size shows the interquartile range, and the whiskers show the top 25% range excluding outliers.

Effect of shower

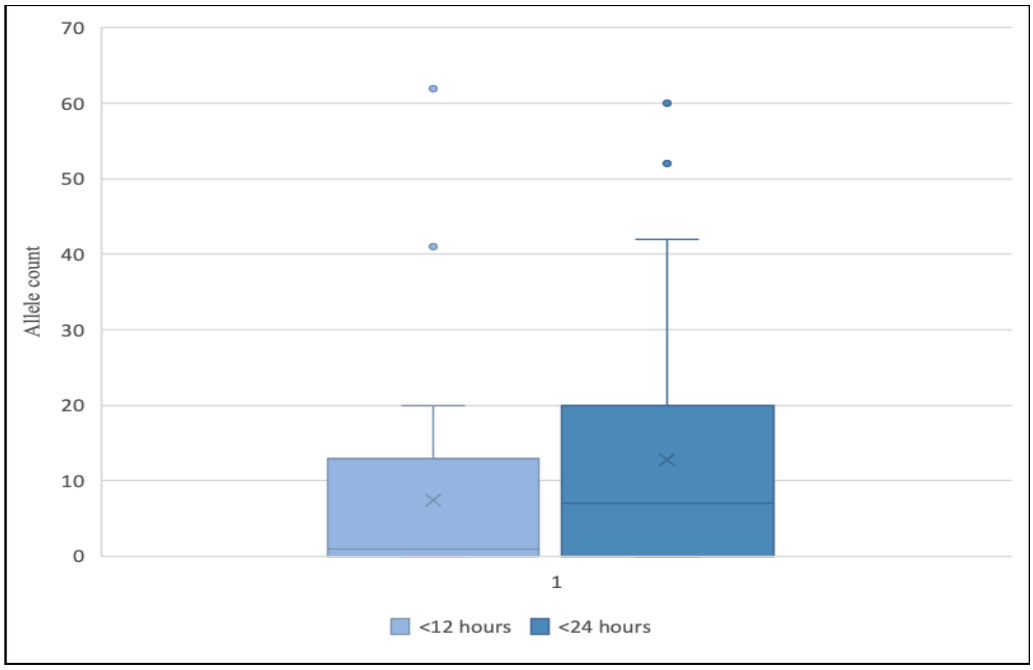

Figure 5. A box and whisker plot showing the non-self alleles based on different shower times (<12 hours n = 35, <24 hours = 28). Horizontal lines show the median, x shows the mean, the box size shows the interquartile range, and the whiskers show the top 25% range excluding outliers.

Skin samples were collected on three different occasions. For every occasion, shower times were classified into three groups: <12 hours, <24 hours where shower times were equal to or after 12 hours and less than or equal to 24 hours, and >24 hours. During the first occasion, 12 <12 hours were recorded, 9 <24 hours, and 1 >24 hours. During the second occasion, 11 <12 hours were recorded, 10 <24 hours, and 1 >24 hours. During the third occasion, 12 <12 hours were recorded, 9 <24 hours, and 1 >24 hours. The calculated non-self allele averages for the two shower times <12 and <24 are 7 and 13, respectively (see figure 5). Since only one sample was recorded for >24 hours for every occasion, this category was excluded from the analysis. ANOVA was performed to determine whether there is a significant difference in the detection of non-self alleles based on

the different shower times. At $p < 0.05$, the calculated f-ratio value was 2.02 (p-value = 0.16). This shows that time since last shower does not significantly affect the detection of non-self alleles.

Determining a pattern over the three collections

The presence of an allele pattern was determined for all skin locations where non-self alleles were detected for more than one collection occasion. Thirty-two samples were identified as mixture DNA profiles where non-self alleles were present for at least two collection occasions. The majority, 24 of these samples were from the upper arm area, and only four samples each were collected from the neck area just below the ear and the nape area. Out of the 32 samples, 20 samples had repeating non-self alleles on at least one locus position. Overall, 48 loci positions were identified to have at least one repeating non-self allele. Twenty percent of those alleles were observed to repeat three times while 80% were observed to repeat twice. Many of these repeating alleles were peaks in stutter positions or were common alleles and thus cannot necessarily be attributed to a repeating contributor.

