THE OPTIMIZED USE AND RECOVERY OF DNA FROM TAPE LIFTS

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ABSTRACT

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The use of tape lifts for DNA recovery is becoming increasingly common in forensic laboratories, however there is no consensus on the best type of tape to use or how it should be processed. The first goal of this study was to optimize the tape lift procedure. Six swabbing solutions, including adhesive removers, were used on eight types of tape to determine which solution and tape was most suited to recovering DNA. The swabbing procedure was also compared to soaking the tape in chloroform and soaking the tape in digestion buffer (1% SDS). Real Time PCR was used to quantify the amount of DNA recovered from each tape or recovery method. All types of tape were equally effective, and digestion buffer and water yielded more DNA than adhesive removers. Swabbing the tape with digestion buffer was more successful at recovering cells than soaking in chloroform, but no more effective than soaking in digestion buffer. The second goal of this study was to determine if tape lifts of the skin could serve as an alternative to buccal swabs for collecting reference samples. Based on preliminary results, three of seven body areas (behind the ear, the back of the hand, and the combination of the fingertips and between the fingers) were sampled on 20 volunteers using tape lifts and swabs. Short tandem repeat (STR) analysis revealed swabs to be more effective than tape lifts of the skin, and the region behind the ear to be a possible alternative source of reference DNA to buccal swabs.

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INTRODUCTION

DNA analysis is an invaluable forensic tool. The likelihood of two people having the same DNA profile is often over one in a quadrillion (Song et al., 2009), a number many orders of magnitude higher than the human population of the Earth. This allows DNA to uniquely identify individuals, tying suspects to crime scenes and exonerating the innocent. Not only is DNA highly discriminating, but it is also abundant within the human body, with every nucleated cell containing a copy. Simply touching an object can deposit skin cells, creating potential DNA evidence, although the amount of DNA left behind on evidence varies (Phipps and Petricevic, 2006). Valuable DNA sources like touch samples contain minute amounts of DNA (Castella and Mangin, 2008) and therefore require a sensitive and effective collection technique. Methods for DNA analysis have improved over the years; while 5 µg of DNA used to be required to obtain a genetic profile using restriction fragment length polymorphism (RFLP) analysis, with the development of polymerase chain reaction (PCR) based methods, a quantity 50,000 times less is now needed (NIST, 2011). However, artifacts, such as the appearance (drop-in) or loss (drop-out) of alleles that cannot be repeated, occur when such a small amount of DNA is analyzed, making collection techniques that maximize DNA yields essential to the analysis process.

Methods for recovering DNA from an object:

In order to recover DNA from a scene, cells containing it must be collected. A common collection method involves swabbing the area in question with a moist swab. The head of the swab is then cut off and placed in a solution designed to free the cells from the swab material and break them open, while simultaneously preventing the degradation of the DNA.

A variation of this method is the double swab technique, in which a dry swab follows the application of the wet swab. This technique was first developed to collect salivary DNA from skin, and was shown to yield higher DNA quantities than the single swab method (Sweet et al., 1997). In later studies, the single and double swab techniques were compared on other materials, including cloth and various office surfaces, such as a stapler and computer mouse (Castella and Mangin, 2008; Pang and Cheung, 2007). The authors of both studies concluded that the double swab technique produced higher DNA yields. In fact, Pang and Cheung (2007) showed that DNA profiles could be generated from the dry swab alone, in some cases even when the respective wet swab gave no profile. The theory behind the double swab technique is that the moisture from the wet swab will rehydrate and loosen dried skin cells, with the time between swabs allowing for the rehydration process to occur. Pang and Cheung (2007) hypothesized that this is why the dry swab sometimes yielded a profile when the wet swab did not.

Not all analysts agree that the double swab technique is better, however. Graham and Rutty (2008) showed that the double swab technique did not yield significantly higher amounts of DNA when the back of the neck was swabbed following simulated manual strangulations. The authors noted that a single swab approach is also beneficial because it requires fewer manipulations, creating less opportunity for contamination.

Other methods of DNA recovery have also been examined. One involves soaking the material believed to hold cells (Bright and Petrocevic, 2004; Petrocevic et al., 2006). This loosens the cells from the substrate, allowing them to enter into solution. Gomez (2009), however, reported higher DNA yields and more complete genetic profiles with stronger peaks were obtained when deflagrated pipe bombs were swabbed instead of soaked. Gomez hypothesized that Fe^{2+} ions from the metal fragments entered the solution when the bombs were

soaked, overwhelming the EDTA that would normally bind the divalent cations necessary for exonuclease activity, allowing nucleases to break down the DNA. Another recovery method, described by Farber and colleagues (2010), involved a casting material that was successfully used to simultaneously create a cast of an impression, such as a fingerprint, and collect DNA. These are just a few examples of currently utilized methods, and new techniques are constantly being developed.

