A COMPARISON OF TRACE DNA COLLECTION TECHNIQUES FROM WORN AND HANDLED CLOTHING

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ABSTRACT

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The sensitivity of current forensic techniques makes it feasible to obtain a DNA profile from very few cells, including those collected from items that have come into contact with one or more individuals. Therefore, it is important to collect as many cells, and hence as much DNA, from the item as possible in order to increase the chances of making an identification. Limited comparative research has been conducted on the common techniques used in forensic laboratories to collect DNA from fabric evidence. The goals of this study were to determine which method(s) is most favorable for collection of owner DNA from worn T-shirts and handler DNA from shirts that were touched during a simulated assault. Three standard methods for collecting touch DNA from fabrics were compared in this research: cutting, swabbing, and tape-lifting. Participants wore T-shirts for a minimum of six hours, some of which underwent a simulated assault. Areas that contained the most DNA and the area of assault handling were targeted for DNA recovery. DNAs were quantified, amplified, and typed using commercial STR kits. The collection methods were compared in terms of DNA quantity and quality, measured using a profile statistic. The findings demonstrate that cutting recovers the most cells, increasing DNA yields and the profile statistics of STR profiles, with Y-STR analysis being the most informative in identifying male T-shirt handlers. Implementing protocols that enhance DNA yield based on this research, supported by court-admissible profile statistics, may increase the number of biological exhibits that could be used in criminal prosecution.

To my family, especially Ben and Cate, for without them, this work (and my sanity) would not have been possible.

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INTRODUCTION

A violent crime, such as murder, forcible rape, robbery, or aggravated assault, occurs in the U.S. every 25.3 seconds, and property crimes occur more frequency with one every 3.5 seconds (FBI Uniform Crime Report, 2010). In cases of violent crime, situational characteristics, such as the amount of physical evidence available, are strong predictors of the likelihood of crime clearance (Roberts, 2007). This is also true of non-violent crimes, where research has shown that the amount of evidence following preliminary investigation greatly influences police action with more evidence corresponding with a more extensive investigation (Bynum et al., 1982). Therefore, the collection of physical evidence is of extreme importance to the investigative process.

In order for a crime to be cleared, at least one individual must be arrested, charged, or turned over to the courts for prosecution (FBI Uniform Crime Report, 2010). The foundation of crime clearance rests on the identification of the perpetrator, which is often accomplished through the analysis of friction ridge impressions, generally fingerprints, left at the crime scene. Fingerprints, which are considered unique to every individual, were used for identification as early as 300 B.C. with the first murder to be solved solely on this type of evidence occurring in 1902 (Barnes, 2011). The use of fingerprints that tie a criminal to a crime through a database, a collection of known profiles, or to a suspect is among the strongest type of individualizing evidence.

Individual identification can also be accomplished using biological material. Early tests that examined biological evidence included ABO bloodtyping and enzyme profiling. While these tests may eliminate many suspects, numerous individuals could still be implicated in the crime as

many individuals have a given trait (i.e., blood type A) or set of genetic markers. Over the years, technological advancements have made it possible to use a much more powerful source of evidence: DNA, which has the ability to be individualizing.

Biological evidence, such as blood, semen, or saliva, which are rich sources of DNA (Giusti et al., 1986; Dimo-Simonin et al., 1997; Sweet and Shutler, 1999), are not always required to link individuals to a crime scene through genetic analysis. Another source is from epithelial cells left behind on handled items, termed "touch DNA" or "contact DNA." At non-violent crime scenes, touch DNA is likely to be found at a higher frequency than rich sources of DNA, such as body fluids, however, it is a comparatively poor source of cells. Therefore, touch DNA evidence, which requires careful collection procedures that maximize cell recovery, can be used to increase the clearance rate for non-violent crimes. This can have a great affect on the clearance rate of property offense, which tends to be much lower than the rate for violent crime, 18.3% and 47.2% respectively in 2010 (FBI Uniform Crime Report, 2010).

Roman et al. (2008) found an increase in the percentage of perpetrators identified when DNA evidence was processed compared to cases where other investigative techniques, such as eyewitness testimony or fingerprint analysis, were used. In Denver, specifically, perpetrator identification increased from 18% to 58% and cases accepted for prosecution rose from 17% to 46% when DNA evidence was the first investigative tool (Roman et al., 2008). The proper collection and analysis of DNA evidence, which has now been admitted in all U.S. jurisdictions (NIJ, 1999), can lead to higher clearance rates for both violent and non-violent crimes.

Studies need to be conducted on locating and collecting biological evidence. This study was designed to address the lack of research regarding the recovery of contact DNA from fabric evidence and focused on determining the optimal technique(s) for obtaining DNA from T-shirt

wearers and from individuals who come into contact with T-shirt wearers. Improvements in DNA collection will facilitate the criminal investigation process by providing probative evidence that can lead to the identification of the perpetrator of a crime in cases where other methods failed to do so. This research strategy combined the use of DNA quantification data and profile evidentiary values, which are directly applicable in the legal system, to assess differences among the collection methods.

Forensic DNA Analysis Methods

Alec Jeffreys, a British geneticist, and his team were the first to describe repetitive DNA elements that varied in the number of repeats, used as a tool in human identification (Jeffreys et al., 1985). These DNA regions are known as variable number of tandem repeats (VNTRs), and result in distinctive fragment numbers and sizes, producing an individual's "DNA fingerprint" (Fig. 1).

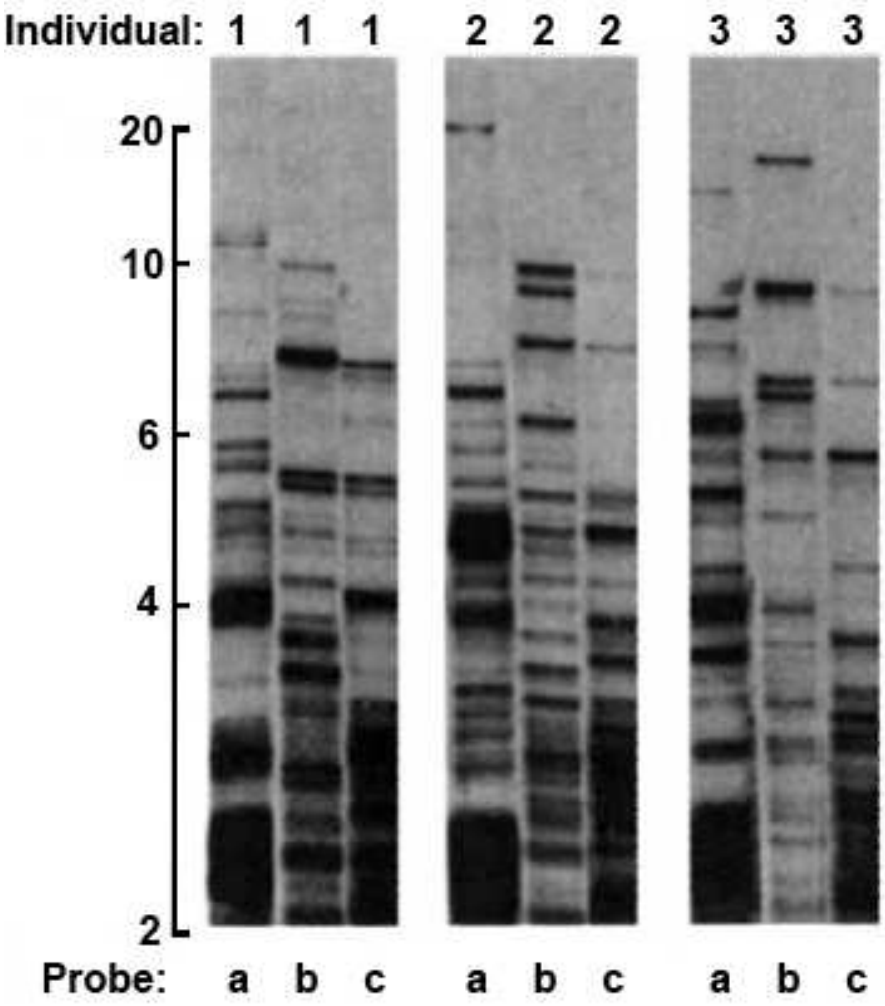

Figure 1. First human DNA fingerprint from Jeffreys et al. (1985). DNA samples from individuals (1, 2, 3) were digested with restriction enzymes and hybridized with probes (a, b, c). Fragment size measured in kilobases (kb) of DNA.

In 1986, another advancement made it possible to copy small amounts of DNA: the polymerase chain reaction (PCR). This mimics the cell's own replicative process, targeting specific regions of the DNA (loci) and making billions of copies of the starting material. The development of PCR was pivotal in the field of forensics as small amounts of biological material are often found at crime scenes. Today, forensic laboratories use PCR to amplify short tandem repeats (STRs) (Fig. 2). STRs are similar to VNTRs in that they are repetitive sequences of

DNA, but they have fewer bases in the repeat units. Individuals have two alleles, or variants, at a given STR locus, with one allele inherited from each parent. These markers have a high degree of variability among individuals and using them in combination, it is possible to obtain an individualizing DNA profile (Fig. 3). Genetic profiles have been successfully obtained on DNA from cigarette butts (Hochmeister et al., 1991), single human hair roots (Higuchi et al., 1988), fingernail scrapings (Wiegand et al., 1993), and bite marks (Sweet et al., 1997b). Amplification and typing of DNA has also been achieved from degraded or burnt bone fragments and tissue specimens (Clayton et al., 1995; Whitaker et al., 1995; Holland et al., 2003).

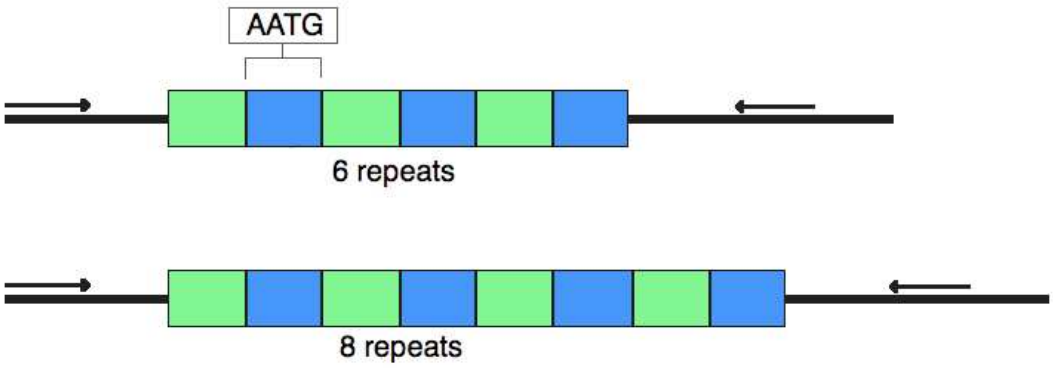

Figure 2. Representation of short tandem repeats (STRs). Colored boxes denote a DNA repeat unit, in this case, AATG with 6 and 8 repeats inherited from the mother and father. Arrows indicate position of primers used in PCR amplification. For interpretation of the references to color in this and all figures, the reader is referred to the electronic version of this thesis.

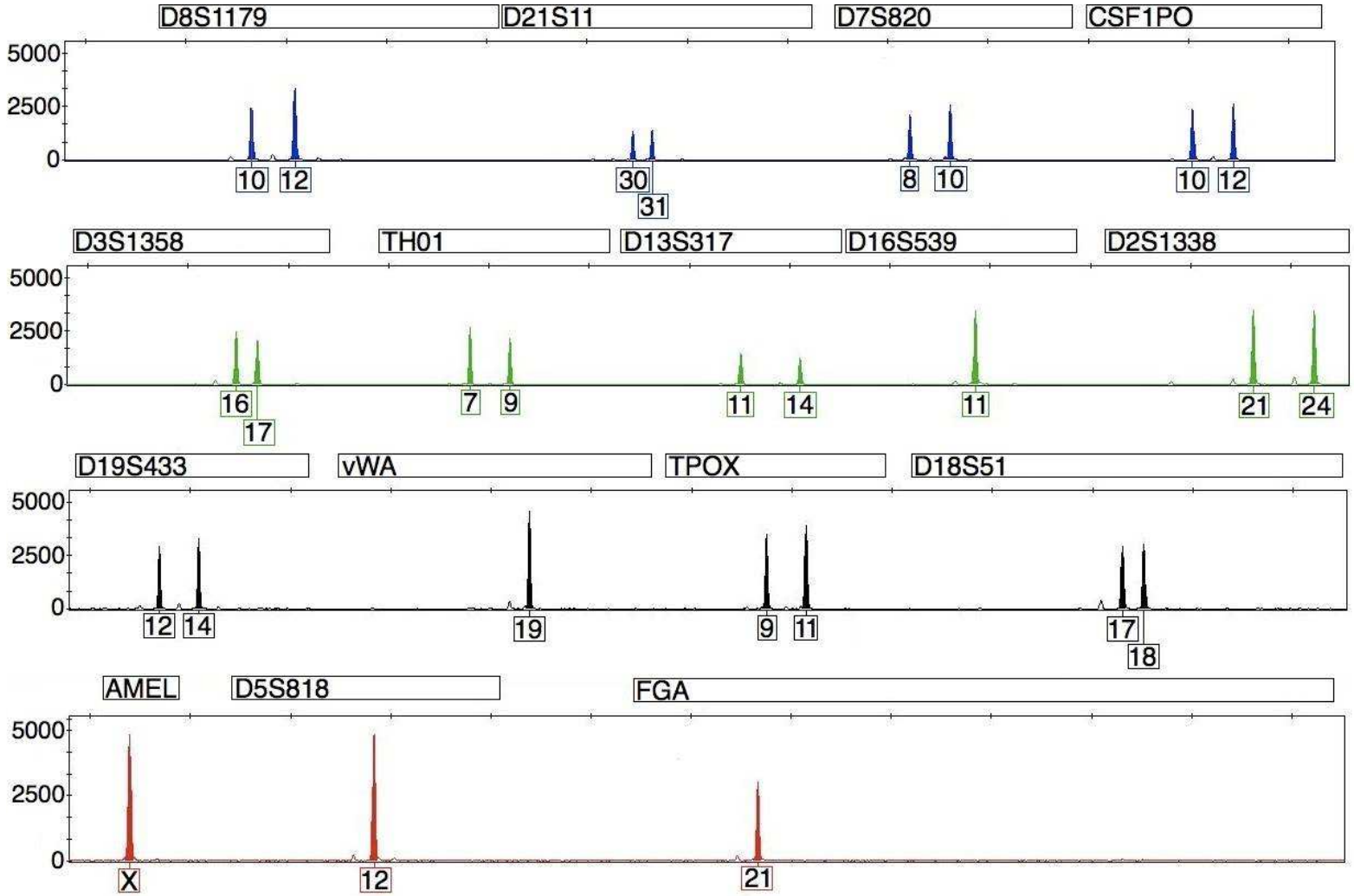

Figure 3. Complete STR profile showing the peaks of the fluorescently labeled loci using the Identifiler® PCR Amplification Kit (Applied Biosystems, 2011). Electropherograms showing allele peaks (measured in relative fluorescent units, shown on y-axis) for the following loci: D8S119, D21S11, D7S820, CSF1PO, D3S1358, THO1, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, Amelogenin, D5S818, and FGA. DNA size measured in bases (x-axis).

Although it is possible to amplify miniscule amounts of DNA, such quantities can cause analysis and interpretation problems. Low quantity DNA is often classified as trace, defined as, "any sample which falls below [the] recommended thresholds at any stage of the analysis, from sample detection down to profile interpretation, and can not [sic] be defined by a precise picogram amount," (van Oorschot et al., 2010). A small quantity of DNA is also considered low copy number (LCN), defined by Gill et al. (2000) as less than 100 pg, or approximately 20 human cells worth of DNA. The LCN DNA level is five to ten times less than the amount of input DNA recommended for most commercial STR kits.

Analysis of trace levels of DNA can lead to STR artifacts in profiles, including increased stutter, drop-out, and peak imbalance (Fig. 4; Budowle et al., 2009). The first of these, stutter, is from template strand slippage during PCR, resulting in a product that is typically one repeat unit smaller than the actual template size. In the case of trace DNA, stutter products may be much more pronounced, and can be larger than the true allele (Gill et al., 2000). Second, drop-out occurs when expected alleles are not present in a profile because they are not well represented in the PCR reaction, owing to the small amount of input DNA. Profiles that contain allelic drop-out are problematic because heterozygous individuals can falsely be reported as homozygous. Third, unequal amplification of alleles at a heterozygous locus results in peak imbalance. This may produce profiles that appear to be mixtures, extracts that contain DNA from more than one source; in some extreme cases, peak imbalance can cause drop-out of the underrepresented allele (e.g., 33 allele in Fig. 4). While retesting of trace DNA may resolve the problems with profile interpretation generated by PCR artifacts, this is not always possible when a portion of the DNA extract must be kept for retesting, which is standard for quality control purposes in criminal laboratories.

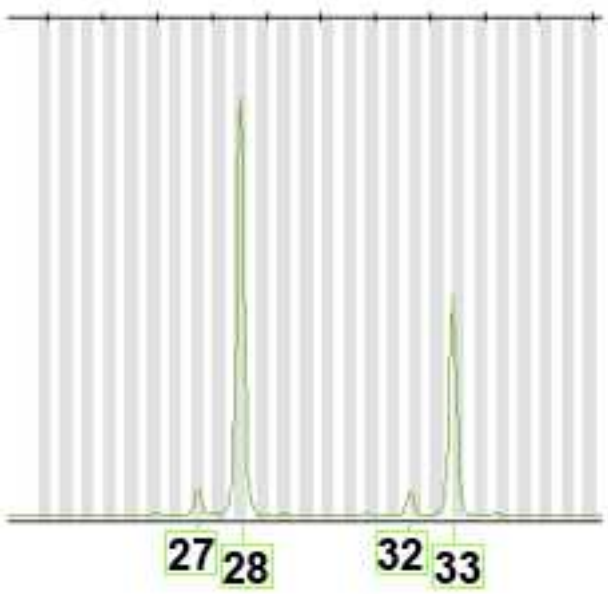

Figure 4. Example of STR artifacts at locus D21S11. Amplification of true alleles, 28 and 33, results in stutter peaks at 27 and 32, respectively. Difference in height between allele 28 and 33 indicates peak imbalance. Drop-out may result if the height of allele 33 falls below the threshold minimum.

Using DNA for Identification: Profile Statistics

The Combined DNA Index System (CODIS), a database created by the Federal Bureau of Investigation (FBI) to help identify perpetrators of crimes within the United States, is a compilation of STR profile data. The 13 core CODIS loci, typically named after their chromosomal locations or genes they are associated with, include D3S1358, THO1, D21S11, D18S51, D5S818, D13S317, D7S820, D16S539, CSF1P, vWA, D8S1179, TPOX, and FGA (Anker et al, 1992; Kimpton et al, 1992; Mills et al, 1992; Neeser and Liechti-Gallati, 1995; Budowle et al, 1996; Tamaki et al, 1996). The CODIS loci have been highly studied to determine allelic and genotypic frequencies within various ethnic groups.

The polymorphic nature of STRs makes DNA profiling very powerful in that definitive discrimination among individuals can be made by comparing the frequency of alleles at several

loci. The power of discrimination using STR kits is so high that it can tie a criminal to the crime scene to the exclusion of all other individuals on earth, with the exception of an identical twin. For example, the discriminatory power for the Identifiler® PCR Amplification Kit, which assays 15 STR loci, is 5.01×10^{-18} for U.S. Caucasians (Applied Biosystems, 2011). This means that there is a 1 in 5 quintillion chance that two individuals selected at random will have identical genotypes. Given that this is many orders of magnitude greater than earth's population (approximately 7.03×10^{9}; U.S. Census Bureau, 2012), a given profile is largely considered an unequivocal identification.

The autosomal markers examined as a part of standard STR kits are independently inherited, meaning that the chance of having a given allele at one locus is not tied to the genotype at another locus. Therefore, profile statistics for autosomal STRs can be determined by applying the product rule, which is calculated using the genotypic frequency—the proportion of individuals with a given genotype (e.g., how common is the combination of the 6 and 8 alleles, Fig. 4)—for each locus. The single locus genotypic frequencies are then multiplied together (the chance of having the 6 and 8 alleles at one locus *and* the 10 and 11 alleles at another), producing a multi-locus frequency.

Different statistical formulae, based on the product rule, have been developed to assess the probative value of a DNA profile. A random match probability (RMP) is the probability of selecting, from random unrelated individuals, a potential contributor to the profile, whereas a combined probability of inclusion (CPI) is the expected frequency of individuals who could have contributed to the DNA profile. Although the RMP formula has been modified to take into account DNAs with more than one genetic donor, it includes an assumption about the number of individuals included in the profile; therefore, RMP is commonly used for profiles derived from a

single-source, while CPI can be used for mixtures without an assumption as to the number of contributors (SWGDAM, 2010).

The product rule cannot be applied to the STRs on the Y chromosome or other assays that examine only a single hereditary unit because genetic markers are linked and are inherited together. Y chromosome STRs, which are inherited through the paternal line, are used to assay male DNA. The counting method must therefore be used to calculate Y-STR profile statistics (Budowle et al., 2003), which estimate the frequency of a particular haplotype, a group of alleles, in a given database. Compared to STRs, Y-STRs have reduced discriminatory power due to the difference in inheritance.

A further matter that can complicate STR analysis and result in decreased evidentiary value is the presence of DNA mixtures, which contain three or more alleles at a given locus (Clayton et al., 1998) that are repeatable in multiple amplifications. Due to the complexity of mixtures, the FBI's Scientific Working Group on DNA Analysis Methods (SWGDAM) formed a Mixture Committee in January 2007 to standardize the way forensic laboratories interpret mixed DNA profiles. They proposed that "the laboratory must perform statistical analysis in support of any inclusion...irrespective of the number of alleles detected and the quantitative value of the statistical analysis," (SWGDAM, 2010). Because DNA analyses typically have great discrimination capabilities, there is concern that even weak DNA results could be given high probative value in court. Profile statistics in a forensic biology report safeguards against distorted interpretation of any conclusions and ensures that results are stated with a scientifically defensible probability.