Therefore, the mixture DNA profiles across all datasets were closely examined for the presence of a repeating non-self genotype. Alleles in a -4bp stutter position were excluded from this evaluation. Samples were deemed to have repeating genotypes if more than three loci have a common repeating allele, and at least one locus has a repeating non-self heterozygote genotype. Samples collected from the upper arm area presented the highest number of mixtures where repeating non-self alleles genotypes were present according to this definition (see appendix 2 for a list of all samples with mixture status). Overall, the DNA profiles of two volunteers (donors 38 and 43) suggest a two-person mixture with a repeating contributor. A third donor (donor 46) displayed two at least three contributor mixtures where a common contributor could not be

excluded. For female donor 38 the repeating genotype was from a male individual and present at more than one location. For donor 43 another female volunteer, the repeating genotype was also from a male individual and found on the area below the ear in weeks 1 and 2. Without information on the volunteers living arrangements and no comparison samples from domestic partners or family members, no further conclusions can be drawn. In summary, most of the mixtures had individual alleles detected more than once but only a few were found to have a pattern of non-self genotypes repeating over the three-week period. This was only observed for three out of 22 different volunteers with mixtures detected.

Discussion

Profile Quality

A novel approach of skin sampling using tape lifts was used in the study. By sampling directly from the skin using tape lifts, an overall increase in the DNA yield can be expected. The method worked well and most samples contained sufficient DNA for successful DNA profiling. Only 2% of the samples generated a negative profile and only 1% was deemed not suitable for analysis. One hundred sixteen DNA profiles were classified as partial profiles (61 high partial and 55 low partial) and 127 DNA profiles were classified as full profiles. Profile quality was overall consistent with DNA concentrations (Chen, 2021) and the variations in the quality of DNA profiles correlate to the range of DNA amounts. Variation in the amount of DNA individuals have on their finger and palm surface is well documented and known as shedding propensity (Lowe et al., 2002; Oleiwi et al., 2015). This study shows a similar variation on sebaceous skin. A study by Chen (2021) reveals that there is no correlation between the DNA amounts on an individual's palmar skin and the three sebaceous areas tested here. Fantinato et al. (2020) found similar inter-person variation in DNA concentrations for the samples they took from three areas from the front of the neck.

Across the entire dataset, male participants generated higher quality profiles than the female participants (data not shown). This supports the observation previously shown by Kanokwongnuwut et al. (2018) and Giacomoni et al. (2009). The scientists suggest that gender plays a key role in the shedding propensity of an individual. In this research, there were more male participants than female. The DNA yield variations can be attributed to the biological differences between genders since males produce more sweat and oil than females which may cause an increased accumulation of DNA on the sebaceous skin surface (Giacomoni et al., 2009). Since the

quantity of DNA deposited usually corresponds to the quality of the DNA profile generated, it was expected that the male participants will yield more high-quality profiles. This shows that certain individuals exhibit a higher fluctuation in the self allele count over the three-week collection period.

Mixture Status

Twenty-two percent of the samples that were analyzed generated a mixture DNA profile. Out of the 252 samples analyzed, non-self alleles were recovered in 56 samples. This confirms the presence of non-self alleles at levels detectable with standard forensic methods. There was an overall fluctuation in the non-self allele count over time where the highest counts were detected on the second occasion. The highest number of non-self alleles was obtained from the samples collected from the upper arm area while the lowest overall non-self allele count was obtained from the area below the ear for the three collection occasions. There are two factors that may have caused the donor to donor variability on the non-self allele count—the hygiene practices of the volunteers and their daily activities such as commute, social interaction, and cohabitation. The project questionnaire did not account for the daily practices and the living situations of the volunteers. Without these pieces of information, it will be difficult to determine a correlation between, e.g. time since last wash and the mixture status of their DNA profiles. A similar result can be observed in the investigation conducted by Graham et al. (2014), where the daily practices of the volunteers did not correlate to the quality of DNA deposited. The study involved little children and their daily practices such as feeding and washing were evaluated. The presence of non-self alleles was consistently low across the entire dataset. In a recent investigation involving cohabitation, Fantinato et al. (2022) demonstrated an increase of the non-self allele count over time. They found a higher percentage of mixtures (62%) and found an effect of living arrangement

and relationship status on mixture detection. A small amount of DNA was from unknown sources which the authors attributed to the possible transfer of DNA from the workplace. The lack of consistency on the presence of non-self alleles across the three collection occasions suggests that several factors including the ones that have not been accounted for in this project may affect the quantity and quality of DNA deposited.