Touched Items as a Source of DNA

Touch DNA is found on objects that are handled as a regular part of their use, such as drinking containers (Abaz et al., 2002) and firearms (Horsman-Hall et al., 2009; Richert, 2011). Fingerprints can serve as a source of DNA in cases where no other biological evidence is recovered. Van Renterghem et al. (2000) found that genetic profiling of fingerprints from glass objects was successful even after visual enhancement failed. Archived latent fingerprints have also been useful in touch DNA typing, but in controlled experiments, archived prints contained less DNA than prints collected directly (Schulz and Reichert, 2002). Offenders, knowing that their fingerprints can tie them to illegal activity, often wear gloves during the commission of a crime. Recovered latex gloves, discarded beyond the crime scene, are another potentially valuable source of touch DNA (Pizzamiglio et al., 2000).

Van Oorschot and Jones (1997) found that even brief contact between an individual and an object was sufficient for depositing cells, and with the DNA from a touched item, they were able to obtain a genetic fingerprint. Having the ability to analyze touched objects has major implications in the field of forensics, but the profile generated by van Oorschot and Jones (1997) consisted of a single STR locus and amplifications used over a nanogram of DNA. Other researchers have expanded this work, increasing the number of loci examined and reducing the amount of input DNA. Findlay et al. (1997) were able to amplify a single cell's worth of DNA to generate a six-locus STR profile. However, buccal cells, which are much more robust source of nuclear DNA than the sometimes nuclei-free cells found on handled items (Balogh et al., 2003), were used as a genetic source. Using touch DNA, 10 loci were amplified by Lowe et al. (2002) from DNA collected on items held for as little as 10 seconds; however, the DNA was not quantified during this study and a set volume was added to each STR amplification reaction,

hence, the precise amount of DNA being used to generate profiles was unknown. Pang and Cheung (2007) quantified DNA from touched objects and used 100 pg to successfully generate 15-locus profiles. While advancements have been made in the analysis of handled items, research regarding the collection of this type of DNA is ongoing.

DNA Recovery Methods for Fabric Evidence

Porous surfaces can harbor epithelial cells left behind during physical contact. Fabric items such as bedding (Petricevic et al., 2006) and those worn on the head (Herber and Herold, 1998) have been shown to contain DNA transferred from an individual during normal activity. Collecting as many cells as possible from items is paramount in obtaining DNA from the object and increasing the chance of identification. Most studies in which fabric evidence was analyzed have only used a single technique for the collection of touch or contact DNA. However, several methods are commonly used to recover touch DNA from fabric items, and it is unknown which one results in the greatest DNA yields or the best quality profiles.

Although it is possible to microscopically locate individual cells on clothing (Herber and Herold, 1998), it is not feasible when areas of transferred cells are unknown. Due to the impracticality of precisely locating epithelial cells from large fabric items, the procedure commonly used in forensic laboratories for collecting biological material is the cutting method (Petricevic et al., 2006) where recovery is targeted in an area that may contain a high cell number. To produce a cutting, a small piece of a fabric item is cut out and soaked in an extraction solution that contains a cell-lysing detergent and a protein-digesting enzyme, initiating the cellular digestion process. Cutting may be optimal for collecting cells because the entire portion of fabric is exposed to the solution, loosening most or all cells adhering to it. However,

some substrates contain substances that can be released during the soaking process, inhibiting DNA amplification procedures and interfering with its analysis (Bright and Petricevic, 2004). Additionally, if an item contains cells from more than one person, a cutting can produce a complex DNA mixture that includes alleles from multiple individuals, making interpretation difficult.

The cutting method is also limited by the size of the test tube generally used for DNA procedures (2 milliliters). Schulz and Reichert (2000) overcame this by using a wash and filtration method where 100 milliliter collection vessels were used, followed by filtration through a membrane, producing a final 50 microliter volume, with contact DNA being isolated from large pieces (25 x 25 cm) of a bandage and T-shirt collar. Therefore, this technique could be used to collect DNA from large items of fabric evidence.

An alternative recovery procedure is the collection of cells from evidence by rubbing the area in question with a swab wetted with water or another solution. Following collection, the head of the swab is placed into extraction solution. For items that may contain trace levels of DNA, Wickenheiser (2002) proposed that this method would minimize mixtures, perhaps because it can be used in a localized area, thereby lessening the chance of collecting cells from multiple individuals, which in turn maximizes the statistical value of the evidence.

The collection of biological material using various types of swabbing materials or more than one swab has also been examined. Mulligan et al. (2011) investigated DNA recovery by swabbing using multiple polyester and cotton fabric squares (1 x 1 cm). Although DNA yields from the two material types were not different when used to remove saliva cells, one type of cotton did produce greater DNA yields when the fabrics were used on touch DNA exhibits. The double swab technique, in which a swab wetted with water is followed by a dry swab, was

developed by Sweet et al. (1997a) and was useful in increasing the amount of salivary DNA recovered from skin. Pang and Cheung (2007) utilized this technique and produced genetic profiles with touch DNA from various items. Some researchers have studied the value of using a single swab for the entire item (cumulative swabbing) versus performing multiple swabbings of an item (Richert, 2011; Orlando, unpublished). Cumulative swabs had higher DNA yields and resulted in more complete profiles (Richert, 2011). While instrumental in supporting the collection of touch DNA for genetic analysis, these studies have focused on non-porous substrates such as staplers, light switches, firearms, and fired cartridge casings, and did not assess the application of swabbing for collecting touch DNA on porous surfaces.

Tape-lifting is a third method utilized for recovering cells from evidence. They have routinely been applied in forensic settings to collect trace evidence such as hairs, fibers, fingerprints, and gun shot residue. Therefore, it might be easy for laboratories to develop protocols that implement tape-lifts for the collection of biological material. A non-destructive alternative to other collection methods (Sewell et al., 2008), tape-lifts may avoid the extraction of inhibitors from the substrate. When trace DNA was recovered from the insole of shoes, tape-lifting resulted in higher DNA recovery without the added difficulty of collecting matrix contaminants, which did occur when DNA was collected via cutting or swabbing (Bright and Petricevic, 2004).

Several researchers have investigated cell collection using various types and sizes of tape-lifts. Kenna et al. (2001) compared the effectiveness of tape-lifts (20 x 20 mm) to that of swabbing for recovering DNA from saliva stains, and found that yield was greater when tape-lifts were used. May and Thomson (2009) designed a method to collect and concentrate salivary DNA from larger tape-lifts (17 x 5 cm), in which swabs were used to collect cellular material on

selected areas of the tape; they found that swabbing the tape with a xylene solvent as well as adding the solvent to the extraction solution during the DNA isolation was best at dissolving the adhesive of the tape and recovering the bound cells. They also generated mock casework exhibits including T-shirts that were processed via this tape-lift xylene treatment, and compared it to evidence processed using a taping technique (2.5 x 6 cm), where the entire tape was processed in the DNA extraction. The results demonstrated that the xylene treatment was better than tape-lifting with full profiles produced in all cases from the former procedure and only 50% of the time from the latter. However, the results of this study were limited because the sample size was extremely small (n=4) and the only result reported regarding the quality of the DNA was the presence or absence of a full 10-locus DNA profile. May and Thomson (2009) were only able to state that the xylene treatment was "comparable" to the taping technique.

The use of tape-lifts to collect touch DNA from fabrics was more thoroughly examined in subsequent studies. Daly et al. (2012) compared the recovery of touch DNA using 7 x 1.5 cm tape-lifts on three substrates: glass, cotton fabric, and wood. Lifts from wood had the highest DNA yields, although they contained mixed profiles more often than those from glass or fabric. Lifts from the cotton fabric contained more DNA than glass, but useful profiles (those with all the donor's alleles present with no additional alleles) were obtained from only 23% of fabrics. In a casework study by Barash et al. (2010), DNA was collected from three evidentiary items: a ski mask, a sock, and a woolen glove. Tape-lifts were used to collect biological material and yielded sufficient DNA for profiling. In some cases, tape-lifting selected areas of the item resulted in two different DNA profiles. However, it is not clear whether other methods may have yielded higher quantity or quality DNA because none were tested.

Current Study: Determination of Optimal Collection Technique(s) for T-shirt Evidence

Forensic science begins at the crime scene with the recovery of evidence, and in order to maximize crime clearance, biological materials needs to be recognized and properly collected at the scene. While several researchers have examined DNA retrieval from handled items, none has adequately assessed the effectiveness of collection techniques for contact DNA from fabrics. The aim of this study was to determine which collection method is best for obtaining DNA of a T-shirt wearer ('owner') or that of an individual who touches a T-shirt during a simulated assault ('handler'). Three collection methods were compared: cutting out portions of fabric that were added directly to the extraction solution, using cotton swabs to recover cells in selected areas of contact, and using adhesive tape to lift cells from the fabric.

Based on previous research, it was hypothesized that 1) the methods would differ in effectiveness and 2) the technique used to collect owner DNA would not necessarily be the most successful at recovering handler DNA. For instance, the highest DNA quantity from T-shirt owners may be obtained using the cutting method because cells from the entire fabric substrate are exposed to the digestion, and thus all have the potential to be collected. On the other hand, when genetic material from multiple sources is present on a fabric item, collection techniques such as swabbing or tape-lifting may preferentially recover the outer layer of cells left by the handler on the surface of the fabric, decreasing the need for the interpretation of DNA mixtures. The outcomes of this research will impact the process by which perpetrators are identified during criminal investigations and influence the way crime laboratories determine which method to use to collect trace DNA from worn and handled fabric evidence.

In the research presented here, study participants wore T-shirts for a minimum of six hours, some of which underwent a simulated assault at the end of the wearing period. Various

locations on the T-shirt were assayed to determine which contained the most DNA. This area and the area of assault handling were targeted for DNA recovery using the cutting, swabbing, or tape-lift methods. DNAs were quantified, amplified, and typed using a commercial STR kit. Selected DNAs from the simulated assault study were also assayed using a Y chromosome STR kit. The collection methods were compared in terms of the quantity of DNA obtained and by the quality of the DNA, measured using a profile statistic, which represented an objective calculation similar to those used in the courtroom for biological evidence.

MATERIALS AND METHODS

T-shirt Substrate Preparation

T-shirts (Fruit of the Loom®, Bowling Green, Kentucky) were removed from their original packaging and placed into brown paper bags, which were given to volunteers (n=20) along with instructions to wear the T-shirt for a minimum of 6 h. There were no activity restrictions while wearing the T-shirt other than no shirt should be worn underneath the experimental one. For the simulated assault study, after female volunteers (n=20) wore the T-shirt for at least 6 h, a male volunteer ('handler') was instructed to grab the right shoulder area for approximately 15 s while the female volunteers "struggled" and pulled away without the male volunteers losing contact. Male handlers had not recently washed their hands preceding assault simulation. After wearing and/or simulated assault, volunteers replaced the T-shirt into the bag and returned it. Buccal samples were obtained from all volunteers to serve as references. The use of human subjects was approved by the University Committee on Research Involving Human Subjects (IRB # 10-410).

Cell Recovery from Worn and Handled T-shirts

Lab coat, sleeves, facemask, and gloves were worn during all pre-amplification procedures. All solutions, tubes, scissors, cotton swabs (860-PPC, Puritan Medical Products Co., Guilford, Maine), and tape (3M™ Transpore™ Medical Tape, St. Paul, Minnesota) were UV irradiated in a Spectrolinker XL-1500 UV Crosslinker (Spectronics Corporation, Westbury, NY) for 5 min (approximately 2.5 joules/cm^2) per side (if applicable) prior to collection of cells;

swabs were placed at a angle to the UV light and were rotated after the initial 5 min irradiation. The type of tape selected for use was based on the success of previous research (Peters, 2011).

A preliminary assay of T-shirt locations was conducted by cutting out 1 x 1 cm sections from nine different locations on a worn T-shirt, including: shoulder seam, sleeve hem, sleeve seam, neck seam, arm seam, side (below arm seam), top of the collar, collar seam, and below the collar (Fig. 5). Another preliminary study included comparing shirt locations that had the highest DNA quantities using the three different collection techniques: cutting, swabbing, and tape-lift. For each collection location, a 1 x 3 cm section was cut out of the fabric. This section was then cut into three, and one piece was randomly used for each collection method. Cuttings were placed directly into 400 µL of digestion solution (20 mM Tris pH 7.5, 50 mM EDTA, 0.1% SDS; 5 µL of proteinase K; 20 mg/mL). Cells were collected with a swab wetted with digestion buffer by rolling the swab over the fabric surface; the tip of the cotton swab was cut off and placed into digestion solution to produce a swabbing. For tape-lifts, the adhesive side of the tape was pressed against the fabric surface until it no longer adhered to the fabric; the portion of the tape that had come into contact with the fabric was cut out and placed into digestion solution. Subsequent studies seeking to identify the owner (Part I) or handler (Part II) focused on the T-shirt locations with greatest DNA yields.

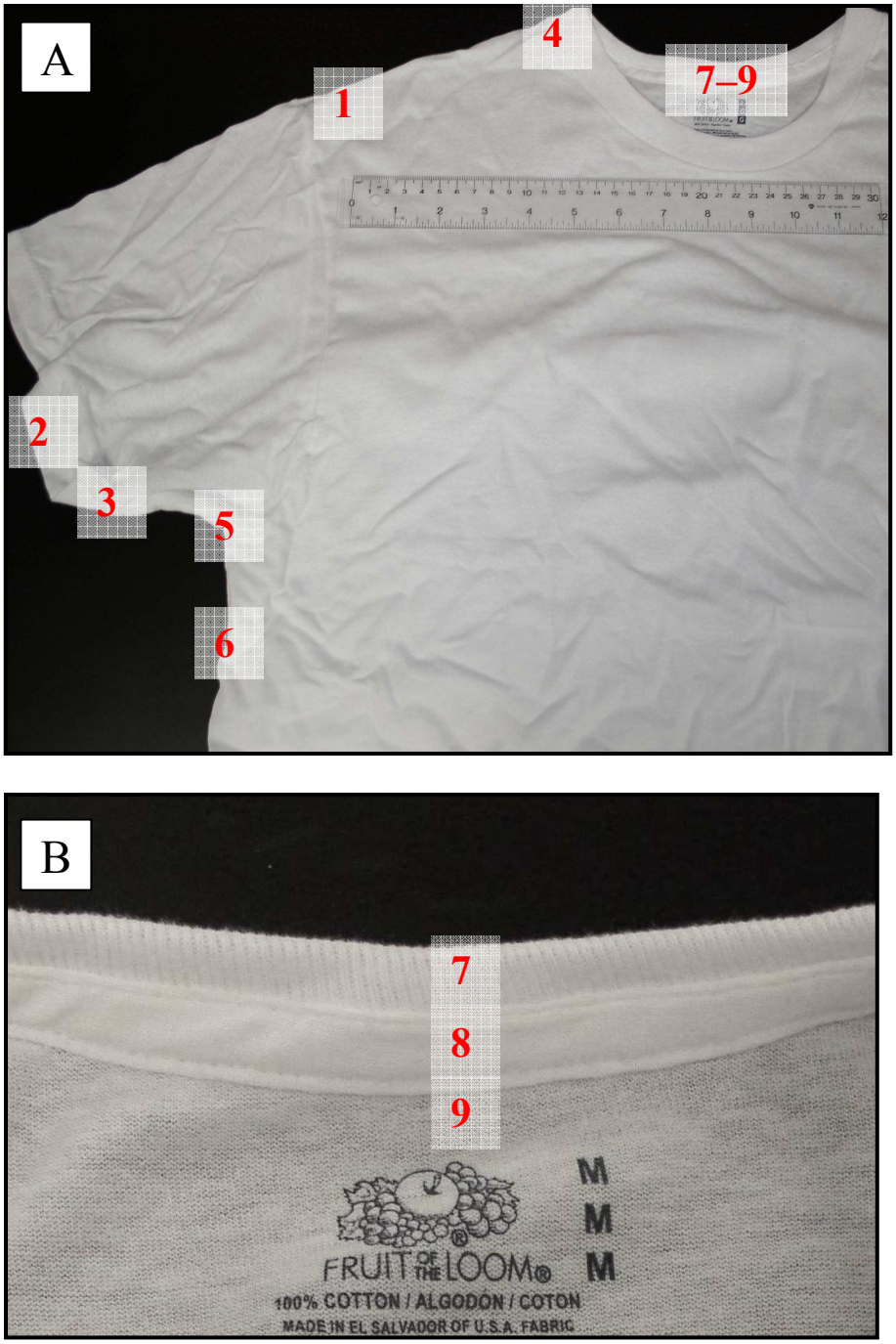

Figure 5. Locations tested during preliminary assay of T-shirt locations, including A) 1) shoulder seam, 2) sleeve hem, 3) sleeve seam, 4) neck seam, 5) arm seam, and 6) side; B) 7) top of the collar, 8) collar seam, and 9) below the collar.

Controls were analyzed concurrently with T-shirt DNA isolations: a negative reagent blank was created by adding all reagents together without adding a source of DNA; a swab reagent blank was produced by adding the cotton tip of a swab directly to digestion buffer; a tape reagent blank was made by adding tape directly to digestion buffer. A substrate control was created by placing an unworn T-shirt in a brown paper bag for the same period of time as the worn T-shirts, which was later processed as if it had been worn, with potential cells being collected via each of the methods.

Cell Digestion and DNA Extraction

T-shirt samples (cuttings, swabbings, and tape-lifts) in digestion buffer were vortexed and incubated at 55°C. A cursory digestion time study was conducted on DNA isolations (n=5) incubated at 3 h or 24 h; all subsequent cell digestions were incubated for 24 h prior to DNA extraction. Fabric cuttings, cotton swab tips, and tape sections were transferred to spin baskets and centrifuged at 15,000 x *g* for 3 min then discarded. The collected liquid from each sample was combined and DNA was extracted using an equal volume of phenol followed by an equal volume of chloroform. The aqueous layer from each extraction was transferred to an Amicon® spin column (Millipore Corporation, Billerica, MA), which was centrifuged at 14,000 x *g* for 10 min and flowthough was discarded. The DNAs were washed with 300 µL of low TE (10 mM Tris, 0.1 mM EDTA, pH 7.5) and centrifuged at 14,000 x *g* for 10 min. Flowthough was discarded and the wash step was repeated. Columns were inverted into new tubes followed by 3 min centrifugation at 1000 x *g*, resulting in a final extract volume of approximately 25 µL.

DNA Quantification

DNA was quantified using a Quantifiler™ Human DNA Quantification Kit (Applied Biosystems, Life Technologies, Carlsbad, CA). Amplification and detection of fluorescence was performed on an iQ™5 Real Time PCR Detection System (Bio-Rad, Hercules, CA) using persistent well factors. Dye calibrations were completed for VIC and FAM as instructed in the iQ™5 Optical System Instruction Manual. Reactions were carried out in 0.2 mL flat-capped strips (USA Scientific®, Ocala, Florida) at 15 µL volumes consisting of 7.5 µL of Quantifiler™ PCR Reaction Mix, 6.3 µL of Quantifiler™ Human Primer Mix, and 1.2 µL of sample, 007 positive control DNA (Applied Biosystems), or DNA standard. The DNA standard series was made by diluting Quantifiler™ Human DNA Standard (200 ng/µL) in TE buffer plus 20 µg/mL glycogen according to the manufacturer's recommendations, producing eight standards with concentrations ranging from 50 ng/µL to 0.023 ng/µL. Standards were run in duplicate. Thermal cycling parameters consisted of a 10 min incubation period at 95°C followed by 40 cycles of 15 s at 95°C and 1 min at 60°C.

STR Amplification

STR analysis was conducted on DNA extracts using an Identifiler® PCR Amplification Kit (Applied Biosystems), in 10 µL volumes, consisting of 4 µL PCR Reaction Mix, 1 µL Primer Set, 0.5 µL of AmpliTaq Gold® DNA polymerase (5 U/µL), and approximately 1.0 ng or a total of 4.5 µL of sample DNA or control. A negative PCR control contained water instead of DNA. Thermal cycling parameters consisted of 11 min incubation at 95°C followed by 28 cycles of 1

min denaturation at 94°C, 1 min annealing at 59°C, 1 min extension at 72°C, then a 60 min final extension at 60°C.

Handler DNA was detected when the Y chromosome-specific allele at the amelogenin locus was present in autosomal STR profiles. Y-STRs were amplified from a subset of the simulated assault study extracts, those that contained handler DNA, using a Yfiler™ PCR Amplification Kit (Applied Biosystems). Reactions were carried out at 10 µL volumes, consisting of 1.85 µL PCR Reaction Mix, 1 µL Primer Set, 0.6 µL of AmpliTaq Gold® DNA polymerase (5 U/µL) with 0.5 µL AmpliTaq Gold® Buffer, 0.5 µL $MgCl_2$, and approximately 1.0 ng or a total of 5.55 µL of sample DNA or control. A negative PCR control contained 1 µL of water instead of DNA. Thermal cycling parameters consisted of 11 min incubation at 95°C followed by 30 cycles of 1 min denaturation at 94°C, 1 min annealing at 61°C, 1 min extension at 72°C, then an 80 min final extension at 60°C.

STR Capillary Electrophoresis

One microliter of amplified DNA or allelic ladder was combined with 8.7 µL of deionized formamide and 0.3 µL of GeneScan™ LIZ 500 Size Standard (Applied Biosystems) and analyzed on an AB® 3500 Genetic Analyzer (Applied Biosystems). Run parameters for Identifiler® samples consisted of an injection period of 8 s, injection voltage of 19.5 kV, run voltage of 15.0 kV, run temperature of 60°C, and run time of approximately 22 min (1330 s). Run parameters for Yfiler™ samples consisted of an injection period of 15 s, injection voltage of 1.2 kV, run voltage of 15.0 kV, run temperature of 60°C, and run time of 30 min (1800 s). Data analysis was done using GeneMapper® v4.1 (Applied Biosystems). Alleles that exceeded a

threshold of 150 relative fluorescence units (RFUs) were recorded. At each locus, the allele with the highest RFU was denoted the base peak; any callable allele that had an RFU less than 50% of the base peak was denoted as part of a minor profile.

Profile Statistic Determination

Autosomal STR profile statistics (the CPI) were generated with GenoStat® 1.4.3 (Forensic Bioinformatic Services, Inc., Fairborn, Ohio) using a Caucasian database (Butler et al., 2003) with theta=0. When only one allele was present at a given locus, statistics for the T-shirt profile at that locus included the potential for an undetected allele. Reference profile CPI's were calculated similarly. CPI values were log transformed, and the transformed values were used for all analyses.

Y-STR profile statistics were generated using the Yfiler Haplotype Database (Applied Biosystems). Only alleles that were consistent with the handler were assessed for profile frequency. When only one allele was present at DYS385, the profile frequency calculation excluded this locus. Reference profile frequencies were calculated using all loci.

Statistical Analyses

Differences in DNA quantities and profile statistics among collection techniques were compared. Analyses were performed to test for a difference in centers of distribution. Normality was assessed using the Kolmogorov-Smirnov (K-S) test: if groups to be compared had $p<0.05$, non-parametric tests were performed, which test for a difference in group medians. To compare differences between independent groups, the Mann Whitney U test (comparison of two groups) or Kruskal Wallis test (comparison of more than two groups) were used. To compare differences

between dependent or related groups, the Wilcoxon Signed Rank test (comparison of two groups) or Friedman test (comparison of more than two groups) were used.

A simple liner regression was performed to assess the relationship between DNA quantity and profile statistic. DNA extracts derived from T-shirt samples (cuttings, swabbings, or tape-lifts) were quantified for DNA concentration and are the unit of analysis for this model, with DNA quantities as the independent variables and profile statistics (CPI or haplotype frequency) as the dependent variables for the regression. The correlation coefficient (R), which measures the strength and linear relationship between the variables, and the coefficient of determination (R^2), which indicates the proportion of variation in the dependent variable that can be explained based on the independent variable (or how well the regression line fits the data), were also calculated. Statistical tests were conducted using Statistical Package for Social Sciences® (SPSS, IBM, Armonk, NY). A p-value below 0.05 (α=0.05) was considered significant.

All results in text are presented as mean ± standard error. Results are visually presented as boxplots, which display the median (black line within the box) and spread of data over the quantitative variable (Fig. 6). The box denotes the data between quartile 1 (Q1) and quartile 3 (Q3), or the interquartile range (IQR)—the middle 50% of the data. The whiskers extend to the largest and smallest non-outlier data values. Mild and extreme outliers are designated with a circle or asterisk, respectively. In this study's data set, outliers were defined as values that surpassed the inner fence (mild outlier, Q3 + 1.5IQR) or outer fence (extreme outlier, Q3 + 3IQR).

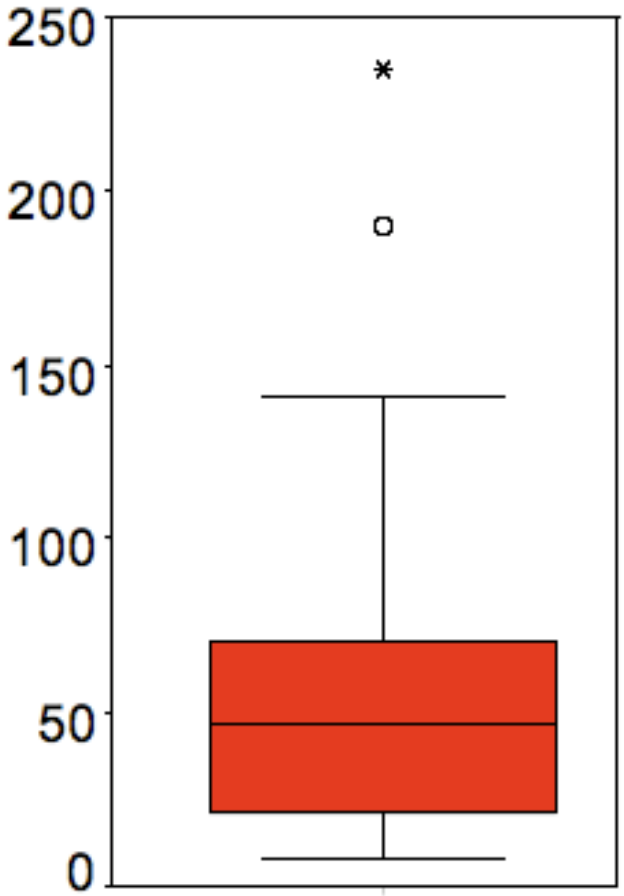

Figure 6. Representative boxplot with the y-axis representing the quantitative variable. The x-axis represents a categorical variable.

RESULTS

The cell collection methods differed in their ease of use. Cutting was the simplest technique and required the least manipulation compared to swabbing and tape-lifting. During cell collection using tape-lifts, the adhesive pulled the thread out of the seam of the T-shirt, creating two smaller pieces of fabric that were more difficult to work with. It was also difficult to keep the cut portion of the tape fully submerged in the digestion buffer. Quantifiable DNA was recovered from every worn T-shirt with DNA ranging from 0 to >1000 pg/µL. Low but detectable levels of DNA were found in four of ten substrate controls (11.1 ± 3.4 pg/µL) and in four of nine negative controls (4.85 ± 1.8 pg/µL); STR amplification of these DNAs resulted in no allelic activity. Quantification data (Appendix A) as well as profile statistic data (Appendix B and C) were assessed for normality, where data skewness necessitated the use of nonparametric statistical tests.

DNA Yields from Preliminary Assay of T-shirt Locations

DNA quantities obtained via cutting from the nine T-shirt locations varied from 40–305 pg/µL (Table 1). The highest DNA concentrations were from the top of the collar (286 pg/µL) and the shoulder seam (305 pg/µL).

Table 1. DNA quantities (pg/µL) recovered from different locations of a worn T-shirt (numbers in parentheses refer to those designated in Fig. 5).

T-shirt Area	DNA Quantity (pg/µL)
shoulder seam* (1)	305
top of collar* (7)	286
sleeve hem (2)	152
collar seam (8)	139
neck seam (4)	111
sleeve seam (3)	102
below collar (9)	96
arm seam (5)	55
side (6)	40

* indicates areas that were used for subsequent testing

The next study compared the two areas for cell collection from worn T-shirts using each of the three collection methods, resulting in 120 DNA extracts. Average DNA yields were 87.9 ± 20 pg/µL and 64.4 ± 12 pg/µL for the collar and shoulder regions, respectively, which were significantly different (Wilcoxon Signed Rank, $p=0.025$; Appendix A). Likewise, DNA yield was significantly different (Friedman, $p<0.001$) among the three collection techniques for both locations (Fig. 7). Similar pairwise relationships between the DNA recovery methods (n=20 per method at both the collar and shoulder) existed at both collection locations (Wilcoxon Signed Rank): a significantly greater yield from cutting versus swabbing (collar, $p<0.001$; shoulder, $p=0.001$) and from tape-lifts versus swabbing (collar, $p<0.001$; shoulder, $p=0.008$) and no significant difference between cutting and tape-lifts (collar, $p=0.070$; shoulder, $p=0.102$).

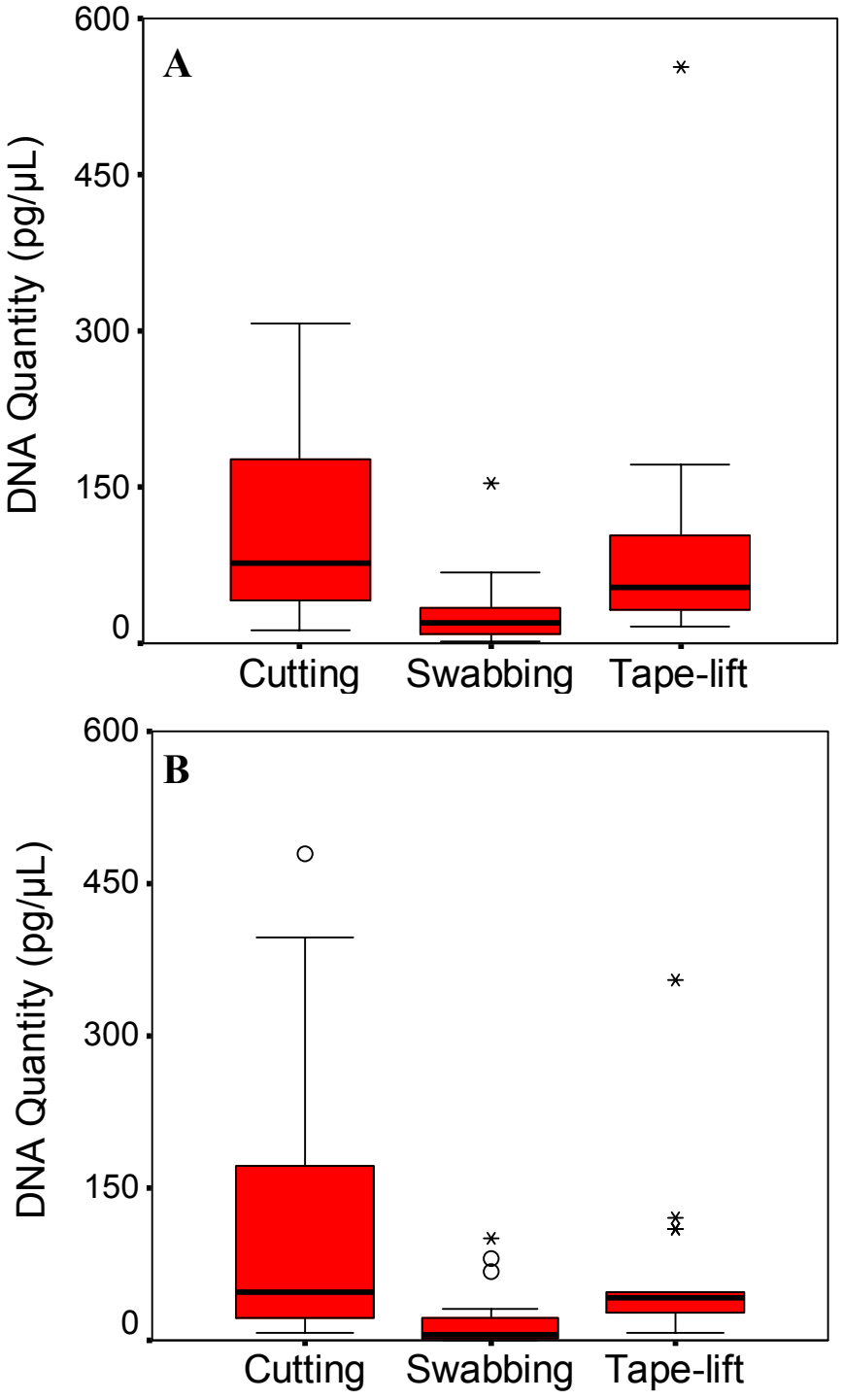

Figure 7. DNA quantities (pg/µL) from T-shirts for A) collar and B) shoulder locations. One outlier (1050 pg/µL) from a cutting of the collar is not shown.

Part I: T-shirt Owner Identification

DNA Quantification

Processing the shoulder seams of the 20 worn T-shirts using the three collection techniques produced five DNA extracts per shirt, including one cutting, two swabbings (inside and outside), and two tape-lifts (inside and outside), for a total of 100 DNA extracts. There was no significant difference in DNA quantities from the inside and outside of the T-shirt for either the swabbing (Wilcoxon Signed Rank, $p=0.227$) or tape-lift ($p=0.550$) methods (Fig. 8A; Appendix A). Total DNA retrieved via each method (inside and outside DNA quantities summed, if applicable) was significantly different (Friedman, $p<0.001$; Fig. 8B). Cutting and tape-lifting recovered significantly more DNA than swabbing (Wilcoxon Signed Rank, $p=0.001$ for both); there was no difference between cutting and tape-lifting ($p=0.412$).

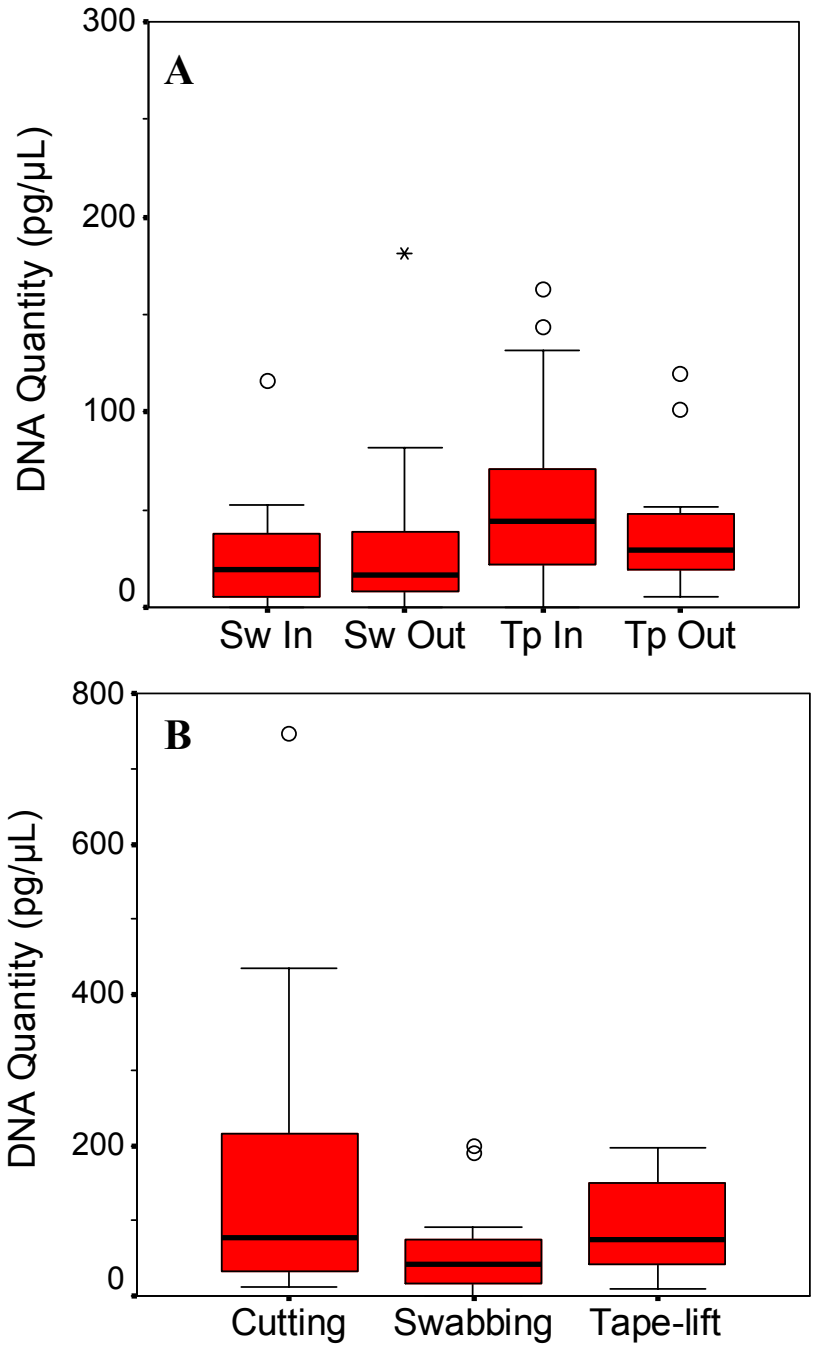

Figure 8. DNA quantities (pg/µL) from T-shirts for owner identification. A) Quantities from the inside and outside using the swabbing and tape-lift methods (n=20 per each method; Sw=swabbing, Tp=tape-lift, In=inside, Out=outside). One outlier (782 pg/µL) for a tape-lift of the outside of the T-shirt is not shown. B) Quantities for each method with inside and outside values summed (n=20 for cutting, n=40 for swabbing and tape-lift). Two outliers (cutting, 1420 pg/µL; tape-lift, 926 pg/µL) are not shown.

STR Profiles: Non-owner Alleles and Drop-out

It was apparent that the major profile from every T-shirt was that of the owner (Appendix B). The extent of non-owner alleles and locus drop-out in T-shirt profiles is shown in Table 2. Allelic activity below the 150 RFU threshold existed in DNAs from every T-shirt. Non-owner alleles were present in 50% of profiles including 60% of cuttings, 30% of swabbings, and 65% of tape-lifts. In some instances, the non-owner alleles were consistent among profiles obtained from the same T-shirt (see Appendix B; T-shirt #4, 9, 15, and 18). Collection of cells from the outside of T-shirts resulted in more non-owner alleles than collection from the inside. Fewer non-owner alleles were generated when the T-shirt was swabbed (42 total alleles from inside and outside) whereas tape-lifting (inside or outside) resulted in the greatest number of non-owner alleles (73 and 67 alleles, respectively). Of the profiles where non-owner alleles were present, those collected via a tape-lift of the outside had an average of 7.0 non-owner alleles per profile, whereas the other methods resulted in 3.0–4.9 non-owner alleles per profile.

Table 2. Non-owner alleles and the absence of alleles at loci (drop-out) in T-shirt profiles (n=20 profiles for each method; Al=non-owner alleles, Lo=loci, Pr=profile, Af=affected).

		Cutting	**Swabbing**		**Tape-Lift**		**Average**
			Inside	**Outside**	**Inside**	**Outside**	
Non-owner Alleles	# of Al	49	18	28	73	67	47
	Al/Pr	2.5	0.9	1.4	3.7	3.4	2.4
	% of Af Pr	60%	30%	30%	75%	55%	50%
	Al/% Af Pr	4.1	3.0	4.6	4.9	7.0	4.7
Drop-out	# of Lo	106	179	151	131	131	140
	Lo/Pr	5.3	9.0	7.6	6.6	6.6	7.0
	% of Af Pr	70%	75%	85%	70%	65%	73%
	Lo/% Af Pr	7.6	11.9	8.9	9.4	10.1	9.6

Seventy-three percent of profiles (73/100) had drop-out of alleles at one or more loci, involving 70% of cuttings, 80% of swabbings, and 67.5% of tape-lifts (Table 2). The DNA quantity of profiles that had one or more loci with drop-out (33.9 ± 5 pg/μL) was significantly less than that of profiles with no drop-out (199.1 ± 60 pg/μL; Mann Whitney, $p<0.001$). Drop-out at eight or more loci occurred in 47% of profiles (40% of cuttings, 55% of swabbings, 42.5% of tape-lifts) and the average DNA quantity of these profiles (21.1 ± 3 pg/μL) was significantly less than that of profiles where drop-out occurred at 1–7 loci (57.0 ± 12 pg/μL; Mann Whitney, $p<0.001$). Less drop-out was present in cuttings, however all methods had a similar percentage of profiles that had at least one locus with no allelic information.

Combined Probability of Inclusion Statistic for T-shirt Profiles

Profile statistics (CPI) from all DNAs were generated and compared to known reference profiles with DNAs from the T-shirts, generating significantly lower CPI values than the corresponding known reference profiles (Wilcoxon Signed Rank, $p<0.001$, Fig. 9A). There was also a significant difference among collection methods (Friedman, $p=0.012$, Fig. 9B). CPIs from cuttings were, in general, higher than those from swabbings or tape-lifts. The only significant difference was between tape-lifting the inside and swabbing the inside with greater CPI's when items were tape-lifted (Table 3).

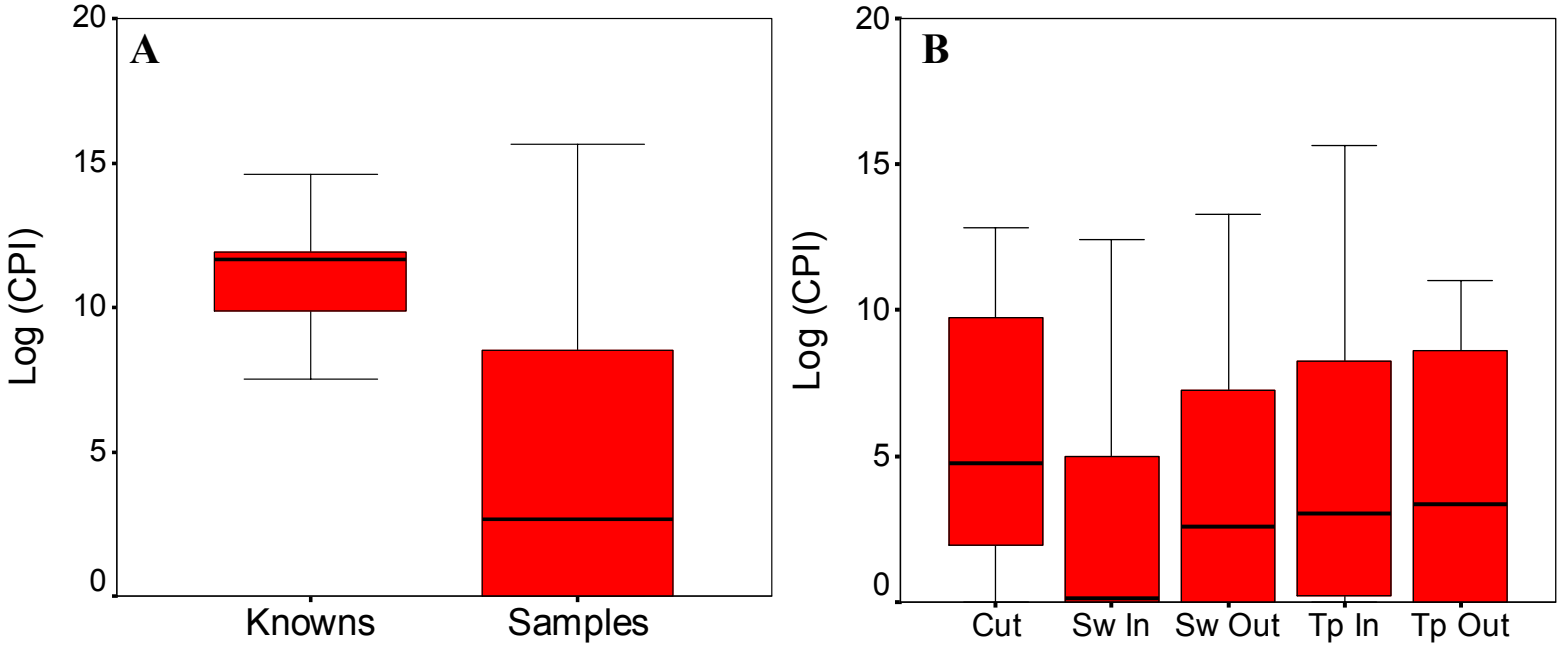

Figure 9. Combined probability of inclusion (CPI) statistics for known and T-shirt profiles (displayed as log of CPI). A) Comparison of CPI's between known reference profiles and profiles from T-shirts (n=20 for knowns, n=100 for samples). B) CPI's from T-shirt samples using the different collection methods (n=20 per each method; Cut=cutting, Sw=swabbing, Tp=tape-lift, In=inside, Out=outside).