Effect of skin location

Variations in the STR profile qualities due to the anatomical origin of the DNA have been observed by Oleiwi et al. (2015) and Quinones & Daniel (2012). These variations can be attributed to the differences in oil and sweat production in different anatomical locations (Zoppis et al., 2014). High quality profiles were obtained from the samples collected from the neck area below the ear while low quality profiles were obtained from the samples collected from the upper arm. Most non-self alleles were detected from the upper arm area while there were only very few non-self alleles detected from the neck below the ear. It was shown that generally, skin location does not have a significant effect on the detection of non-self alleles. However, the high prevalence of non-self alleles on the upper arm area may be due to its unique anatomical position where there is an increased probability of contact with other surfaces or skin surfaces. Most of the samples were collected in the months of June, July, and August when the donors would have worn short-sleeve shirts. Clothing and hairstyle were not documented and cannot be used to evaluate these findings. Contact may have occurred through social interactions and on their way to commute. The project questionnaire did not take into account the relationship status and daily practices of the participants prior to testing therefore the variations of the non-self allele count based on skin location will require further investigation.

Effect of gender

Previous research by Fonneløp et al., 2017, Goray et al., 2016, and Gosch & Courts, 2019 demonstrated that the quantity and the quality of DNA from hands are significantly different between males and females. In a study by Giacomoni et al. (2009), the authors listed gender-linked differences which included sweat and sebum production. Men were discovered to produce more sweat and oil than women where DNA can accumulate. Additionally, in a study conducted by Fonneløp et al. (2017), the scientists demonstrated an indirect relationship between the shedding propensity of an individual and the presence of non-self alleles. In this study, more high-quality profiles were obtained from male participants while there were more low-quality profiles observed from the samples that were collected from the female participants. It can then be expected that there are fewer non-self alleles from the samples that were collected from the male participants than from the female. One of the reasons is that an increased self allele count could mask the detection of non-self alleles (Fonneløp et al., 2017). Across the three collection occasions, there was an overall increase in the non-self allele count from the samples that were collected from the female participants (data not shown). The same pattern was observed in a study conducted by Lacerenza et al. (2016) where there was a lower prevalence of mixtures from the male participants. However, in our study, the difference in the detection of non-self alleles between males and females was not statistically significant. This could be due to the collection method of the study using tape lifts. This novel approach collects DNA from the skin surface at a uniform diameter therefore the quantity and the quality of DNA are unaffected by variations in the sampling area. Studies conducted by swabbing the hands or the fingerprints may be affected by the difference in size of the resulting sampling areas for males and females.

Effect of time since last shower

To test whether time since last shower would affect non-self allele count, the participants were asked to record it prior to testing. Time since last shower was classified into three: <12 hours, <24 hours, and >24 hours. Samples across all datasets were equally distributed between <12 hours and <24 hours, with only one sample each recorded at >24 hours on each collection occasion. Most volunteers kept their shower times consistent over the three collection occasions. Only four volunteers changed their time since last shower from <12 hours to <24 hours or vice versa however, there was no pattern in the allele count over the three collection occasions. It was determined that in this study, time since last shower did not have a statistically significant effect on the detection of non-self alleles. The highest non-self allele count was observed on the second collection occasion however, there was no general pattern on the non-self allele count over the three collection occasions Additionally, most of the high non-self allele counts can be traced back to the same donor. This donor, where possible repeating genotypes were detected, kept the time since last shower at <24 hours over the three collection occasions. One factor that may have caused this increase in the non-self allele count could be the living arrangement. This same donor may have co-habited with a partner or other family members therefore, more non-self alleles were detected during analysis. This could also mean that the other participants may be living alone or with roommates with less social interactions as indicated by Fantinato et al. (2022).

Determining a pattern for repeating non-self genotypes

In order to determine an allele pattern, each DNA profile was closely examined for a non-self genotype that appeared in at least two collection occasions. The presence of these repeating non-self genotypes in more than one collection occasion suggests that it could come from the same individual. This further suggests that the participants may have cohabited with a partner or other

family members. Although cohabitation was not accounted for in the project questionnaire, a similar finding on the persistence of non-self DNA was observed by Fantinato et al. (2022). The authors investigated the effect of cohabitation on STR yield in a time-course study. Their study revealed that the donors' partners' DNA accumulated over time in at least two collection intervals. The authors attributed the persistence of non-self DNA to a close relationship and physical contact between persons in the same household. Apart from direct possible transfer from close family members, an indirect transfer from communal areas can also play a role in the persistence of non-self DNA, but it's less likely to cause a repeating pattern over several days.