Table 3. Wilcoxon Signed Rank pairwise comparisons (2-tailed) for CPI values using different collection methods (those with higher mean rank sum listed first).

Pair		P-value
Cutting	Swab Inside	0.076
Cutting	Swab Outside	0.156
Cutting	Tape Inside	0.709
Cutting	Tape Outside	0.062
Tape Inside	Swab Inside	0.017*
Tape Inside	Swab Outside	0.064
Tape Inside	Tape Outside	0.681
Tape Outside	Swab Inside	0.177
Tape Outside	Swab Outside	0.811
Swab Outside	Swab Inside	0.569

* indicates a significant difference

Model of DNA Quantity and Combined Probability of Inclusion

There was a positive linear relationship (R=0.421) between DNA quantity and log of the CPI when all DNA extracts were included in the regression analysis (Fig. 10). The DNA concentration of the extract explained 17.7% of the variation in the CPI. For profiles that were generated with less than 1 ng (DNA quantity <222 pg/ pg/µL), there was an even stronger positive linear correlation (R=0.643) and more of the variation (41%) of the profile statistic was explained by the DNA concentration of the extract (Fig. 11).

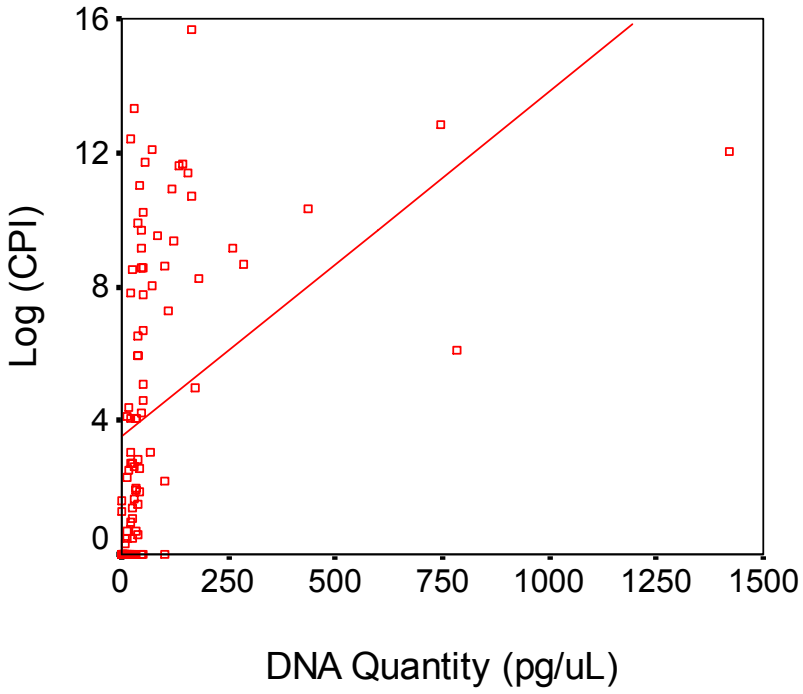

Figure 10. Linear regression of DNA quantity (pg/µL) versus log CPI for T-shirts (y=0.0103x+3.5156, R=0.421, R^2=0.177).

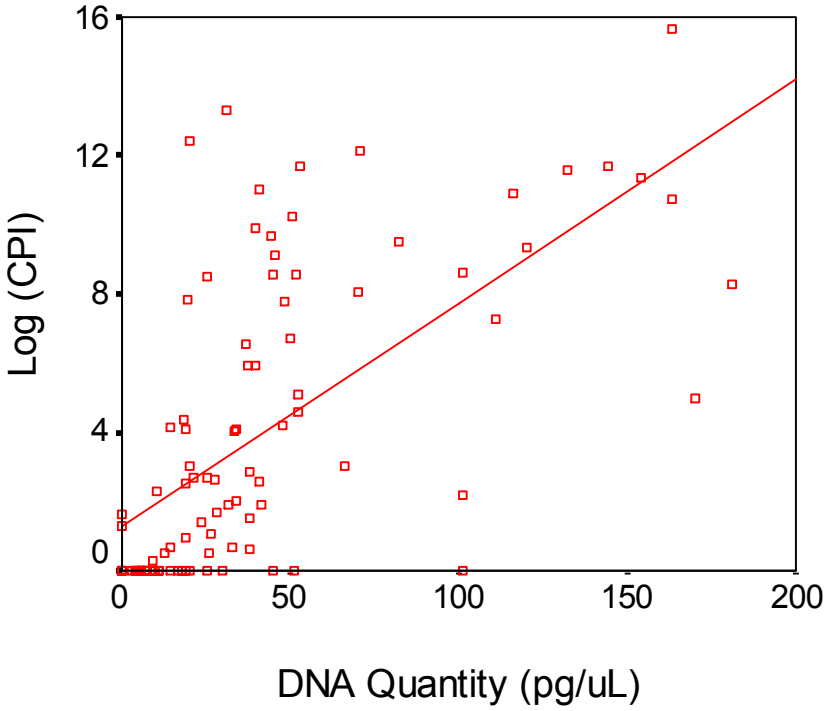

Figure 11. Linear regression of DNA quantity (pg/µL) versus log CPI for T-shirts with suboptimal DNA added to STR amplification reactions (y=0.0646x+1.2793, R=0.643, R^2=0.413).

Part II: T-shirt Handler Identification Following Simulated Assault

DNA Quantification

Processing the 20 shoulder seams of worn and handled T-shirts using the three collection techniques produced one cutting, two tape-lifts (inside and outside), and two swabbings (inside and outside), for a total of 100 DNA extracts. There was no significant difference in DNA quantities from the inside and outside of the T-shirt for either the tape-lift (Wilcoxon Signed Rank, $p=1.000$) or swabbing ($p=0.852$) methods (Fig. 12A, Appendix A). There was a significant difference (Friedman, $p<0.001$) among the methods used (inside and outside DNA quantity summed, if applicable; Fig. 12B): cutting resulted in significantly more DNA than tape-lifting (Wilcoxon Signed Rank, $p=0.003$) and swabbing ($p<0.001$); tape-lifts resulted in significantly more ($p=0.006$) DNA than swabbing.

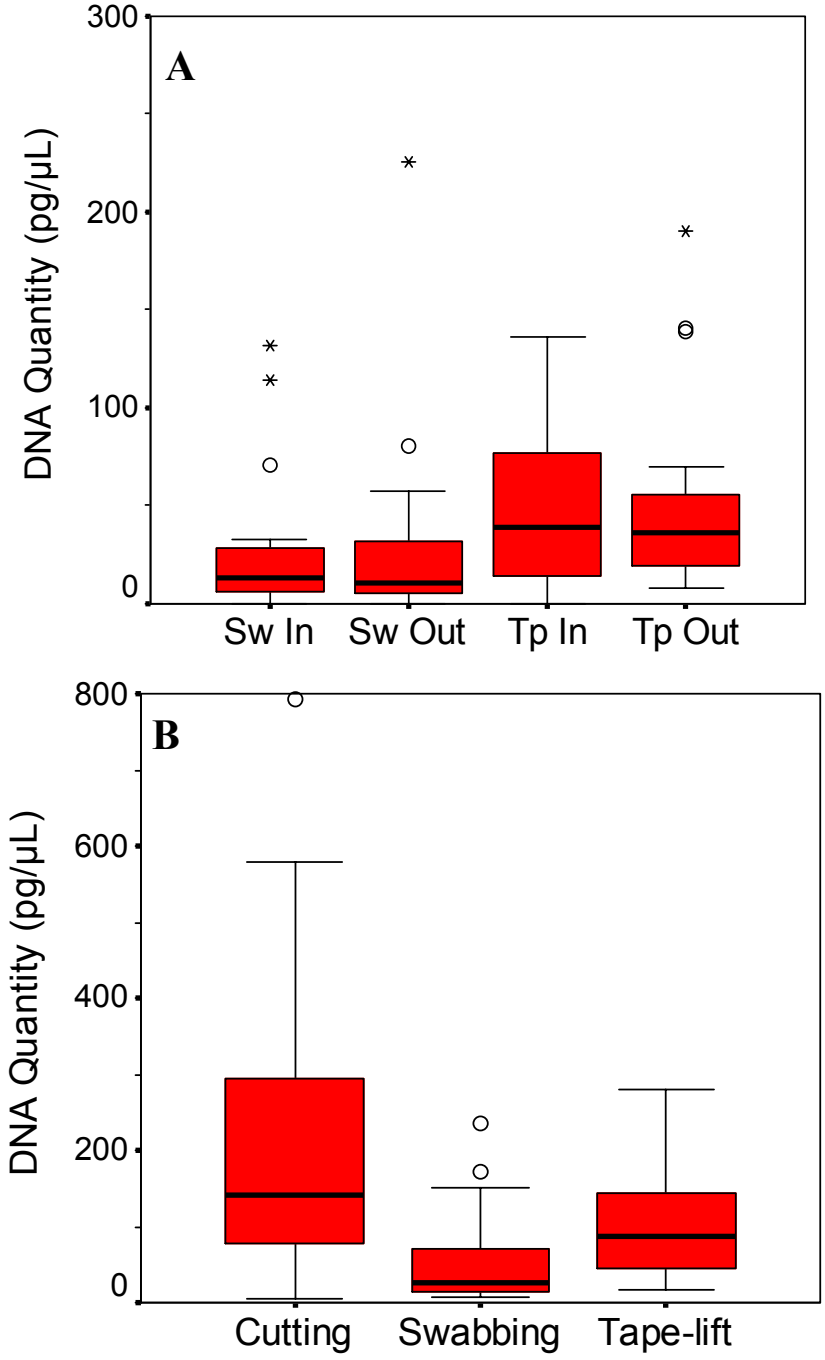

Figure 12. DNA quantities (pg/µL) from T-shirts following a simulated assault. A) Quantities from the inside and outside using the swabbing and tape-lift methods (n=20 per each method; Sw=swabbing, Tp=tape-lift, In=inside, Out=outside). B) Quantities for each method when inside and outside yields were summed (n=20 for cutting, n=40 for swabbing and tape-lift).

STR Profiles: Non-owner Alleles and Drop-out

The major profile from every T-shirt was that of the owner, even after simulated assault (Appendix B). The extent of non-owner alleles and locus drop-out from handled T-shirt profiles is shown in Table 4. Allelic activity below the 150 RFU threshold was present in every T-shirt and in extracts from every collection method. Non-owner alleles existed in 49% of profiles including 85% of cuttings, 27.5% of swabbings, and 52.5% of tape-lifts. Profiles from cuttings contained at least twice the number of non-owner alleles per profile than did swabbings or tape-lifts. Slightly more non-owner alleles were present in profiles when cell recovery occurred on the outside of the T-shirt compared to collection of cells from the inside. Cuttings resulted in more alleles that were consistent with the handler (125 alleles) than when using all the other methods combined (105 alleles).

Table 4. Non-owner alleles and the absence of alleles at loci (drop-out) in T-shirts profiles following a simulated assault (n=20 profiles for each method; Al=non-owner alleles, Lo=loci, Pr=profile, Af=affected, Ha=handler alleles).

		Cutting	**Swabbing**		**Tape-Lift**		**Average**
			Inside	**Outside**	**Inside**	**Outside**	
Non-owner Alleles	# of Al	201	22	26	55	95	80
	Al/Pr	10.1	1.1	1.3	2.8	4.8	4.0
	% of Af Pr	85%	20%	35%	50%	55%	49%
	Al/% Af Pr	11.8	5.5	3.7	5.5	8.6	7.0
	# of Ha	126	16	15	20	54	46
	% of Ha/Al	62.7%	72.7%	57.7%	36.4%	56.8%	57.3%
Drop-out	# of Lo	45	210	170	133	117	135
	Lo/Pr	2.3	10.5	8.5	6.7	5.9	6.8
	% of Af Pr	45%	90%	70%	100%	65%	74%
	Lo/Af Pr	5.0	11.7	12.1	6.7	9.0	8.9

Profiles that contained drop-out (74%) included 45% of cuttings, 80% of swabbings, and 82.5% of tape-lifts (Table 4). The DNA quantity (37.6 ± 6 pg/μL) of profiles that had drop-out at one or more loci was significantly less than that of profiles with no drop-out (180.4 ± 39 pg/μL; Mann Whitney, $p<0.001$). Drop-out at eight or more loci occurred in 45% of profiles (10% of cuttings, 72.5% of swabbings, 40% of tape-lifts) and the average DNA quantity of which (22.7 ± 7 pg/μL) was significantly less than that of profiles where it occurred at 1–7 loci (60.7 ± 9 pg/μL; Mann Whitney, $p<0.001$). Drop-out occurred less frequently in cuttings (45%) than the other methods; the most profiles that experienced drop-out (100%) were collected from tape-lifts of the inside. Of profiles that had any drop-out, those that were swabbed had the greatest average number of loci (11.7, swabbings of the inside; 12.1, swabbings of the outside) without allelic information.

Handler DNA Detection and Haplotype Frequency of T-shirt Profiles

The Y amelogenin allele was most often detected in cuttings or in profiles that were collected from the outside of the T-shirt (Table 5). Yfiler haplotype frequencies from T-shirt DNAs were significantly lower than reference profile frequencies (Wilcoxon Signed Rank, $p<0.001$, Fig. 13A). There was also a significant difference in haplotype frequency among the collection methods (Kruskal Wallis, $p=0.011$, Fig. 13B). Cuttings resulted in significantly greater frequencies than both swabbings (inside and outside) and tape-lifts from the inside but not tape-lifts of the outside; tape-lifting the outside resulted in significantly higher haplotype frequencies than analysis of profiles collected by swabbing the outside or tape-lifting the inside (Table 6). Haplotype frequencies from these tape-lifts tended to be high; however, of the autosomal STR profiles, only 33.3% (4/12) had Y alleles above the 150 RFU threshold.

Table 5. Detection of the Y chromosome amelogenin allele in T-shirt profiles following simulated assault (Pr=profile, Above Threshold=Y allele peak above the 150 RFU threshold, Above Background=Y allele peak above background).

		Cutting	Swabbing		Tape-Lift		Total
			Inside	Outside	Inside	Outside	
Above Threshold	# of Pr	13	0	2	0	4	19
	% of Pr	65%	0%	10%	0%	20%	19%
Above Background	# of Pr	15	2	6	3	12	38
	% of Pr	75%	10%	30%	15%	60%	38%

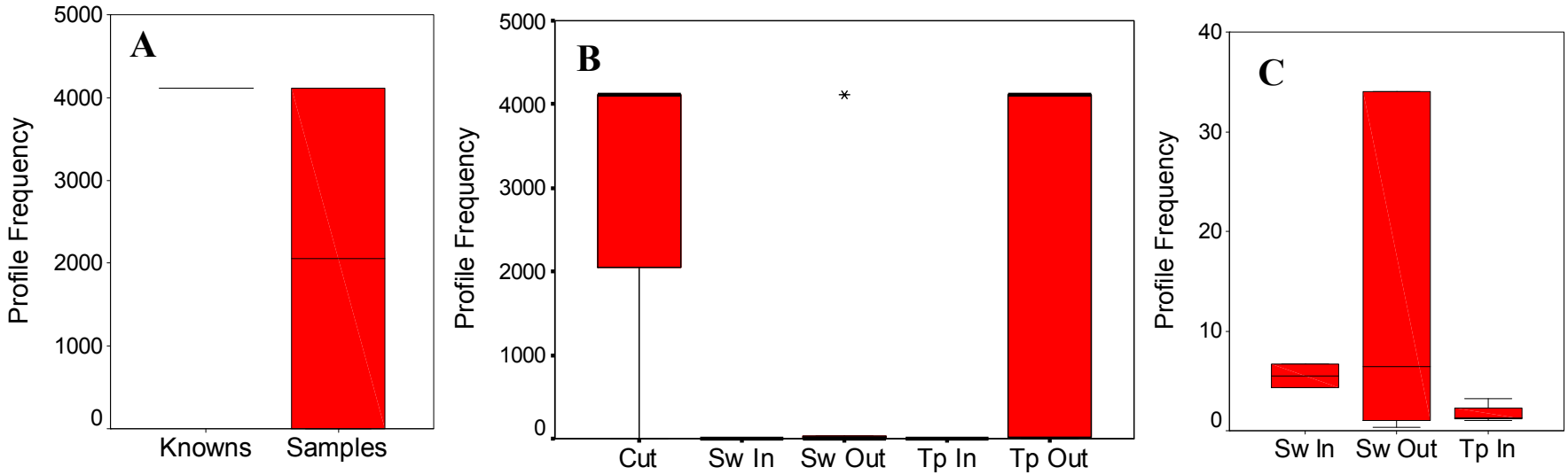

Figure 13. Yfiler haplotype frequency statistics for known and T-shirt Yfiler profiles. A) Comparison of Yfiler profile frequencies between known reference profiles and profiles from T-shirt DNAs (n=16 for knowns, n=38 for samples). B) Comparison of Yfiler profile frequencies from T-shirts using the different collection methods (Cut=cutting, Sw=swabbing, Tp=tape-lift, In=inside, Out=outside; Cut, n=15; Sw In, n=2; Sw Out, n=6; Tp In, n=3; Tp Out, n=12).

Table 6. Mann Whitney pairwise comparisons (2-tailed) for haplotype profile frequencies using different collection methods (those resulting in lower mean rank sum listed first).

Pair		P-value
Cutting	Swab Inside	0.030*
Cutting	Swab Outside	0.011*
Cutting	Tape Inside	0.009*
Cutting	Tape Outside	0.447
†Swab Inside	Swab Outside	1.000
Swab Inside	Tape Inside	0.083
Tape Outside	Swab Inside	0.242
Swab Outside	Tape Inside	0.793
Tape Outside	Swab Outside	0.044*
Tape Outside	Tape Inside	0.027*

* indicates a significant difference

† mean rank sum equal between methods

Model of DNA Quantity and Combined Probability of Inclusion

There was a positive linear relationship (R=0.469) between DNA quantity and log(CPI) when all DNA extracts were included in the regression analysis (Fig. 14). The DNA concentration of the extract accounted for 24.5% of the variation in the CPIs. For profiles that were generated with less than 1 ng (DNA quantity <222 pg/μL), there was an even stronger positive linear correlation (R=0.670) and more of the variation (45%) of the CPI was explained by the DNA concentration (Fig. 15).

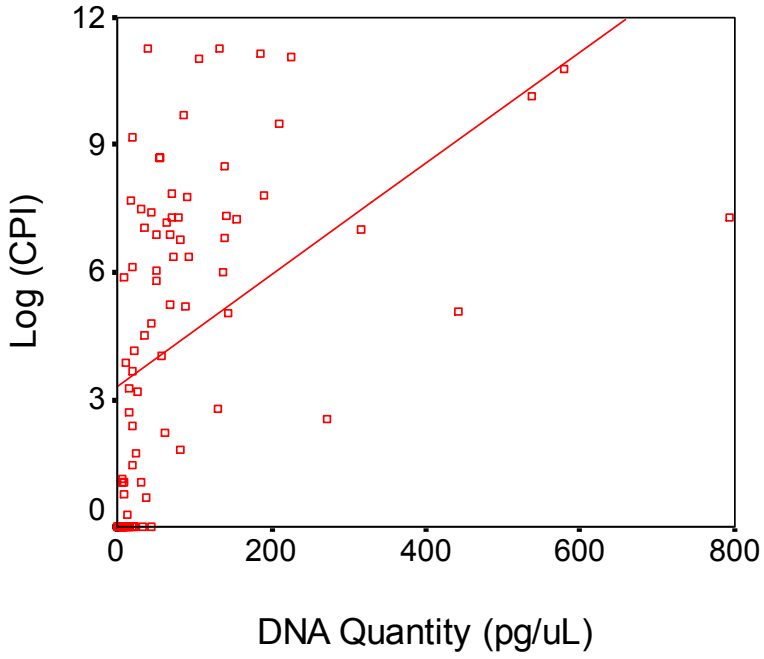

Figure 14. Linear regression of DNA quantity (pg/µL) versus log CPI for worn and handled T-shirts (y=0.0148x+2.6947, R=0.469, R^2=0.245).

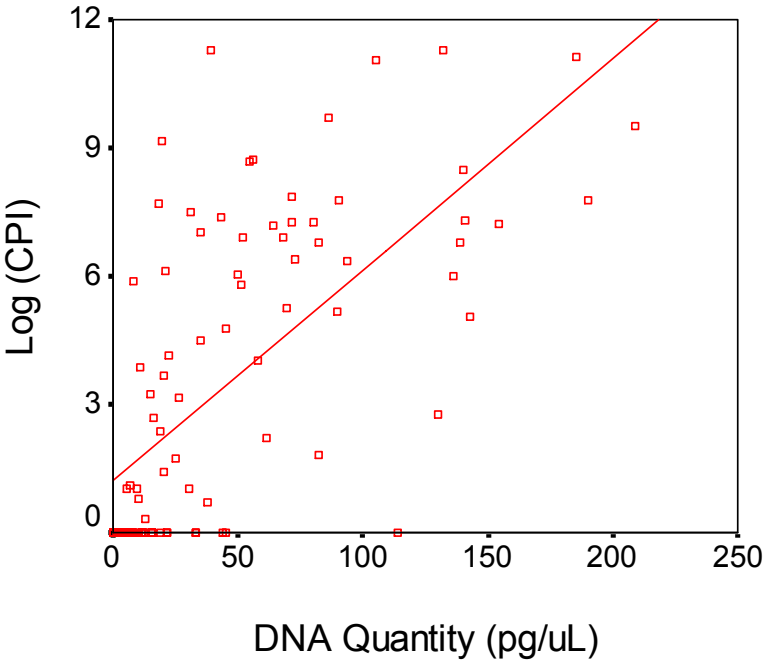

Figure 15. Linear regression of DNA quantity (pg/µL) versus log CPI for worn and handled T-shirts with suboptimal DNA added to STR amplification reactions (y=0.0492x+1.2335, R=0.670, R^2=0.449).

DISCUSSION

The analysis of DNA can be critical in linking a victim and suspect to each other or to a crime scene. The success of such analyses is dependent on the collection of as many cells and hence, as much DNA as possible. In order to determine the optimal technique for processing evidence and maximizing DNA yield, cell recovery methods need to be thoroughly investigated for all types of evidence.

Various forms of fabric evidence, such as carpets, beddings, and articles of clothing, are commonly found at crime scenes and may contain DNA from the victim or perpetrator. Several methods have been used to collect trace DNA from fabric items: cutting out sections of the evidence, swabbing the evidence directly, or performing tape-lifts. The goals of this study were to determine which method(s) are most favorable for collection of owner DNA from worn T-shirts and handler DNA from shirts that were touched during a simulated assault. The findings demonstrate that cutting recovers the most cells, increasing DNA yields and the profile statistics of STR profiles with Y-STR analysis being the most informative in identifying T-shirt handlers.

Collecting cells from the every area of a fabric item is impractical, therefore, it is necessary to target those that will likely yield full STR profiles and result in the most genetic information. In this study, the top of the collar and shoulder regions were the only ones from which DNA concentrations surpassed the recommended level of DNA to add to a STR amplification reaction (approximately 1 ng). These regions, compared to many of the others tested (e.g., sleeve and arm), are in constant contact with the skin of the wearer, increasing the potential for shed epithelial cells to be transferred to the fabric. Friction caused by movement of the head and neck may also cause additional cells to be deposited in the collar region.

The shoulder region was used in this research in order to make direct comparisons between the T-shirt owner portions of the study. However, profiles with more allelic information, and potentially higher profile statistics, may be generated from DNA from the upper collar region compared to those from the shoulder based on the difference in yield between the two. More DNA (approximately 100 pg) could be added to amplifications from collar DNA extracts, which greatly affects the STR profiles. Unlike the other regions, the fabric of the collar was folded over and sewn into the seam, which provided an extra area for trapping cells, thus collection from this region may provide the best chance of identifying the T-shirt owner.

Given the variability in DNA yields from different shirt areas found in this study, the collection location of owner and handler DNA should be determined on a case-by-case basis. Sampling locations for the collection of owner DNA in areas of lesser contact, such as the arm or sleeve hem, while not producing the highest DNA yields, may avoid areas where cells have been transferred through ordinary activity. However, a decrease in DNA quantity also results in more allelic drop-out, which decreases the CPI of a STR profile. The sampling location of handler DNA from another individual's clothing should be based on statements made by victims or eyewitnesses if available. For example, cell collection from the shoulder would not be useful when the victim described an incident where the perpetrator grabbed the fabric at the back of his neck.

The fact that cutting recovered more DNA from the fabric than the other methods from both the T-shirt owner and handler indicates that the swabbing and tape-lift methods have limitations. This is evidenced by that fact that non-owner alleles in handled T-shirts were present in almost equal number to those in non-handled T-shirts for the swabbing and tape-lift methods, but non-owner alleles in cuttings from handled shirts more than quadrupled compared to non-

handled. The swab and tape may have collected only the surface cells and not those from within the fabric in a manner that was as effective as recovery using the other methods. The range in DNA quantity was twice as large for cuttings than for tape-lifts, which may be due to a 'maximum binding/collection capacity' of the tape. Once a layer of material had been collected after applying and reapplying the tape to the shirt, it no longer adhered to the fabric surface, and may not have been able to recover additional cells. The cutting method has the capability to recover more cells without the collection restrictions of swabbing or tape-lifting.

The fact that non-owner alleles were present in half of all STR profiles during the T-shirt owner study indicates that cell transfer happens readily, even when it is not purposeful. Although the length of contact between the T-shirt and other individuals in this study is unknown, brief contact has been shown to deposit cells on touched items (van Oorschot and Jones, 1997; Lowe et al., 2002; Daly et al., 2012), thus it is not surprising that other alleles were present after wearing a shirt for six hours or more. In some cases (see T-shirt #4, 9, 15, 18 in Appendix B) minor profiles from non-owners existed that were consistent in DNA extracts from the same shirt collected using different methods. Despite the prevalence of non-owner alleles however, a full profile from a non-owner was never developed. This means that finding a full profile from a non-owner in a forensic setting indicates a strong association between the T-shirt owner and the other individual.

Preparation of collection materials used to collect cells is of great importance in the recovery and analysis of trace DNA. Swabbings had the fewest non-owner alleles, which may be due, in part, to the fact that swabs were rotated during irradiation and placed at an angle to the light. In contrast, only the adhesive side of the tape-lift was exposed to UV light with all components, including the backing, added to the digestion solution, which may have introduced

non-owner cells. However, non-owner alleles were not present in amplifications of negative controls although low levels of DNA were detected in some extracts; therefore, the origin of these alleles is unknown. Ideally, collection materials would be irradiated or autoclaved prior to cell collection, as was the case with swabs.

Individuals wearing shirts are in constant contact with the fabric, which seemed to be enough to deposit cells throughout the thin T-shirt fabric, therefore material thickness should be considered when collecting biological material from fabric evidence. When the swabbing and tape-lift methods were used to collect owner cells, there was no difference in DNA recovery based on whether the inside or outside of the shirt was tested. With very thin fabrics, it is feasible that sampling the outside collects cells that have been lodged into it from the inside. In this study, the location of handler alleles (inside or outside surface) differed between the methods with more handler alleles collected on the inside for swabbing and on the outside for tape-lifts. After simulated assault, the number of non-owner alleles in cuttings quadrupled over those present in cuttings from the T-shirt owner study, while the number using the other two methods stayed relatively the same, supporting the idea that handler cells are not collected solely on the surface of shirt but also from within the fabric. However, results from this research may not apply to fabrics that are thicker or have a tighter weave. Thick or dense fabrics, such as wool, leather, rainwear, etc., could easily provide more of a barrier to cells, preventing the deposition and collection of owner cells from the outside or handler cells from the inside. For these fabrics, autosomal STR analysis may prove to be more informative for profiling DNA from a single surface because less owner DNA would be collected from the outside and less handler DNA from the inside, resulting in more genetic information.

The influence of non-owner alleles and allelic drop-out on profile interpretation is not effectively measured without the use of profile statistics, which were utilized in this research to assess differences among the collection techniques. In other research, DNA profiles have often been placed into categories such as full, strong partial, weak partial, and no profile (Horsman-Hall et al., 2009). These groupings, while potentially informative, are based on arbitrary boundaries and may separate profiles that are extremely similar. For example, two profiles could be placed in separate categories based on a single allele in one of the profiles (e.g., strong partial=more than 50% of expected alleles present, poor=less than 50% of expected alleles present). However, the distinction between the two may have very little practical difference. Additionally, profiles that include multiple sources of DNA can have more than the expected number of alleles and cannot be placed into one of the aforementioned categories. Using profile statistics, the presence of non-owner alleles and drop-out at a given locus will decrease the CPI compared to that of the known reference profile, with drop-out having a greater effect. This is because the lack of allelic information causes that locus to be excluded in the calculation of the profile statistic whereas the addition of additional alleles only increases the probability that an individual could be a contributor. Profile statistics that take into account the number of alleles as well as the allelic frequency provide an objective method of comparison and are based on calculations that are applicable in the legal system.

The overall wide range in CPI for all DNA collection methods may be explained by the fact that there was a corresponding range in known owner profile CPIs. Drop-out of one allele at a heterozygous locus in T-shirt profiles resulted in the appearance of homozygosity, altering the genotypic frequency of that locus in the CPI calculation. In order to make comparisons between known and T-shirt profiles unbiased, CPIs were generated by excluding homozygous loci, which

artificially decreased the CPI of the known profiles. The lowest and highest reference CPIs calculated were separated by many orders of magnitude, 1 in 10 million (10^7) and 1 in 100 trillion (10^{14}), respectively, the former value originating from an individual with a high level of homozygosity. Because the maximum CPI possible for T-shirt profiles depends on the frequency of the known STR profile of the owner, the varying levels of homozygosity among volunteers artificially increased the range in CPIs, regardless of any actual differences among the methods used to collect cells.

Another possible explanation for the wide range in CPIs is that DNA quantity and quality is influenced by the variable nature of this type of DNA. The amount of DNA on a touched object is not consistent due to the characteristics of the individual depositing the cells and the environmental conditions the cells are exposed to that may lead to degradation. Previous research has shown that the propensity of an individual to shed epithelial cells, handwashing, personal habits (e.g., face touching, perspiration), and type of contact all affect the cells available for transfer to handled objects (Lowe et al., 2002; Wickenheiser, 2002; Phipps and Petricevic, 2006; Goray et al., 2012; Quinones and Daniel, 2012). Conditions, such as substrate type and exposure to the environment also have an impact on the persistence of trace DNA with the collection of cells from outdoor surfaces decreasing substantially two weeks after cell deposition (Raymond et al., 2009; Linacre et al., 2010).

The factors that influence the quality of cells that are deposited during contact may explain much of the variation in profile statistics not attributable to DNA quantity. The regression models demonstrated that CPIs from the T-shirt owner and handler studies were related to DNA yields. However, the relationships were not strong, which was likely due to the variability of touch DNA. For practitioners, this means that a low DNA quantity should not

exclude STR analysis from being conducted because many amplification reactions that contained less than the recommended DNA input resulted in informative profiles.

Unlike the T-shirt owner study, Y chromosome haplotype frequencies were used to compare any differences between the DNA collection methods for handled T-shirts due to the widespread presence of owner DNA. For example, the "best" method for collecting handler DNA would recover a full profile both from the owner and handler, resulting in a two-person mixture. However, another collection method may fail to collect handler cells, recovering only owner DNA; the CPI from this would be higher than that from the mixture, even though the first method did a comparatively better job at collecting the handler's cells. Therefore, separating the male component of the DNA was necessary to assess which technique was best for collecting handler DNA from T-shirts. While this method of analysis is less informative than using an autosomal STR statistic, assaying Y chromosome-specific alleles is common and very useful in context of the male perpetrator–female victim scenario.

Cutting results in the greatest likelihood of obtaining handler DNA from worn and handled T-shirts, along with higher haplotype frequencies than the other methods. The haplotype statistics also demonstrate that there is utility in re-examining DNA extracts that do not surpass the RFU threshold by using additional assays such as Yfiler that can target specific components of the mixture as several extracts with Y alleles below the 150 RFU threshold were analyzed and resulted in informative Y-STR profiles. However, crime laboratory protocols limit continued analysis on DNAs that do not meet certain requirements, in this case, the peak height minimum. While alleles below threshold should not be used in profile statistics such as CPI, guidelines from SWGDAM (2010) state that these alleles can be used to establish the presence of a mixture. Below threshold alleles that are reproducible indicate that the genetic source is in low quantity;

such alleles are useful for excluding suspects but may not provide substantial evidence for the inclusion of an individual as a contributor to the biological material analyzed.

The successful analysis of trace DNA evidence depends on many individuals involved in its collection and processing including police officers, evidence technicians, and laboratory analysts. Communication between investigators is critical for the proper identification and collection of biological material. Responding officers are responsible for conducting preliminary interviews with those present during or shortly after the commission of a crime. The statements from victims and eyewitnesses are then used by detectives to recover physical evidence, providing support for statements that identify a suspect (Bayley, 1994). Therefore, detectives should be prompted to inquire about potential areas of biological information; noting details regarding the precise location of any contact between the criminal and his or her environment will enable those collecting the evidence to recognize areas or items that may contain touch DNA more easily.

Obtaining and packaging items that potentially contain contact DNA should be done with utmost care. Personnel need to be trained how to wear and use gloves and other protective gear that prevent their cells from being transferred to evidentiary items during crime scene processing. The development of training programs to inform officers about touch DNA, including collection, storage, and the likelihood of success (Caddy et al., 2008) will enable police to fully understand the importance of preventing biological evidence contamination, especially in cases that might involve trace DNA. This research found that transfer of cells occurred through the normal activity of T-shirt owners, therefore, fabric evidence that may contain trace DNA from the perpetrator of an assault should be stored and transported in a way that prevents the transfer of cells from the initial area of contact. In some cases, it may be advantageous to cut out the portion

that was touched and package it separately from the rest of the item. This would allow forensic technicians to sample the contact area without having to interpret the details found in the report, increasing the chance of obtaining only the cells related to the case.

In terms of laboratory policy, this research indicates that standard operating procedures should list the cellular collection method to use based on the evidence type and situational characteristics of the crime. The methods to be performed can be differentiated based on thick or thin fabrics, and protocols could detail which assays, e.g. autosomal or sex-specific STR analysis, should be used in the context of the criminal case. Additional studies that examine other types of fabrics, varying in thickness and composition, will shed further light on how to collect DNA evidence, providing investigators with the best chance to solve crimes.

Expanding the surface area that is sampled may increase the number of cells collected, but the disadvantage of collecting more non-owner or other extraneous DNA may outweigh the benefits of greater DNA quantity. In this study, all collection techniques were conducted on cut T-shirt sections of equal size. While this made the cutting method easier (the T-shirt section was added directly to the digestion buffer), it made the other methods more difficult, which extended the time spent handling the fabric, both increasing the potential for contamination and decreasing productivity. In casework, however, tape-lifts and swabs could be used on intact pieces of fabric evidence. If cell collection occurred over a larger area, recovering more owner DNA would reduce drop-out, but other sources of DNA may also be attained. In the case of T-shirt owner identification, this is not problematic, as the number of owner cells greatly outnumbers those from non-owners. However, when trying to identify a T-shirt handler, the availability of more owner cells may cause handler alleles to drop-out in STR profiles due to the overwhelming amplification of the major DNA source.

Although this research was conducted under controlled conditions where volunteers wore the T-shirts for a single day or handled the T-shirts in a prescribed manner, in casework, it is probable that a victim or perpetrator's T-shirt will have been worn more than once. Previous research has shown that, even following laundering, cells are retained on clothing and that cells from one item can be transferred to another during machine washing (Kafarowski et al., 1996). Owner cells might accumulate following continued wearing and washing, making identification of the owner easier but increasing the difficulty in discerning the T-shirt handler, creating a similar problem as sampling larger areas. Therefore, the possibility of shared laundry or similar clothing contact need to be considered in the processing and interpretation of trace DNA evidence.

PCR inhibition of DNA extracts did not occur with the T-shirt substrates used for this study. Other articles of clothing, such as colored T-shirts or treated fabrics (e.g., water resistance, fire retardant), may contain dyes or compounds that interfere with PCR. Even though the greatest DNA yields were found using the cutting method, this may also collect inhibitors, preventing successful genetic typing. Cell recovery using tape-lifts has already been shown to avoid inhibitors in some substrate types (Bright and Petricevic, 2004; Sewell et al., 2008). Alternative techniques should be employed if known inhibitors, such as indigo dye, are present in the clothing evidence being analyzed.

Due to the allelic information below threshold found in many T-shirt profiles, these data are classified as trace and should be analyzed carefully. Some researchers have advocated the exclusion of this type of DNA profiling in criminal cases because the chance of adventitious matches are increased from such extremely sensitive analysis techniques (Budowle, 2009; Raymond et al., 2009). However, DNA evidence can be given a statistical value that can be used

justifiably even in cases of trace DNA typing. This means that comprehension of the statistical values given to DNA evidence is of great importance, especially by those that adjudicate legal proceedings. Implementing protocols that enhance DNA yield based on this research, supported by court-admissible statistics like CPI and haplotype frequency, may increase the number of biological exhibits that could be used in criminal prosecution.

CONCLUSION

This study showed that cutting, swabbing, and tape-lifting are viable techniques for collecting DNA from fabric evidence, however, these techniques are not equally effective. An ideal cell recovery method collects as many cells as possible and requires only a few manipulations, limiting the possibility for contamination. The cutting method should be used to collect cells when attempting to identify a T-shirt owner. This method consistently had the greatest DNA yields, resulting in the most allelic information and the highest CPIs. For identifying T-shirt handlers, the cutting method was also most successful at collecting handler cells, leading to Y-STR profiles with the highest haplotype frequencies.

Genetic profiles can be generated from low quantities of DNA, therefore, comparing the techniques used to collect cellular material from briefly touched objects, which generally contain very few cells, is of great value. It is well established that deposition of cells varies considerably depending on many factors, but forensic examiners have the ability to maximize cellular collection by using the technique most appropriate for the substrate. Improving the way trace amounts of biological materials are collected can greatly affect the approach of a criminal investigation and the result of criminal prosecution, resulting in higher clearance of crimes where physical contact between individuals has occurred.

APPENDICES

APPENDIX A

DNA QUANTIFICATION DATA

Table A1. DNA quantities (pg/µL) from T-shirt owner samples recovered from the collar and shoulder regions. Positive controls averaged 85.3 pg/µL (n=3).

T-shirt #	Collar			Shoulder		
	Cutting	Swabbing	Tape-lift	Cutting	Swabbing	Tape-Lift
1	23.2	1.91	15.5	10.0	67.3	9.97
2	48.0	20.1	29.6	14.5	5.25	18.7
3	93.0	42.1	99.7	41.4	0.00	7.76
4	62.7	40.2	32.9	195	18.1	355
5	43.2	23.3	65.2	103	2.60	35.2
6	176	22.5	69.9	150	31.2	41.0
7	89.1	30.9	67.2	480	80.1	45.1
8	42.8	4.24	54.8	33.8	0.00	29.0
9	1050	153	133	397	26.1	109
10	39.7	33.8	22.3	63.2	7.98	110
11	307	34.3	122	55.5	4.09	28.6
12	32.1	2.21	25.4	28.3	99.8	47.8
13	60.3	10.4	15.3	6.64	1.02	44.9
14	277	18.4	46.9	37.7	11.7	121
15	141	12.2	171	210	0.00	44.8
16	12.2	8.62	106	20.9	1.19	27.0
17	13.7	1.85	39.6	17.1	2.61	6.52
18	176	11.6	47.3	233	4.53	47.8
19	155	15.7	54.1	22.8	4.95	28.8
20	186	67.7	553	78.7	9.76	47.0

Table A2. DNA quantities (pg/µL) from T-shirt owner samples recovered from the shoulder seam using the various methods. Positive controls averaged 96.2 pg/µL (n=3).

T-shirt #	Cutting	Swabbing		Tape-Lift	
		Inside	Outside	Inside	Outside
1	33.0	7.50	181	41.2	7.66
2	52.2	6.03	0.00	16.5	26.2
3	101	34.1	14.4	25.0	51.8
4	434	116	81.9	144	782
5	154	45.2	27.7	132	40.8
6	170	37.3	19.8	47.8	120
7	284	21.0	50.2	111	18.3
8	10.6	17.9	19.1	51.0	20.4
9	1420	52.6	39.7	70.8	39.6
10	101	38.1	36.7	23.8	37.8
11	45.0	20.0	9.60	0.00	25.2
12	12.4	4.28	0.00	4.35	5.41
13	28.2	0.00	0.00	25.7	5.76
14	261	14.3	7.17	8.92	25.3
15	52.3	4.02	14.6	48.3	44.3
16	31.7	2.76	10.7	29.8	19.0
17	33.7	9.03	6.92	20.3	18.9
18	746	44.8	40.8	70.2	101
19	18.8	5.83	10.8	66.3	50.7
20	163	37.8	31.1	163	33.2

Table A3. DNA quantities (pg/µL) from handled T-shirt samples recovered from the shoulder seam using the various methods. Positive controls averaged 182 pg/µL (n=2).

T-shirt #	Cutting	Swabbing		Tape-Lift	
		Inside	Outside	Inside	Outside
21	143	15.6	25.2	30.7	20.0
22	130	32.7	57.7	105	37.4
23	64.0	114	0.00	0.00	69.6
24	271	10.4	226	82.5	43.8
25	82.5	18.9	0.00	33.2	45.1
26	93.9	0.00	11.7	136	12.7
27	140	19.4	20.8	45.0	139
28	794	71.1	80.1	90.2	190
29	185	16.3	4.38	61.2	54.8
30	536	30.4	20.0	51.4	35.1
31	316	26.2	6.57	89.5	55.9
32	442	132	39.2	71.5	35.2
33	11.1	6.81	1.77	12.6	15.3
34	579	1.29	43.4	68.0	21.8
35	209	9.39	7.76	15.0	141
36	3.62	2.02	5.69	2.94	15.7
37	72.7	6.47	11.7	7.70	8.06
38	86.4	6.72	9.93	19.0	18.3
39	51.7	2.04	5.68	12.4	21.5
40	154	5.57	6.26	22.1	49.8

Table A4. DNA quantities (pg/µL) from unworn T-shirt samples recovered from the shoulder seam using the various methods (SC=substrate control).

T-shirt #	Cutting	Swabbing		Tape-Lift	
		Inside	Outside	Inside	Outside
SC1	7.64	17.8	0.00	15.7	0.00
SC2	0.00	3.36	0.00	0.00	0.00

Table A5. Average DNA quantities (pg/µL) from negative controls (n=3 per control for preliminary and owner study; n=2 per control for handler study).

Study ID	Negative	Swab RB	Tape RB
Preliminary	0.00	2.76	5.60
Part I: Owner	0.00	1.34	0.00
Part II: Handler	0.00	0.00	9.69

APPENDIX B

STR PROFILES

*Tables of autosomal STR profiles from T-shirt owner samples recovered from the shoulder region (red number=non-owner allele; *=allelic activity below 150 RFU threshold; …=no allelic activity).*

Table B1. Autosomal STR profiles from T-shirt 1.