Conclusions

Non-self DNA can be present on the exposed areas of an individual's skin as a result of adventitious DNA transfer during normal daily activities. Most of the samples in this study were single source but non-self DNA was detected on all three test locations (upper arm, the neck area below the ear and the nape). Skin location, gender and time since last shower did not have a significant effect on the detection of non-self DNA. However, the daily activities and the living arrangement of the participants should be accounted for in order to make a more meaningful deduction. In addition, even though the difference was not significant, more non-self alleles were detected on the upper arm area. This can be due to the anatomical position of the upper arm that, especially in the summer, is more exposed than the nape and the area below the ear, and increased contact with other individuals and surfaces is more likely. Repeating non-self genotypes were only observed for three donors. This suggests that this DNA could have come from the same contributor as a result of a close living arrangement and physical contact between persons in the same household. When non-self DNA is collected from the exposed areas of a victim such as the upper arm area and the neck area of the neck, a careful evaluation is necessary since DNA from unknown contributors can be transferred to the exposed areas of an individual during public transport and social interactions. Seeing DNA mixtures is less likely for the nape area of the neck. The result of this study can aid in the evaluation of DNA casework results if the victim's upper arm area, the nape area of the neck, or the neck area below the ear had been tested.

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Appendix 1

For Office Use Only:

Sample code: _____

Date: _____

Study Title:
Population distribution and factors affecting individual DNA shedding Propensity

Disclaimer:
Please note that you are not obliged to answer all questions. All answers are confidential and will be stored under the code number.

Questions:

1. What is your gender?

_____

2. Which year were you born?

_____

Please check the box or circle the correct answer:

3. What is your dominant hand?

Right Left

4. When was the last time you took a shower?

□ This morning (<12h ago) □ Last night (<24h ago) □ Yesterday morning (>24h ago)

5. When was the last time you washed your hands?

□ Less than 1 hour ago □ More than 1 hour ago

□ More than 3 hours ago □ Do not remember

Appendix 2

Sample mixture status for all collections

Donor		EAR w1	EAR w2	EAR w3	NAPE w1	NAPE w2	NAPE w3	ARM w1	ARM w2	ARM w2
31	S	N	N	N	N	N	N	N	N	N
32	S	N	N	N	N	N	N	N	N	N
35	SM	N	N	N	N	N	N	N	N	MXB
36	MM	N	MX	N	N	MXB	N	N	MXB	MX
37	MM	MXB	N	N	N	N	N	N	MXB	N
38	REP	N	MX	N	N	MX	MXB	MX	MXDM	MX
39	SM	N	N	N	N	N	N	N	MXB	N
40	MM	N	N	N	MX	N	N	MXDM	N	N
41	MM	N	N	N	N	MXDM	N	MXB	MX	MX
42	S	N	N	N	N	N	N	N	N	N
43	REP	MX	MXDM	N	N	MXB	N	NA	MXDM	N
44	SM	N	N	N	N	N	N	MXB	N	N
45	MM	N	N	N	N	MXB	NA	MXB	MXDM	MXDM
46	REP	N	N	N	MXB	MXB	MXB	N	MXDM	N
47	SM	N	N	N	NA	N	N	MX	N	N
48	MM	N	MXB	MX	N	N	N	MXB	MX	MX
49	SM	N	MXDM	N	N	N	N	N	N	N
50	S	N	N	N	N	N	N	N	N	N
51	MM	N	N	N	MX	NA	NA	N	MX	N
52	S	N	N	N	N	N	N	N	N	N
53	S	N	N	N	N	N	N	N	N	N
54	MM	MX	N	N	MXDM	N	N	MXB	N	MXB
55	SM	N	N	N	N	N	N	N	MX	NA
56	MM	N	N	MXB	N	N	N	N	MX	MX
57	SM	N	N	N	N	N	N	N	N	MXB
58	MM	N	N	N	MXB	N	N	N	N	MXB
59	MM	N	N	N	N	N	N	N	MXDM	MXDM
60	SM	N	N	N	N	N	N	N	MXDM	N

Legend

S	only single source samples
SM	only one sample with non-self alleles
MM	multiple mixtures, no repeating non-self contributor
REP	mixtures with repeating non-self contributor

N	single source result
MXB	mixture with low background peaks
MX	mixture with donor as major contributor
MXDM	mixture with donor as minor contributor