Locus	Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
Amel	XX	XX	…	XX	XX	…
D8	10, 12	*	…	10, 12	*	*
D21	28	*	…	28	*	…
D7	10, 11	*	…	10, 11	*	*
CSF	10, 12	*	…	10, 12	*	*
D3	16, 17	*	…	16, 17	*	*
THO	9, 9.3	9, *	…	9, 9.3	*	*
D13	8, 12	*	…	*	*	*
D16	11, 13	*	…	*	*	…
D2	17, 20	*	…	20, *	*	*
D19	12, 15.2	12, *	…	12, 15.2	12, 15.2	…
vWA	16, 17	16, 17	…	16, 17	*	*
TPOX	8, 9	9, *	…	8, 9	8	*
D18	15, 17	14, *	…	15, 17	*	*
D5	12, 13	*	…	12, 13	*	*
FGA	22, 24	*	…	*	*	…

Table B2. Autosomal STR profiles from T-shirt 2.

Locus	Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
Amel	XY	XY	*	*	*	* Y
D8	11, 15	10, 11, 15	*	15, *	*	15, *
D21	28, 29	28	*	*	*	28, *
D7	8, 11	8, *	*	*	*	11, *
CSF	12	12	*	*	*	12, *
D3	14, 17	17, *	*	*	*	17, *
THO	9	9	*	*	*	9
D13	11	*	…	*	*	*
D16	12, 13	13	…	*	…	*
D2	18, 22	(17) 22, *	*	22, *	17, *	22, *
D19	12, 14	12, 14	12, *	12, *	*	12, *
vWA	14, 15	15, 17, *	*	*	*	14, *
TPOX	11, 12	11, 12, *	6, *	*	*	12, *
D18	13, 14	14, *	*	14, *	*	13, 14
D5	11	11, *	*	*	*	*
FGA	21, 22	*	…	*	*	22, *

Table B3. Autosomal STR profiles from T-shirt 3.

Locus	Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
Amel	XX	XX	XX	X *	XX	XX
D8	10, 15	10, 15, *	10, *	10, 15, *	10, 15, *	10, 15 (12) *
D21	28	…	28, *	28	28 (29) *	28
D7	11	…	11, *	11, *	11, *	11
CSF	10, 12	…	*	10, *	10, 12	10, 12
D3	17	…	17, *	17, *	17, *	17, *
THO	9	*	9	9	9	9 (9.3)
D13	11, 12	…	11, *	*	11, 12, *	11, 12, *
D16	12, 13	…	*	*	12, 13, *	12, 13
D2	17, 22	…	17, *	17 (22)	17, 22, *	17, 22, *
D19	12	…	12 (14) *	12	12 (14,14.2,15.2) *	12 (15.2)
vWA	15, 17	15, 17	15, 17, *	*	15, 17 (16, 19)	15, 17 (16)
TPOX	8, 11	*	8, 11	8, 11	8, 11	8, 11
D18	13, 15	…	13, 15	15, *	13, 15	13, 15
D5	11, 12	…	11, 12	11, 12	11, 12	11, 12 (13)
FGA	21, 22	*	21, 22	*	21, 22	21, 22, *

Table B4. Autosomal STR profiles from T-shirt 4.

Locus	Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
Amel	XX	XX	XX	XX	X (Y)	XX
D8	10, 13	10, 13 (14)	10, 13 (16) *	10, 13 (14)	(8) 10, 13	10, 13 (14)
D21	29, 32.2	29, 32.2	29, 32.2	29, 32.2	29, 32.2	29 (32,32.2)
D7	12	(10) 12	12	(10) 12	12	(9,10) 12
CSF	10	10	10	10 (12)	10	10 (12)
D3	14, 18	14, 18	14, 18	14, 18 (17)	14, 18	14, 18 (15,17)
THO	7, 9.3	7, 9.3 (9)	7, 9.3, *	7, 9, 9.3	7, 9.3	7, 9.3 (9)
D13	8, 12	8, 12	8, *	8, 12, *	8, 12	8, 12 (11,13)
D16	11, 12	11, 12	11, 12	11, 12, *	11, 12	11, 12 (13)
D2	18, 26	18, 26 (20,25)	18, 26	18, 26 (20,24)	18, 26	17, 18, 26 (20,24)
D19	14	14	14	14	14	(13) 14
vWA	16, 19	16, 19 (18)	16, 19	16, 19 (18)	16, 19	16, 19 (17,18)
TPOX	8, 11	8, 11	8, 11	8, 11	8, 11	8, 11 (12)
D18	11, 18	11, 18	11, 18	11, 18 (12)	11, 18	11, 17, 18 (12)
D5	11, 12	11, 12	11, 12	(10) 11, 12	11, 12	10, 11, 12, 13
FGA	24, 27	24, 27	24, 27	24, 27, *	24, 27	(21) 23, 24, 27

Table B5. Autosomal STR profiles from T-shirt 5.

Locus	Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
Amel	XX	XX	XX	XX	XX	XX
D8	8, 14	8, 14	8, 14	*	8, 14	8, 14
D21	32.2, 33.2	32.2, 33.2	32.2, 33.2	33.2, *	32.2, 33.2	32.2, 33.2
D7	10, 11	10, 11	10, 11	11, *	10, 11	10, 11
CSF	12, 14	12, 14	12	12, *	12, 14	12, 14
D3	15	15	15	15	15	(14) 15
THO	7, 9.3	7, 9.3	7, 9.3	7, 9.3, *	7, 9.3	7, 9.3
D13	12	12	12	12	12	12
D16	9, 12	9, 12	9, 12	12, *	9, 12	9, 12
D2	19, 25	19, 25	19, 25	25, *	19, 25	19, *
D19	12, 14	12, 14	12, 14	12, 14	12, 14	12, 14
vWA	15, 17	15, 17	15, 17	17, *	15, 17	15, 17
TPOX	8	8	8	*	8	8
D18	12, 16	12, 16	12, 16	12, 16	12, 16	12, 16
D5	12	12	12	12	(11) 12	(11) 12
FGA	23, 25	23, 25	23, *	23, *	23, 25	23, 25

Table B6. Autosomal STR profiles from T-shirt 6.

Locus	Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
Amel	XY	XY	XY	XY	XY	XY
D8	13, 15	13, 15	13, 15	13, 15	*	13, 15
D21	29	29, 30.2	29, *	29, *	*	29
D7	10, 11	*	10, 11	10 (8,11,12)	*	10, 11
CSF	11	*	11	11	*	11
D3	14, 16	14, 16	14, 16 (18)	14, 16	14, 16	14, 16
THO	7	7	7	7	7	7
D13	12	*	12	12	12	12
D16	12, 13	*	12, 13	12, 13	*	12, 13
D2	23, 24	…	17, 23 (24)	(18) 23, 24	23, 24	23, 24
D19	12, 13	12, 13	12, 13	12, 13	12 (13)	12, 13
vWA	17, 18	17, 18	(16) 17, 18	17, 18	17, 18	17, 18
TPOX	8	8	8 (11)	8 (11)	8	8
D18	14, 15	…	(13) 14, 15	14, 15	14, *	14, 15
D5	11, 13	11, 13	11, 13	11, 13	11, 13	11, 13
FGA	22, 25	…	22	22, 25	22	22, 25

Table B7. Autosomal STR profiles from T-shirt 7.

Locus	Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
Amel	XY	XY	X *	X *	XY	XY
D8	10, 11	10, 11	*	10, 11, 12	10, 11, *	10, *
D21	28	28	*	28, *	28, *	28, *
D7	8, 10	8, 10	*	8, 10, *	8, 10	*
CSF	10, 12	10, 12	12, *	10, 12, 13	10, *	*
D3	16, 17	16, 17	17	16, 17, *	16, 17	16, 17
THO	9	9	9	9 (9.3)	9	9
D13	8, 11	*	*	11, *	*	*
D16	13	13	*	13, *	13	*
D2	17, 22	17, 22	22, *	17, 22	17, 22	*
D19	12, 14	12, 14	12, 14	12, 14 (13,15)	12, 14	12, 14
vWA	15, 17	15, 17	15, 17	14, 15, 17	15, 17	15, 17, *
TPOX	8, 11	8, 11	8, 11	(8) 11	8, 11	8, *
D18	14, 17	14, 17	14, 17	12, 15, 17	14, 17, *	14, 17, 20
D5	11, 12	11, 12	*	11 (12)	11, 12	11, 12
FGA	22, 24	22, 24	*	22, *	24, *	22, 24

Table B8. Autosomal STR profiles from T-shirt 8.

Locus	Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
Amel	XX	X *	XX	XX	XX	*
D8	13	13 (10,14)	*	13, *	13	*
D21	29, 33.2	*	*	*	*	…
D7	8, 11	11, *	*	8, 11	*	*
CSF	12, 13	*	*	12, 13, *	*	*
D3	17, 18	17, *	*	*	*	*
THO	9, 9.3	*	9, *	9.3, *	9.3, *	*
D13	11, 12	*	*	11, 12	…	…
D16	12, 13	*	*	13, *	*	…
D2	17	17, *	*	17	*	*
D19	12, 15	12, 15, *	*	13, 15, *	13, *	*
vWA	14, 19	17, 18, *	…	19, *	*	*
TPOX	8, 11	8, *	*	11, *	*	*
D18	12, 15	15, *	*	12, 15	*	*
D5	11	11, *	…	11, *	*	…
FGA	19, 22	19, *	…	*	*	….

Table B9. Autosomal STR profiles from T-shirt 9.

Locus	Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
Amel	XY	XY	XY	XY	XY	XY
D8	14, 16	14, 16 (10,13)	14, 16 (10,13)	14, 16	10, 14,16 (13)	14, 16, *
D21	29	29 (32.2)	29	29	29 (32.2)	29, *
D7	10	10 (12)	10 (12)	10	10 (12)	10, *
CSF	10, 12	10, 12	10 (12)	10 (12)	10, 12	10, 12
D3	17, 18	(14) 17, 18	17, 18 (14)	17, 18	17 (14, 18)	17, 18
THO	9	9 (7,9.3)	9 (7)	9	9	9
D13	10, 11	(8,12) 10, 11	10, 11 (12)	10, 11	10, 11, 12	10, *
D16	9, 12	9, 12 (11)	(9) 12	*	9, 12, *	*
D2	20, 24	20, 24 (18,26)	(17) 20, 24	*	20, 24 (18,26)	20, *
D19	14, 19.2	14, 19.2	14, 19.2	14, 19.2	14 (19.2)	14 (12, 19.2)
vWA	16, 18	16, 18	16 (18, 19)	16, *	16, 18	16, 18
TPOX	8, 11	8, 11	8, 11	8, 11	8, 11	8, 11
D18	12, 20	12, 20	12, 20	12, 20, *	12, 20 (11)	12, 20, *
D5	10, 11	10, 11	10, 11	10, 11	10, 11	10, *
FGA	17, 26	17, 26	17, 26	17, *	17, 26, *	*

Table B10. Autosomal STR profiles from T-shirt 10.

Locus	Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
Amel	XY	*	* Y	XY	(X)Y	X(Y)
D8	13, 14	13, *	13, *	*	*	13, *
D21	31, 32.2	…	*	*	*	*
D7	10, 11	…	10, *	10, 11	*	*
CSF	11, 12	…	*	12	*	*
D3	15, 16	…	16, *	15, 16	*	16, *
THO	6, 7	…	*	6, 7	7, *	7, *
D13	12	…	*	*	*	*
D16	9, 11	…	*	*	*	*
D2	23, 25	…	*	23 (25)	*	*
D19	14, 15	…	14, 15	14, *	13, 16.2, *	13, 16.2, *
vWA	15, 16	*	16, *	16, *	15, 16	*
TPOX	8, 10	*	*	8, 11	11, *	8, 11
D18	16, 18	…	…	*	*	*
D5	11, 12	…	11	11	11	11
FGA	23, 25	…	…	…	*	23, 25

Table B11. Autosomal STR profiles from T-shirt 11.

Locus	Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
Amel	XX	XX	XX	XX	XX	X *
D8	8, 11	8 (11,14)	8, 11	*	*	*
D21	32.2, 33.2	*	33.2, *	*	*	*
D7	10, 11	10, 11, *	10, 11	*	*	*
CSF	10, 14	10, 12, 14	10, 14	*	*	*
D3	14, 15	14, 15	14, 15	15	*	*
THO	7, 9.3	(6) 7, 9.3	(7) 9.3	7, *	*	*
D13	8, 12	8, 12	12, *	*	*	*
D16	12	12, *	12	*	…	*
D2	17, 19	17, 19	17, 19	*	*	*
D19	12, 14.2	12, 14.2 (14)	12, 14.2	12, *	*	14, *
vWA	14, 17	14, 17 (15)	14, 17	*	*	*
TPOX	8, 11	8, 11	8, 11	*	8, *	8, *
D18	12, 13	12, 13, 16	12, 13, 16	*	13, 16, *	*
D5	12	12	12, 13	*	…	12
FGA	20, 25	20, 23	20, 25	…	…	*

Table B12. Autosomal STR profiles from T-shirt 12.

Locus	Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
Amel	XX	*	*	*	*	…
D8	13, 15	15	*	15	*	*
D21	27, 29	*	…	…	*	*
D7	8, 10	*	*	*	*	*
CSF	12, 13	*	*	…	*	*
D3	16, 18	16, *	…	*	*	*
THO	9	7, *	*	7, 9	9, *	*
D13	8, 12	*	*	*	…	…
D16	13	*	*	…	*	…
D2	17	*	*	…	*	…
D19	14, 15	13, 14	*	13, *	*	*
vWA	16, 19	19, *	…	*	…	…
TPOX	11	8, *	…	8, *	…	*
D18	12, 17	*	*	…	…	…
D5	11	11	…	10, 11	11, *	…
FGA	19, 22	25, *	…	…	…	…

Table B13. Autosomal STR profiles from T-shirt 13.

Locus	Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
Amel	XY	XY	…	…	*	Y
D8	13, 15	*	*	…	13, *	*
D21	27, 31.2	27, 31.2	…	…	*	*
D7	10, 11	*	…	*	10	*
CSF	12, 13	*	*	…	*	*
D3	15, 16	16, *		…	15	*
THO	8, 9	9	*	*	*	*
D13	8, 13	*	…	…	…	*
D16	13, 14	*	…	…	…	*
D2	17, 23	*	…	*	*	*
D19	12, 14	14, *	*	…	13, 14, *	*
vWA	16, 18	18, *	*	…	*	…
TPOX	8, 11	*	…	*	*	…
D18	15, 17	*	*	…	15	17
D5	11	*	…	…	…	…
FGA	22, 25	…	…	*	…	…

Table B14. Autosomal STR profiles from T-shirt 14.

Locus	Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
Amel	XY	XY	…	*	X *	X
D8	15	15	*	*	*	10, 15, *
D21	29, 32.2	29, 32.2	*	*	*	32.2, *
D7	8, 11	8, 11	*	*	*	*
CSF	10, 11	10, 11	*	*	*	*
D3	14, 16	14, 16	*	*	*	15, *
THO	7	7 (9)	*	*	9, *	7, 9
D13	12	12	*	*	*	*
D16	11, 13	11, 13	*	*	*	*
D2	23, 24	23, 24	*	*	*	17, *
D19	13	13 (14,15)	13	*	13	13, 14
vWA	17, 18	17, 18	*	*	*	19, *
TPOX	8	8 (11)	8	*	8, *	12, *
D18	14, 15	14, 15 (12,17)	*	*	*	*
D5	11, 12	11, 12	…	…	*	*
FGA	22, 25	22, 25	…	*	…	…

Table B15. Autosomal STR profiles from T-shirt 15.

Locus	Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
Amel	XX	XX	XX	XX	XX	X (Y)
D8	10, 12	10, 12	*	12, *	10 (12,13)	10, 12
D21	30, 31	*	…	*	(29) 30, 31, 32	(29) 30, 31
D7	8, 10	*	*	*	8, 10, 12	8, 10
CSF	10, 12	*	…	*	10, 12 (11)	10, 12
D3	16, 17	*	*	*	14, 15, 16, 17	(14) 16, 17
THO	7, 9	7, 9	…	9, *	9 (7,9.3)	7, 9
D13	11, 14	*	…	…	11, 12, 13 (14)	11, 14
D16	11	*	11, *	*	11 (13)	11 (13)
D2	21, 24	*	*	*	17 (19,21,24)	(17) 21, 24
D19	12, 14	12, 14	12, *	12, 14	12, 13, 14	12, 14
vWA	19	19	*	19	17, 19	(17,18) 19
TPOX	9, 11	9, 11	11, *	11	8, 9, 11, 12	(8) 9, 11
D18	17, 18	*	*	…	17, 18	17, 18
D5	12	8, 12	12	12	10, 12, 13	(11) 12
FGA	21	*	21	21	21 (23)	21

Table B16. Autosomal STR profiles from T-shirt 16.

Locus	Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
Amel	XX	XX	…	*	*	XX
D8	13, 15	11, 13, 14, 15	*	*	*	*
D21	30, 31	*	…	*	*	*
D7	10	…	…	…	*	10
CSF	11	…	…	*	*	*
D3	14, 17	*	…	*	*	*
THO	6, 9.3	6, 9.3	…	*	…	9.3, *
D13	11, 12	…	…	…	…	*
D16	11	*	*	…	…	*
D2	19, 25	19, *	…	*	…	*
D19	12, 13	*	…	…	*	12, 13
vWA	15, 16	15, 16	…	*	*	*
TPOX	9 ,11	(8) 9, 11	…	*	*	*
D18	16, 18	…	…	*	…	*
D5	13, 14	…	…	…	…	*
FGA	21, 23	…	…	…	…	*

Table B17. Autosomal STR profiles from T-shirt 17.

Locus	Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
Amel	XY	XY	X *	…	X *	XX
D8	12, 14	12, 14	*	*	14, *	*
D21	28, 29	*	*	…	*	…
D7	8, 12	*	…	…	*	*
CSF	9, 11	*	*	…	*	*
D3	14, 18	*	*	…	17, 17.2, 18	*
THO	8, 9	8, 9	*	…	(7) 8, 9	*
D13	11, 14	*	…	…	…	*
D16	13	*	*	*	*	…
D2	19, 25	19	*	…	25, *	*
D19	15	15, *	*	…	(14) 15	*
vWA	18, 19	18, *	*	…	19, *	*
TPOX	8, 11	8, *	8, 11, *	*	(8) 11	*
D18	14, 16	*	…	…	14, 15, 16	…
D5	11, 12	12, *	*	*	12, *	…
FGA	21	…	…	…	…	…

Table B18. Autosomal STR profiles from T-shirt 18.

Locus	Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
Amel	XX	X (Y)	XX	XX	X (Y)	X (Y)
D8	12, 13	12, 13	12, *	12, 13	(11,12) 13	12, 13 (14)
D21	28, 30	28, 30	*	*	28 (30)	28, 30 (31)
D7	9, 10	9, 10	*	*	*	9, 10, *
CSF	10, 11	10, 11	*	*	10, *	10, 11
D3	15, 16	15, 16	*	*, 16	14, 15, 16	(14) 15, 16
THO	8, 10	8, 10	*	*	6, 8, 9, 9.3, 10	8, 10 (6,9.3)
D13	8, 12	8, 12 (10)	*	…	*	8, 12
D16	11, 13	11, 13	…	*	11,13	11, 13
D2	19, 20	19, 20	…	19, *	(18,19) 20	19, 20 (17,25)
D19	13	13	13	13	13, 14	13 (14)
vWA	16, 19	16, 19	*	16, 19	15, 16, 18	(15) 16, 19
TPOX	9, 11	9, 11	*	9, 11	9, 11	9, 11 (8,10)
D18	15, 18	(12) 15, 18	*	*	12, 13, *	12, 15, 18
D5	10, 13	10, 13	*	*	10, 11, *	(9,10,12) 13
FGA	20, 22	20, 22	…	*	21	20, 22, *

Table B19. Autosomal STR profiles from T-shirt 19.

Locus	Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
Amel	XX	XX	*	XX	X (Y)	XX
D8	14, 15	15, *	*	*	11, 13, 14, 15	14, 15
D21	31.2, 32.2	*	*	…	31.2, *	31.2
D7	10	10	*	*	10, *	10
CSF	11, 12	*	*	*	10, 12, *	11, 12
D3	16, 17	16, *	*	*	15, *	16, 17
THO	8, 9.3	8, 9.3	*	*	9.3, *	8, 9.3
D13	11, 12	*	…	…	11, *	11, 12
D16	11, 13	*	*	*	13, *	11, 13
D2	20, 24	*	20, *	*	20, *	20 (19, 24)
D19	12, 14	12, 14	*	*	(12) 14, *	12, 14
vWA	16, 19	16, 19	*	*	16, 18, *	16, 19
TPOX	9	9	9	9	9	9
D18	17, 20	*	…	*	13, *	17, 20
D5	10, 12	12, *	*	…	10, 12	(10) 12
FGA	19, 23	*	…	…	*	19, 23

Table B20. Autosomal STR profiles from T-shirt 20.

Locus	Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
Amel	XY	XY	XY	XY	XY	*
D8	11, 13	11, 13	11, 13	11, 13	11, 13	11, 13
D21	28	28	28	28	28	*
D7	7, 10	7, 10	*	7, 10	7, 10	10, *
CSF	10	10	10	10	10	10
D3	14, 15	14, 15	14, *	14, 15	14, 15	14, 15
THO	6, 9	6, 9	6, *	6, 9	6, 9	6, *
D13	10, 13	10, 13	*	10, *	10, 13	*
D16	11, 13	11, 13	*	11, 13	11, 13	*
D2	18, 20	18, 20	20, *	18, 20	(17) 18, 20	18, *
D19	14	14	14	14	14	14
vWA	15, 18	15, 18	15, 18	15, 18	15, 18	15, 18
TPOX	9	9	9	9	9	9
D18	12, 13	12, 13	12, 13, *	12, 13	12, 13	12, 13
D5	11, 12	11, 12	12, *	11, 12	11, 12	11, 12
FGA	19, 21	19, 21	*	*	19, 21	…

*Tables of autosomal STR profiles from T-shirt owner samples recovered from the shoulder region following a simulated assault (red number=non-owner allele; bold number=handler allele; *=allelic activity below 150 RFU threshold; …=no allelic activity).*

Table B21. Autosomal STR profiles from T-shirt 21 following a simulated assault.

Locus	Owner Buccal	Handler Buccal	Cutting	Swabbing		Tape-Lift	
				Inside	Outside	Inside	Outside
Amel	XX	XY	X (**Y**)	*	X *	XX	X *
D8	11, 13	10, 14	11, 13 (**10,14**)	*	11, *	13 (11) *	*
D21	30, 30.2	30	30, 30.2	*	30.2, 31.2	30, 30.2, *	*
D7	9	12	*	*	*	9, *	*
CSF	9, 10	10, 12	…	*	*	9, 10, *	*
D3	18	15, 18	18	*	*	18, *	*
THO	7, 9.3	7, 8	7, 9.3	*	*	7, *	*
D13	11, 12	9, 12	*	*	*	*	…
D16	9, 12	11, 12	9, 12	…	*	*	*
D2	17, 24	19, 24	17 (24)	*	17	17, 24, *	17, *
D19	13	13, 14	13 (**14**)	13	13 (*)	13 (14)	13, **14**
vWA	15, 16	16, 17	15, 16 (**17**)	*	*, **17**	15, 16	15, 16
TPOX	8, 11	8, 9	8, 11 (**9**)	…	8, *	8, *	11, *
D18	16, 20	12, 20	…	*	*, 16	16, 20, *	16, *
D5	11, 12	11, 12	11, 12	…	…	11, 12	*
FGA	21, 23	21	*	…	…	*	…

Table B22. Autosomal STR profiles from T-shirt 22 following a simulated assault.

Locus	Owner Buccal	Handler Buccal	Cutting	Swabbing		Tape-Lift	
				Inside	Outside	Inside	Outside
Amel	XX	XY	X (**Y**)	XX	XX	XX	X *
D8	10, 14	13, 15	10, **13**, 14 (**15**)	*	10, 14, *	10, 14, *	10, *
D21	31, 32.2	28, 29	*	31, *	32.2, *	31, 32.2	*
D7	7, 12	8, 10	*	*	12, *	7, 12, *	*
CSF	10, 11	11, 13	*	10, *	10, *	10, 11	11, *
D3	16, 18	15, 18	18, *	*	16, 18	16, 18, *	*
THO	6, 7	6, 9	6, **9** (7)	*	6, 7	6, 7, *	6, *
D13	8, 9	10	*	*	8, *	*	*
D16	11, 13	8, 13	*	*	11, *	11, 13	*
D2	20, 25	19, 24	**19**, 20, **24**, *	20, *	25, *	20, 25	*
D19	13, 14	13	13 (14)	*	13 (14) *	13, 14	13 (14)
vWA	18, 19	16, 17	**16**, 18, 19, *	18, *	19, *	18, 19, *	19, *
TPOX	8, 11	8, 11	8, 11	8	8 (11)	8, 11, *	8, 11
D18	13	13, 14	13, *	13	13	13, *	13
D5	10, 12	10, 12	12, *	*	10, 12, *	10, 11, 12	10, *
FGA	21, 24	22, 26	*	*	21, *	21, 24	…

Table B23. Autosomal STR profiles from T-shirt 23 following a simulated assault.

Locus	Owner Buccal	Handler Buccal	Cutting	Swabbing		Tape-Lift	
				Inside	Outside	Inside	Outside
Amel	XX	XY	X (**Y**)	…	…	*	XY
D8	13, 14	14, 15	13, 14, *	*	…	*	11, 13, *
D21	27	28, 30	27, *	*	…	*	*
D7	9, 11	12	9, 11 (10) *	*	…	*	10, *
CSF	12	11, 12	12, *	…	…	*	**11**, 12
D3	15, 17	15, 16	15, 17	*	…	*	15, **16**, 17
THO	6, 8	7, 8	6, 8	*	…	*	6, 8
D13	10, 11	8, 11	*	…	…	*	11, *
D16	12, 13	12, 13	12, 13	*	…	*	12, *
D2	20, 23	18, 19	**19**, 20, 23	*	…	*	**19**, *
D19	14, 14.2	13.2, 16	(13,**13.2**) 14, 14.2	…	…	…	14 (13,**13.2**,15,**16**)
vWA	16, 18	17, 18	16, 18, *	*	…	*	14, 16 (18)
TPOX	8, 10	8	8, 10	…	…	*	8 (9,10)
D18	11, 16	13, 17	11, **13**, 15, 16, *	*	…	11	11, 12, 15, *
D5	11, 12	9, 10	11 (12) *	…	…	*	**9**, **10**, 11
FGA	21, 25	21, 23	21, *	…	…	*	19, *

Table B24. Autosomal STR profiles from T-shirt 24 following a simulated assault.

Locus	Owner Buccal	Handler Buccal	Cutting	Swabbing		Tape-Lift	
				Inside	Outside	Inside	Outside
Amel	XX	XY	XX	…	XX	XX	XX
D8	14, 15	13, 15	14, 15	*	14, 15	14, 15, *	*
D21	30, 31	28, 29	…	*	30, 31	*	*
D7	11, 13	8, 10	…	*	11, 13	*	…
CSF	12	11, 13	…	*	12	*	*
D3	14, 15	15, 18	…	*	14, 15	*	14, *
THO	6, 9.3	6, 9	6, 9.3	*	6, 9.3	*	*
D13	12	10	…	…	12	*	*
D16	12	8, 13	…	…	12	*	*
D2	16, 18	19, 24	*	…	16, 18	*	16
D19	10.2, 15	13	15	*	15	15, *	15, *
vWA	14, 18	16, 17	14 (18)	*	14, 18	14, *	18
TPOX	11	8, 11	11	11	11	11, *	11, *
D18	12, 15	13, 14	…	…	12, 15	*	15, *
D5	10, 12	10, 12	*	…	10, 12	10, 12	*
FGA	20, 26	22, 26	…	…	20, 26, 31.2	…	…

Table B25. Autosomal STR profiles from T-shirt 25 following a simulated assault.

Locus	Owner Buccal	Handler Buccal	Cutting	Swabbing		Tape-Lift	
				Inside	Outside	Inside	Outside
Amel	XX	XY	XX	XX	*	*	*
D8	10, 13	8, 15	13, *	*	*	*	*
D21	28, 33.2	30, 32.2	28	*	…	*	*
D7	10, 11	9, 10	10, 11	*	*	*	*
CSF	10, 12	11	10, 12	*	*	*	*
D3	14, 16	16, 17	14, 16	*	*	*	*
THO	7, 9.3	10	7	*	*	*	*
D13	8, 11	9, 12	*	*	*	*	…
D16	12, 13	9, 13	*	*	*	*	*
D2	20	18, 25	20, 23	*	*	*	*
D19	13.2, 15	13, 14	(13.2) 15, *	…	*	*	*
vWA	16, 18	15, 17	**15**, 16	*	*	*	*
TPOX	8	8, 11	8	*	*	*	*
D18	13, 14	13, 19	13, 14	14	…	…	…
D5	13	12, 13	13	…	*	*	*
FGA	21, 23	22, 23	…	…	…	*	*

Table B26. Autosomal STR profiles from T-shirt 26 following a simulated assault.

Locus	Owner Buccal	Handler Buccal	Cutting	Swabbing		Tape-Lift	
				Inside	Outside	Inside	Outside
Amel	XX	XY	X (**Y**)	*	XX	XX	X *
D8	14	8, 14	(**8,**13) 14	*	*	14	*
D21	30, 31.2	29, 31.2	(30) 31.2, *	*	*	31.2, *	*
D7	10, 11	8, 11	**8**, 10, *	*	*	10, *	9, *
CSF	11, 12	10, 12	12, *	…	*	12, *	*
D3	15, 18	16, 17	15, 18, *	*	*	15, 18	*
THO	6, 9.3	6, 9	6, 9.3 (**9**)	…	*	6, 9.3	*
D13	8, 10	8, 11	*	…	*	*	*
D16	12, 13	9, 11	**11**, 12, 13	…	*	*	*
D2	19, 24	17, 25	19, 24, *	…	*	19, 24	*
D19	13, 16	14, 15	13, **14**, 16 (**15**)	*	*	13, 16	13, *
vWA	14, 16	15, 19	14, 16, **19** (**15**)	…	*	14, 16	*
TPOX	8	8	8	*	*	8	8, 11
D18	14, 16	15, 16	14, 16 (**15**)	*	*	14, 16	*
D5	11, 12	12, 13	11, 12 (**13**)	…	*	11, 12	*
FGA	24, 25	19, 22	29, *	…	*	*	…

Table B27. Autosomal STR profiles from T-shirt 27 following a simulated assault.

Locus	Owner Buccal	Handler Buccal	Cutting	Swabbing		Tape-Lift	
				Inside	Outside	Inside	Outside
Amel	XX	XY	X (**Y**)	XX	X (**Y**)	XX	X (**Y**)
D8	13, 14	13	13, 14	13, 14	13, 14	13, 14	11, 13, 14
D21	30, 32.2	30.2, 31	(29) 30, 32.2	*	32.2	30, 32.2	30, **31**, 32.2 (**30.2**,31.2)
D7	10, 12	8, 12	10, 12	(**8**,10) 12	(10,11) 12	*	10, 12 (**8**,11)
CSF	10	10, 11	10 (**11**)	10, **11**	10 (12)	10, *	10, **11**
D3	15	15, 16	15	15, *	15	15	15 (**16**,17)
THO	6, 9	9.3	6, 9 (**9.3**)	6, 9	6, **9.3**, *	6, *	6, 9, **9.3** (8)
D13	11, 12	8, 11	…	11, 12	11, 12	*	**8**, 11, 12
D16	12, 14	13	12, 14	**13**, 14	12, **13**	*	12, **13** (14)
D2	23, 24	25	23, 24 (22,**25**)	23, 24	23, 24, *	*	24 (23,**25**,26)
D19	12, 14	13	12, **13**, 14	12, **13** (14)	12, **13**, 14	12, 14	**13**, 14 (12,16)
vWA	14, 17	17	14, 17	17 (14,18)	(14, 15) 17	17	14, 17 (19)
TPOX	11, 12	8	11, 12 (**8**,10,13)	11, 12 (**8**,10)	**8**, 11, 12	11, 12	**8**, 11, 12
D18	12 ,14	16	12, 14 (**16**)	(12) 14	12, 14 (**16**,18)	12, 14	14 (12,**16**)
D5	11, 13	11	11, 13	11, 13	11 (9,12)	11, 13	9, 11 (12,13)
FGA	23, 28	19, 20	23, 28	**19**, 23	28	…	**20**, 23, 28

Table B28. Autosomal STR profiles from T-shirt 28 following a simulated assault.

Locus	Owner Buccal	Handler Buccal	Cutting	Swabbing		Tape-Lift	
				Inside	Outside	Inside	Outside
Amel	XX	XY	XX	XX	XX	XX	XX
D8	9, 13	10, 14	9, 13	9, 13	9, 13	(9,**10**) 13	9, 13 (**10**)
D21	29, 30	30	29, 30	29 (30)	29, 30	29 (32) *	29, 30, *
D7	8	12	8	8	8	8, 9, **12**	8 (9) *
CSF	10	10, 12	10	10	10	10, **12**	10, *
D3	15, 16	15, 18	15, 16	15, 16	15, 16	15, 16	15 (14,16)
THO	6	7, 8	6	6	6	6 (9,9.3)	6 (9,9.3)
D13	11, 12	9, 12	11, 12	11, 12	11, 12	*	11, 12
D16	13	11, 12	13	13	13	13, *	(**11**) 13
D2	17, 19	19, 24	17, 19	17, 19	17 (19)	17 (19)	17 (19)
D19	13, 14	13, 14	13, 14	13, 14	13, 14	13, 14	13, 14
vWA	15, 19	16, 17	15, 19	15, 19	15, 19	(15,**17**) 19	15, 19 (**17**)
TPOX	8, 11	8, 9	8, 11	8, 11	8, 11	8, 11, 12	8, 11 (12)
D18	10, 14	12, 20	10, 14	10 (14)	10, 14	10, 14, 17, 18	10, 14 (17,18)
D5	11	11, 12	11	11	11	11 (13)	11 (10,13)
FGA	23	21, 22	23	23	23	23, *	23, *

Table B29. Autosomal STR profiles from T-shirt 29 following a simulated assault.

Locus	Owner Buccal	Handler Buccal	Cutting	Swabbing		Tape-Lift	
				Inside	Outside	Inside	Outside
Amel	XX	XY	X *	XX	…	X *	X *
D8	10, 13	12, 14	10, 13 (**12**)	*	*	10	10, 13
D21	30, 32.2	28, 29	30, 32.2	**29**, *	*	*	30, 32.2
D7	10	8, 11	10	**8**, 10	…	*	10
CSF	11	10, 11	(**10**) 11	*	…	*	11
D3	16, 17	16, 17	16, 17	*	…	*	16, 17
THO	6, 9	7, 9.3	6, 9	*	*	*	6, *
D13	11, 12	11, 12	*	*	…	…	12
D16	10, 14	11, 12	10, 14	*	…	*	10, 14
D2	18, 20	24	18, 20 (**24**)	**24**	*	…	18, 20
D19	12, 14	14, 16	12, 14	14, **16**	…	*	12, 14
vWA	14, 17	15, 18	14, 17	**15**	…	14, **15**	14, 17
TPOX	9, 11	8, 11	9, 11	**8**, 11	*	9, 11, *	9, *
D18	14, 18	14, 15	14, 18 (**15**)	14, **15**	…	14	14
D5	12, 13	11	(**11**) 12, 13	…	*	*	12
FGA	21, 28	20, 22	21, 28	…	…	…	21, 28

Table B30. Autosomal STR profiles from T-shirt 30 following a simulated assault.

Locus	Owner Buccal	Handler Buccal	Cutting	Swabbing		Tape-Lift	
				Inside	Outside	Inside	Outside
Amel	XX	XY	X (**Y**)	XX	XX	XX	XX
D8	12	11, 12	(**11**) 12	12	12	12, *	12
D21	30, 33.2	29, 32	30, 33.2	*	33.2, *	32.2, *	*
D7	8, 9	9, 11	9 (8,**11**)	9, *	8, 9	9, *	8, 9, *
CSF	9, 13	10, 15	9, 13 (**15**)	*	*	13	9, *
D3	15, 16	17, 19	15, 16 (**19**)	*	16	(15) 16	15, 16
THO	8, 9	9, 9.3	9 (8,**9.3**)	9	8, *	7, 8, 9, *	8, 9
D13	12, 14	11, 13	12, 14, *	12	…	…	*
D16	12, 13	8, 12	12, 13	*	*	…	*
D2	23, 24	24, 25	23, 24 (**25**)	24, *	18, *	*	23, 24
D19	14, 15	15	(13) 14, 15	14, 15	14, 15	14 (15)	14 (15)
vWA	17, 19	16, 18	17, 19 (**16**,**18**)	17	17	(14) 19	17, 19
TPOX	9, 12	8	**8**, 9, 12	(8) 12	**8**, 9, 12	(9,10) 12	9 (12)
D18	14, 15	13, 15	14, 15 (**13**,17)	…	14 (15)	**13**, 14	15
D5	11	11, 12	11 (12)	…	11, 15	11	11
FGA	20, 22	23	20, 22, **23**	…	…	…	20

Table B31. Autosomal STR profiles from T-shirt 31 following a simulated assault.

Locus	Owner Buccal	Handler Buccal	Cutting	Swabbing		Tape-Lift	
				Inside	Outside	Inside	Outside
Amel	XX	XY	X (**Y**)	XX	X (**Y**)	XX	XX
D8	12, 14	9, 14	14 (**9**,12,13)	14, *	12, *	12, *	(12) 14
D21	32.2	29, 30	32.2, *	**29**, 32.2	32.2	32.2, *	32.2, *
D7	9, 11	8	(**8**) 9, 11	9	*	9, *	9, 11
CSF	11, 13	11, 12	11, 13 (**12**)	11	*	13	11, 13 (**12**)
D3	15, 16	15, 16	15, 16	15, *	15, 16	16, *	15 (16)
THO	7, 8	9, 9.3	7, 8 (**9**) *	**9**	8 (7,**9.3**) *	7, 8, **9**, *	7 (8,**9**)
D13	12	11	12	*	*	…	12, *
D16	8, 11	11, 12	8, 11	…	*	…	8, 11
D2	22	18, 25	22, *	22, *	*	*	17
D19	11, 14	15	11, 14 (**15**)	14, **15**	14, *	14 (**15**)	11, 14
vWA	16	14, 18	16	(15) 16	16	(**14**) 19	16 (15,**18**)
TPOX	10	8	(**8**,9) 10	10	10, *	(9,10) 12	(**8**) 10
D18	19, 21	14, 15	(**14**) 19, 21	…	…	13, **14**	19, *
D5	11, 13	11, 13	11, 13 (12)	11, 13	11, *	11	11, 13
FGA	19, 25	22, 26	19, 24, 25	…	…	…	19, 25

Table B32. Autosomal STR profiles from T-shirt 32 following a simulated assault.

Locus	Owner Buccal	Handler Buccal	Cutting	Swabbing		Tape-Lift	
				Inside	Outside	Inside	Outside
Amel	XX	XY	X (**Y**)	XX	XX	XX	X**Y**
D8	13, 14	10, 12	**12**, 13, 14 (8,**10**)	13, 14	(**12**) 13, 14	(8,**12**) 13, 14	(**12**) 13, 14
D21	30, 31.2	28, 33.2	30, 31.2 (29,32.2,**33.2**)	30, 31.2	30, 31.2	30, 31.2	(29) 30, 31.2
D7	10, 12	10	(9) 10, 12	10, 12	10, 12	10, 12, *	10
CSF	12	10	(**10**) 12	12	(**10**) 12	12 (**10**,13)	11, 12
D3	16, 18	15, 17	16, 18 (**17**)	16, 18	16, 18	16, 18	16, 18
THO	7, 9	6, 9	7, 9 (**6**,8)	7, 9	7, 9	7, 9	7 (8,9)
D13	10, 13	11	10 (**11**,13)	10, 13	10, 13	*	*
D16	10, 12	9, 13	10 (11,12,**13**)	10, 12	10, 12	10, 12	**13**
D2	24, 25	18, 24	24, 25 (16,17,**18**)	24, 25	24, 25	24, 25 (17,**18**)	(17) **18**, 24, 25
D19	14	13, 14.2	14 (12,**13**,15.2)	14	(**13**) 14	(**13**) 14	**13**, 14
vWA	16, 18	16, 18	16, 18 (15,19)	16,18	16 (15,18)	(15) 16, 18	16, 18 (15,17)
TPOX	11	8, 10	(**8**) 11	11	11	11	(**8**) 11
D18	14, 17	12, 15	13, 14, 17 (10,**12**,**15**,16)	14, 17	(**12**) 14, 17	14, 17 (**12**,**15**,16)	14, *
D5	11, 16	12, 13	11 (10,**12**,**13**)	11	(10) 11	11	(10) 11
FGA	21, 24	19, 22	**19**, 21, 23, 24 (20,23.2)	21, 24	21, 24	21, 24	24

Table B33. Autosomal STR profiles from T-shirt 33 following a simulated assault.

Locus	Owner Buccal	Handler Buccal	Cutting	Swabbing		Tape-Lift	
				Inside	Outside	Inside	Outside
Amel	XX	XY	XX	*	XX	XX	XX
D8	10, 12	12, 15	10, *	*	*	*	*
D21	28, 31.2	28, 31.2	28, 31.2	…	*	*	*
D7	12	8, 13	12	*	*	*	*
CSF	10, 12	12	12, *	12, *	…	*	*
D3	15, 16	14	15, 16	*	…	*	*
THO	6, 9	8, 9.3	6, *	*	…	…	9
D13	11, 14	9, 13	**9**, 11, *	*	*	*	*
D16	8, 13	9, 12	13, *	…	…	*	*
D2	17, 20	17, 19	*	…	…	*	17, *
D19	15	14	14, 15	…	*	*	*
vWA	17	18, 19	17	*	*	17, *	*
TPOX	8, 11	10	8, 11, *	…	…	*	*
D18	13, 15	17, 19	*	…	…	…	…
D5	11, 13	12, 13	11, 13	…	…	*	*
FGA	19, 24	21, 24	*	…	…	…	*

Table B34. Autosomal STR profiles from T-shirt 34 following a simulated assault.

Locus	Owner Buccal	Handler Buccal	Cutting	Swabbing		Tape-Lift	
				Inside	Outside	Inside	Outside
Amel	XX	XY	X (**Y**)	*	X *	XX	*
D8	9, 13	12, 15	9, 13	*	9 (13)	13, *	*
D21	28, 32.2	30, 33.2	28, 32.2	32.2, *	28, 32.2	30.2, *	*
D7	10, 11	12	10, 11	*	10, 11	8, *	*
CSF	9, 10	11	9, 10	*	9, 10	9,10, *	*
D3	15	15, 16	15	15	15	(15,**16**) 17	*
THO	6	7, 9.3	6 (**9.3**)	6	6, *	6, **7**, *	*
D13	8, 13	9, 13	8, 13	8	(8) 13	12 (13*) *	*
D16	12	9, 12	12	12	12	(**9**) 12	*
D2	19, 23	17, 19	19, 23	…	19, 23	*	…
D19	14	12, 15.2	14	14	14	15, 16	*
vWA	16	16, 18	16 (**18**)	…	16	(15) 16, **18**, 19	*
TPOX	8	8, 11	8	8	8	8 (**11**)	8
D18	13, 19	12, 15	13, 19	…	13, *	*	*
D5	11	11, 13	11	11	11	11, 12	*
FGA	18, 20	21, 23	18, 20	*	18, *	20, 22, *	…

Table B35. Autosomal STR profiles from T-shirt 35 following a simulated assault.

Locus	Owner Buccal	Handler Buccal	Cutting	Swabbing		Tape-Lift	
				Inside	Outside	Inside	Outside
Amel	XX	XY	X (**Y**)	*	*	XX	X(**Y**)
D8	14, 15	10, 13	**10**, **13**, 14, 15	*	*	14, 15, *	(**10**,**13**) 14, 15
D21	29, 30	29, 30	29, 30	*	*	29, *	29, 30
D7	8, 10	11	8, **11** (10)	*	*	*	8, 10 (**11**)
CSF	11, 12	11, 13	11, *	*	…	*	11, 12
D3	14, 18	14, 18	14, 18	(14) 18	14	14, 18	14, 18
THO	9	8, 9	(**8**) 9	9	9	9	9
D13	11, 12	11, 12	11, 12	*	11, *	11, 12	11, 12
D16	10, 11	11	(10) 11	*	*	10, *	10, 11
D2	17	18, 20	17, **18**, **20**	*	…	17	17
D19	13, 15	15, 17.2	13, 15, **17.2**	13	*	13, *	13, 15 (**17.2**)
vWA	18	16, 18	**16**, 18	18	*	18, *	18
TPOX	8, 11	8, 11	8, 11	*	*	8, 11	8, 11
D18	12, 17	15, 16	12, **15**, **16**, 17	*	…	12, *	12, 17 (**15**,**16**)
D5	12, 13	12	12 (13)	12	*	12, 13	12, 13
FGA	20, 21	20	20 (21)	*	…	20, *	20, 21

Table B36. Autosomal STR profiles from T-shirt 36 following a simulated assault.

Locus	Owner Buccal	Handler Buccal	Cutting	Swabbing		Tape-Lift	
				Inside	Outside	Inside	Outside
Amel	XX	XY	XX	…	XX	X *	XX
D8	11, 13	12, 15	*	*	13, *	10, *	*
D21	31	28, 32.2	*	31.2, *	…	*	*
D7	11	8	*	*	*	*	*
CSF	12	10, 12	*	*	*	*	*
D3	14, 16	15, 16	*	*	*	*	*
THO	6, 9	7, 9.3	*	*	*	*	*
D13	12	8, 12	*	11	*	12, *	*
D16	11, 12	12	…	…	…	12, *	*
D2	18, 24	16, 20	*	22, *	20	*	*
D19	14, 16	13, 15	…	…	…	*	…
vWA	16, 19	16, 17	*	*	*	*	…
TPOX	8, 11	8, 10	*	*	11	11, *	*
D18	14, 17	15, 19	*	*	*	*	…
D5	11, 12	11, 12	*	…	11, *	*	…
FGA	22, 26	19, 23	*	…	*	…	…

Table B37. Autosomal STR profiles from T-shirt 37 following a simulated assault.

Locus	Owner Buccal	Handler Buccal	Cutting	Swabbing		Tape-Lift	
				Inside	Outside	Inside	Outside
Amel	XX	XY	X(**Y**)	XX	XY	*	X *
D8	10, 12	12, 13	12, **13** (10,11,14)	…	*	*	12 (**13**) *
D21	30, 31.2	28, 30	30 (**28**,31.2)	…	*	*	30, 31.2, *
D7	9, 10	10	10 (9,11)	…	*	*	9, 10
CSF	12	10, 12	12 (9,**10**)	…	*	*	12, *
D3	15, 17	15, 17	15, 17	…	*	*	15, 17
THO	9.3	6, 7	**6**, 9.3 (**7**)	*	9.3, *	*	**6**, **7**, 9.3
D13	8, 11	8, 10	8, **10** (11,13)	…	*	13, *	8, *
D16	13, 14	12, 13	13 (9,**12**,14)	…	…	*	13 (**12**,14) *
D2	23, 25	17	**17** (21,23,25)	…	*	*	23, *
D19	13	14	13, **14**	…	*	*	13 (**14**)
vWA	16, 17	15, 16	**15**, 16, 17	…	*	*	16, 17 (**15**)
TPOX	11	8	**8**, 11 (12)	*	…	*	**8**, 11
D18	13, 15	14	**14**, 15 (10,13,17)	…	…	*	13, **14**, 15
D5	11, 12	9, 12	**9**, 11, 12 (10,13)	*	*	*	11, 12
FGA	19, 20	23, 24	**23**, **24** (20)	*	*	…	20, *

Table B38. Autosomal STR profiles from T-shirt 38 following a simulated assault.

Locus	Owner Buccal	Handler Buccal	Cutting	Swabbing		Tape-Lift	
				Inside	Outside	Inside	Outside
Amel	XX	XY	X(**Y**)	*	*	XX	X *
D8	11, 15	14	11, **14**, 15 (12)	*	*	11, *	(13) 15, *
D21	30, 31	30, 30.2	30, 31	*	*	*	31, *
D7	10, 11	8, 11	11, *	…	*	*	11, *
CSF	10, 12	10, 11	10, 12	*	*	*	10 (12), *
D3	16	16, 18	16 (**18**)	…	16, *	(15) 16	16 (15) *
THO	8	9.3	8 (7,9)	*	8	8	8, **9.3**, *
D13	12, 13	8, 13	12, 13	…	13, *	13, *	12, 13
D16	9, 13	11	9, 13	…	*	*	9, **11** (13)
D2	16, 24	18, 25	16, 24, *	…	*	16, *	16, 24
D19	13, 15	13, 15	13, 15 (14)	…	13, *	13, 15, *	13, 15, *
vWA	16, 18	14, 18	16, 18 (**14**)	…	16, 18, *	16, 18	16, 18, *
TPOX	8, 11	8, 10	8, 11	…	8, *	8, 11	8, 11, *
D18	17, 18	15, 18	**15**, 17, 18	…	*	*	17, 18
D5	11	11, 12	11 (**12**,13)	…	*	11	...
FGA	22	22, 26	22 (21,25)	…	*	*	22

Table B39. Autosomal STR profiles from T-shirt 39 following a simulated assault.

Locus	Owner Buccal	Handler Buccal	Cutting	Swabbing		Tape-Lift	
				Inside	Outside	Inside	Outside
Amel	XX	XY	X(**Y**)	*	XX	X *	*
D8	13	8, 12	13 (12,15)	*	13	*	*
D21	30.2, 31	29, 31	30, 30.2, 31 (**29**)	*	*	*	*
D7	8, 9	11, 12	8, 9	…	*	*	*
CSF	10	11, 12	10 (**12**)	…	*	*	*
D3	17	15, 17	17 (**15**,16,18)	*	*	17	17, *
THO	6, 7	6, 9.3	6, 7 (9,**9.3**)	*	*	*	*
D13	12	8, 13	12 (11,**13**)	*	12, *	12, *	*
D16	9, 12	12	12 (9,11)	*	*	*	*
D2	23, 25	18, 23	23 (20,25)	…	*	*	*
D19	15, 16	13, 18	15, 16 (14)	…	*	*	*
vWA	18, 19	16, 17	**17**, 18, 19 (15,**16**)	*	18, 19	*	*
TPOX	8, 11	8	8, 11	*	*	8, *	8, *
D18	12, 14	13, 14	12, 14 (11,20)	…	*	…	*
D5	11, 12	12, 13	11, 12 (10)	*	*	11, *	…
FGA	22, 25	21, 23	22 (23,25)	…	…	*	…

Table B40. Autosomal STR profiles from T-shirt 40 following a simulated assault.

Locus	Owner Buccal	Handler Buccal	Cutting	Swabbing		Tape-Lift	
				Inside	Outside	Inside	Outside
Amel	XX	XY	X(**Y**)	XX	*	XX	X *
D8	11	13, 15	11 (14,**15**)	11, *	11, *	11	11, *
D21	28, 30	30	28, 30	*	*	28, *	28, 30, *
D7	9	9, 11	9 (10,**11**)	*	*	9, *	9
CSF	11, 12	11, 12	11, 12	*	*	*	11, 12
D3	14, 15	14, 16	14, 15	14, *	14, *	14, 15	14, 15
THO	9.3	7, 9.3	9.3 (6,9)	9.3	*	9.3	9.3, *
D13	11, 12	8, 10	11, 12	12	12, *	11, 12	11, 12, *
D16	11, 13	10, 13	11, 13 (12)	*	*	11, 13, *	11, 13
D2	23	22, 25	23 (19,21)	*	…	23	23
D19	14	13, 14	14	*	…	14	14
vWA	17, 18	16, 17	17, 18 (**16**)	*	…	17, 18	17, 18 (**16**)
TPOX	8, 11	9, 10	8, 11	*	…	(8) 11	8 (11)
D18	13, 17	13, 17	13, 17	…	*	13, 17	13, 17
D5	11, 12	12	11, 12	*	*	11, 12	11 (12)
FGA	20, 21	20, 23	20, 21 (**23**,24)	…	*	20, *	20, 21

*Tables of Y chromosome STR profiles from T-shirt owner samples recovered from the shoulder region following a simulated assault (red number=non-handler allele; *=allelic activity below 150 RFU threshold; …=no allelic activity). Only T-shirts samples containing a Y chromosome allele in Identifiler were analyzed using Yfiler (see Table 5).*

Table B41. Y-STR profiles from T-shirt 21 following a simulated assault.

Locus	Handler Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
456	16	16		*		*
389I	13	13, 14		*		14
390	25	24, 25		…		…
389II	29	29 (30)		*		…
458	16	16, 17		…		17, *
19	14	14		14		14
385	11, 15	11 (15)		11, *		15
393	13	13		*		*
391	11	11		11		11
439	12	12		12		12
635	24	24, *		*		*
392	13	13		*		*
GATA H4	11	11		11		11
437	15	15		15		…
438	12	12		…		…
448	18	18, 19		*		…

Table B42. Y-STR profiles from T-shirt 22 following a simulated assault.

Locus	Handler Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
456	14	14				14 (15)
389I	13	13				(12) 13
390	23	23				23
389II	29	29				29 (30)
458	18	18				(17) 18
19	14	14				14
385	13, 17	13, 17				13 (11,12,14,17)
393	14	14				(13) 14
391	10	10				10
439	12	12				12
635	27	*				…
392	10	10				10
GATA H4	12	12				12
437	16	16				16
438	11	11				11, 12
448	19	19				19

Table B43. Y-STR profiles from T-shirt 23 following a simulated assault.

Locus	Handler Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
456	16	15, 16				15, 16
389I	13	13, *				13
390	22	24, *				24
389II	30	30				30
458	15	15, 16				15, 16
19	15	16, *				(15) 16
385	14, 14	11, 14				11, 14
393	14	13				13
391	9	9, 11 (10)				(9) 11
439	10	10				10
635	20	23, *				23, *
392	11	11				11
GATA H4	12	12				12
437	15	14, *				14, *
438	10	*				10, 12
448	21	…				20

Table B44. Y-STR profiles from T-shirt 26 following a simulated assault.

Locus	Handler Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
456	16	(14) 15, 16				*
389I	13	13				…
390	24	24				…
389II	30	30				…
458	16	16 (17)				…
19	14	14				…
385	12, 15	12, 15				12
393	12	12				…
391	10	10				…
439	11	11				…
635	23	23				…
392	14	14				…
GATA H4	12	12				…
437	15	…				…
438	12	12				…
448	19	19				*

Table B45. Y-STR profiles from T-shirt 27 following a simulated assault.

Locus	Handler Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
456	17	(14) 17		17		(16) 17
389I	14	*		14		13, 14
390	24	24		*		(23) 24
389II	32	…		32		*
458	15	15		15 (18)		15
19	13	13		*		13
385	16, 18	(15) 16		(15,16,17) 18		16, 18
393	13	13		13		(12) 13
391	10	10		10		10 (11)
439	11	11, *		(10) 11		11
635	22	22		22, 24		22
392	11	11		11		11
GATA H4	12	12		(11) 12		(11) 12
437	14	14		*		14
438	10	…		…		10, *
448	20	…		20		(19) 20

Table B46. Y-STR profiles from T-shirt 29 following a simulated assault.

Locus	Handler Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
456	17	16, 17			14	*
389I	13	13			*	…
390	24	*			*	…
389II	29	29			…	…
458	17	17			17	…
19	14	14			…	*
385	11, 13	11, 13, 14			13, 14	*
393	13	13			*	…
391	10	10, 12, *			*	*
439	12	12, *			…	…
635	24	…			…	…
392	14	…			…	…
GATA H4	12	…			11	8, 12
437	15	…			…	…
438	12	*			…	…
448	18	*			…	…

Table B47. Y-STR profiles from T-shirt 30 following a simulated assault.

Locus	Handler Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
456	16	16				
389I	14	14				
390	24	24				
389II	30	30				
458	16	16				
19	14	14				
385	11, 14	11, 14				
393	13	13				
391	12	12				
439	13	(12) 13				
635	23	23				
392	13	13				
GATA H4	12	12				
437	15	15				
438	10	10				
448	19	19				

Table B48. Y-STR profiles from T-shirt 31 following a simulated assault.

Locus	Handler Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
456	16	(14,15) 16		*		
389I	13	13 (14)		*		
390	24	24		*		
389II	29	29		…		
458	16	16, 18		*		
19	14	14		14		
385	11, 15	11		11, 17		
393	13	(12) 13		*		
391	10	10		10		
439	12	12		12		
635	23	23		…		
392	13	11, 13		…		
GATA H4	12	(11) 12		*		
437	15	15		…		
438	12	*		…		
448	19	…		*		

Table B49. Y-STR profiles from T-shirt 32 following a simulated assault.

Locus	Handler Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
456	13	(13) 14, 16				(13,15) 16
389I	13	13, 14				13
390	23	23				23
389II	30	29, 32				29
458	19	15				15
19	14	15				17
385	13, 17	15				13, 16, 17 (11,15)
393	12	13 (15)				15
391	11	10				10
439	11	11 (12)				11, 12
635	20	21				21
392	11	12				12
GATA H4	11	10, 11 (12)				11
437	14	13, 14				15
438	10	10				…
448	20	20				20, *

Table B50. Y-STR profiles from T-shirt 34 following a simulated assault.

Locus	Handler Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
456	17	(16) 17				
389I	13	12 (13)				
390	24	24				
389II	30	28, 30				
458	17	17				
19	14	(13) 14				
385	11, 15	14 (15)				
393	13	13				
391	11	(10) 11				
439	13	10, 13				
635	23	23, *				
392	13	11, *				
GATA H4	13	11, 13				
437	14	14 (16)				
438	12	10				
448	18	18				

Table B51. Y-STR profiles from T-shirt 35 following a simulated assault.

Locus	Handler Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
456	16	16	…			16
389I	13	13	13			13
390	24	24	*			24
389II	29	29	*			29
458	17	17	17			17
19	14	14	…			14
385	10, 11	10, 11	*			10 (11)
393	13	13	*			13
391	11	11	…			11
439	11	11	…			11
635	23	23	…			23
392	14	14	…			14
GATA H4	12	12	…			12
437	13	14	…			14
438	12	12	…			12
448	18	18	…			18

Table B52. Y-STR profiles from T-shirt 36 following a simulated assault.

Locus	Handler Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
456	16				*	
389I	13				…	
390	23				…	
389II	29				…	
458	17				*	
19	14				…	
385	11, 14				…	
393	13				13	
391	11				…	
439	13				…	
635	23				…	
392	13				…	
GATA H4	12				13	
437	15				*	
438	12				…	
448	19				…	

Table B53. Y-STR profiles from T-shirt 37 following a simulated assault.

Locus	Handler Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
456	17	17		*		17
389I	13	13		*		13
390	23	23		…		23
389II	29	29		…		29
458	16	16		…		16
19	14	14		…		14
385	11, 14	11, 14		…		11, *
393	13	13		…		13
391	11	11		…		11
439	13	13		…		13
635	23	23		…		23
392	13	13		…		13, 14
GATA H4	12	12		*		12
437	14	14		…		14
438	12	12		…		12
448	19	19		…		19

Table B54. Y-STR profiles from T-shirt 38 following a simulated assault.

Locus	Handler Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
456	13	*				13 (15)
389I	12	*				12, *
390	22	*				22
389II	29	*				28, 29
458	14	*				14, 16
19	14	15				14, 15
385	12, 14	*				11 (12) *
393	13	*				13
391	10	11				10
439	10	...				10, 12
635	22	...				23, *
392	11	*				11
GATA H4	11	*				11
437	16	15				15, 16
438	10	...				10
448	21	*				…

Table B55. Y-STR profiles from T-shirt 39 following a simulated assault.

Locus	Handler Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
456	15	16	…		…	*
389I	11	13	*		…	*
390	24	23	24		…	*
389II	27	29	*		…	*
458	17	15	17		…	15
19	14	14	…		…	*
385	11, 13	11, 14	…		…	*
393	13	13	…		…	*
391	9	11	…		…	11
439	11	12	*		…	*
635	24	*	…		…	…
392	13	13	…		…	13
GATA H4	12	12	*		…	12
437	15	15	…		…	…
438	12	12	…		…	…
448	19	19	*		…	*

Table B56. Y-STR profiles from T-shirt 40 following a simulated assault.

Locus	Handler Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
456	16	16, *		…		…
389I	13	13		*		…
390	22	22, *		…		…
389II	29	30		…		…
458	17	16, 17				18
19	15	*		…		…
385	15, 17	*		…		15, 16
393	12	(12) 13		…		*
391	10	10, 11		…		11
439	11	…		…		11
635	20	20, 23		…		20, *
392	11	11, 13		…		…
GATA H4	12	12, *		…		…
437	14	15		…		*
438	9	*		…		…
448	20	*		…		…

APPENDIX C

PROFILE STATISTICS

Table C1. Combined probability inclusion (CPI) profile statistics from worn T-shirts recovered from the shoulder seam using the various methods.

T-shirt #	Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
1	5.00E+11	5.00E+00	0.00E+00	1.80E+08	7.50E+01	0.00E+00
2	5.70E+11	1.20E+05	1.00E+00	1.00E+00	1.00E+00	1.20E+01
3	3.30E+07	1.60E+02	1.20E+04	1.30E+04	3.20E+08	3.80E+08
4	5.20E+11	2.20E+10	8.10E+10	3.20E+09	4.80E+11	1.20E+06
5	2.40E+11	2.40E+11	1.40E+09	4.00E+02	3.90E+11	1.00E+11
6	2.10E+08	9.20E+04	8.30E+05	6.70E+07	1.60E+04	2.20E+09
7	2.80E+09	4.90E+08	5.10E+02	5.00E+06	1.80E+07	2.30E+04
8	7.40E+10	1.90E+02	1.00E+00	1.20E+04	1.00E+00	0.00E+00
9	3.30E+12	1.10E+12	5.10E+11	8.40E+09	1.30E+12	8.50E+05
10	4.40E+11	1.00E+00	4.00E+00	3.50E+06	2.60E+01	3.10E+01
11	4.10E+14	3.50E+08	2.60E+12	1.00E+00	1.90E+01	1.00E+00
12	1.10E+09	3.00E+00	0.00E+00	4.20E+01	1.00E+00	0.00E+00
13	1.80E+13	4.80E+01	0.00E+00	0.00E+00	3.00E+00	1.00E+00
14	2.30E+08	1.40E+09	1.00E+00	0.00E+00	1.00E+00	5.10E+02
15	2.00E+10	3.80E+04	1.00E+00	5.00E+00	5.70E+07	4.60E+09
16	5.30E+11	8.50E+01	0.00E+00	0.00E+00	0.00E+00	9.00E+00
17	6.50E+10	9.90E+01	2.00E+00	0.00E+00	1.10E+03	0.00E+00
18	2.10E+13	6.60E+12	1.00E+00	3.70E+02	1.10E+08	4.20E+08
19	7.40E+11	3.30E+02	1.00E+00	1.00E+00	1.10E+03	1.70E+10
20	8.30E+11	5.30E+10	6.90E+02	2.00E+13	4.50E+15	1.10E+04

Table C2. Combined probability inclusion (CPI) profile statistics from worn T-shirts following a simulated assault recovered from the shoulder seam using the various methods.

T-shirt #	Owner Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
21	1.00E+10	1.10E+05	1.00E+00	5.30E+01	3.20E+07	2.70E+01
22	1.40E+13	6.00E+02	1.00E+00	1.10E+04	1.10E+11	5.00E+00
23	1.00E+11	1.50E+07	0.00E+00	0.00E+00	1.00E+00	1.70E+05
24	5.70E+12	3.50E+02	1.00E+00	1.20E+11	6.40E+01	1.00E+00
25	2.70E+10	5.90E+06	1.00E+00	0.00E+00	0.00E+00	0.00E+00
26	3.80E+11	2.30E+06	0.00E+00	0.00E+00	9.70E+05	2.00E+00
27	1.70E+11	3.10E+08	1.50E+09	1.30E+06	6.00E+04	6.20E+06
28	1.90E+07	1.90E+07	1.90E+07	1.90E+07	5.80E+07	6.20E+07
29	3.20E+12	1.40E+11	4.90E+02	0.00E+00	1.60E+02	4.80E+08
30	6.20E+13	1.40E+10	1.10E+01	4.80E+03	6.30E+05	1.10E+07
31	1.80E+11	1.00E+07	1.50E+03	1.30E+01	1.50E+05	5.20E+08
32	1.50E+11	1.20E+05	1.90E+11	1.90E+11	6.90E+07	3.30E+04
33	9.40E+10	7.50E+03	1.00E+00	0.00E+00	1.00E+00	1.00E+00
34	3.20E+09	6.30E+10	1.00E+00	2.50E+07	7.90E+06	1.00E+00
35	8.50E+08	3.30E+09	1.10E+01	1.00E+00	1.80E+03	2.10E+07
36	8.00E+08	0.00E+00	1.00E+00	1.00E+00	1.00E+00	0.00E+00
37	1.10E+11	2.40E+06	0.00E+00	1.00E+00	1.00E+00	7.90E+05
38	1.40E+10	5.30E+09	0.00E+00	6.00E+00	2.40E+02	5.00E+07
39	7.30E+10	7.80E+06	0.00E+00	1.10E+01	1.00E+00	1.00E+00
40	2.20E+06	1.70E+07	1.00E+00	1.00E+00	1.40E+04	1.10E+06

Table C3. Y chromosome haplotype frequencies of handler DNA from worn T-shirts following a simulated assault recovered from the shoulder seam using the various methods. Frequency is equal to one in the value shown with a profile frequency of 1 in 4114 being unique to the database.

T-shirt #	Owner Buccal	Cutting	Swabbing		Tape-Lift	
			Inside	Outside	Inside	Outside
21	4114	4114		34.00		36.09
22	4114	4114				4114
23	4114	4114				4114
24	4114					
25	4114					
26	4114	4114				1.000
27	4114	4114		4114		4114
28	4114					
29	4114	2057			3.187	1.889
30	4114	4114				
31	4114	822.8		11.89		
32	4114	4114				4114
33	4114					
34	4114	4114		1.000		
35	4114	4114	4.299			4114
36	4114				1.290	
37	4114	4114		0.361		4114
38	4114	1.000				4114
39	4114	6.868	6.668		1.000	3.937
40	4114	2057		1.000		43.77

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