Using tape to recover DNA from an object:

The use of adhesive tapes for DNA recovery has also begun to draw attention. Barash and colleagues (2004) showed that tape is a suitable tool for collecting cells from items collected from crime scenes, including a hat, jacket, gun, and rope. Not only is the technique effective, but it has multiple potential benefits. Tape lifts do not use moistening agents like swabs do, so there is no risk of the moistening agent soaking into the substrate, carrying cells with it. Bright and Petricevic (2004) showed that tape lifts may also pick up fewer potential PCR inhibitors than when the substrate is swabbed or soaked. The authors compared these three methods on shoe insoles, and noted that extracts from the swabbing and soaking methods contained much more dirt and debris than the extract from the tape lifts. They concluded that due to the lack of debris in the tape lift extract, it was much less likely to contain compounds that could interfere with subsequent DNA analysis.

Another benefit of tape lifts is that they are already utilized in the collection of other types of trace evidence, such as hair, fibers, gunshot residue, and fingerprints. A technique for recovering DNA from such lifts would allow fewer of them to be taken at crime scenes, and more information to be gleaned from those collected. Hall and Fairley (2004) recovered DNA

from tape lifts used to recover gunshot residue. In their procedure, small squares of tape were used to lift the residue from articles of clothing, which were then carbon coated and subjected to scanning electron microscopy. Next, the tape was cut into pieces and DNA extracted. The authors reported an 80% recovery rate, though it was unclear if this meant full DNA profiles were obtained or simply that DNA was detected 80% of the time. Zamir and colleagues (2000) investigated the retrieval of DNA from tape lifts that had been subjected to fingerprint enhancement techniques. Fingerprints were laid down on insulation tape and examined with an alternate light source, followed by cyanoacrylate fuming, and staining with BY-40 and crystal violet. The tape was then cut into pieces, placed in a digestion solution, and the DNA organically extracted. A profile was obtained from 75% of the donors for at least five of the six loci examined. These studies show that recovering DNA from tape lifts is not only possible, but could simply be another step in a process that most crime labs already use and are familiar with.

Unfortunately, recovery methods used to collect DNA from tape in previous studies vary greatly, making comparisons difficult. Organic extraction was the most common method of DNA isolation (Zamir et al., 2000; Bright and Petricevic, 2004; Zamir et al., 2004; Bille et al., 2009*a*; Frigolette, 2010). Li and Harris (2003) and Barash and colleagues (2010) utilized chelating resins, while Hall and Fairley (2004) employed a Qiagen QIAmp mini tissue kit plus a 1M solution of dithiothreitol (DTT). The method used to recover cells from the tapes varied as well. Cutting the tape into small pieces and submerging them in a solution was mentioned by a few authors (Li and Harris, 2003; Hall and Fairley, 2004; Barash et al., 2010). Bille and colleagues (2009*a*) and Frigolette (2010) used a foam swab moistened with an adhesive remover for collecting cells from tape. Likewise the types of tape employed in the studies varied, including Scotch® tape, lifting tape, electrical tape, and insulation tape. While the authors all seem to

recognize the potential of tape lifts in DNA collection, there is no consensus on the best type of tape for lifting, or the most efficient processing methods. An optimized tape lifting technique would be useful for recovering DNA from a number of substrates, including crime scene items.

Tape as an alternative method for collecting reference samples:

When a DNA profile from a specific person (a known, or reference sample) is needed, a buccal (cheek) swab is usually obtained. This is because bodily fluids, like saliva, are a rich source of DNA (Walsh et al., 1992). Once isolated, regions (loci) of DNA that are highly variable among individuals are characterized by size or sequence, forming a profile, which can then be compared to profiles generated from evidence. Crime scene items, however, generally contain much less DNA than a buccal swab, and so full profiles may not be obtained (a full profile is one in which all of the variable regions being considered (16 in this study) are successfully characterized, whereas a partial profile results when some of the regions being considered are not ($\leq$15 characterized)). A partial profile may result from a sample containing degraded or low levels of DNA, or from a suboptimal recovery technique.

Tape has been shown to successfully lift cells from an object someone has touched (Barash et al., 2010); it follows, then, that the application of tape directly to the skin could also recover cells. Based on this, tape lifts from skin have been suggested as an alternative method of collecting reference samples (Li and Harris, 2003). Such a method could be extremely useful among certain cultures (e.g., in the Middle East), where the use of buccal swabs can be considered invasive or is otherwise socially unacceptable. Sampling a less intimate area, such as the hands or feet, might provide an acceptable alternative.

DNA profiles can indeed be generated from tape applied to the skin. Zamir and colleagues (2004) reported that cadaver profiles were retrievable from adhesive lifters that had been used to collect gunshot residue from the tissue surrounding bullet wounds. The authors set out to determine whether biological residue that had been transferred to the lifter, such as dried blood and skin cells, could be used to generate the DNA profile of the cadaver. The study showed that a full profile was generated 50% of the time, and a partial profile was obtained 42% of the time.

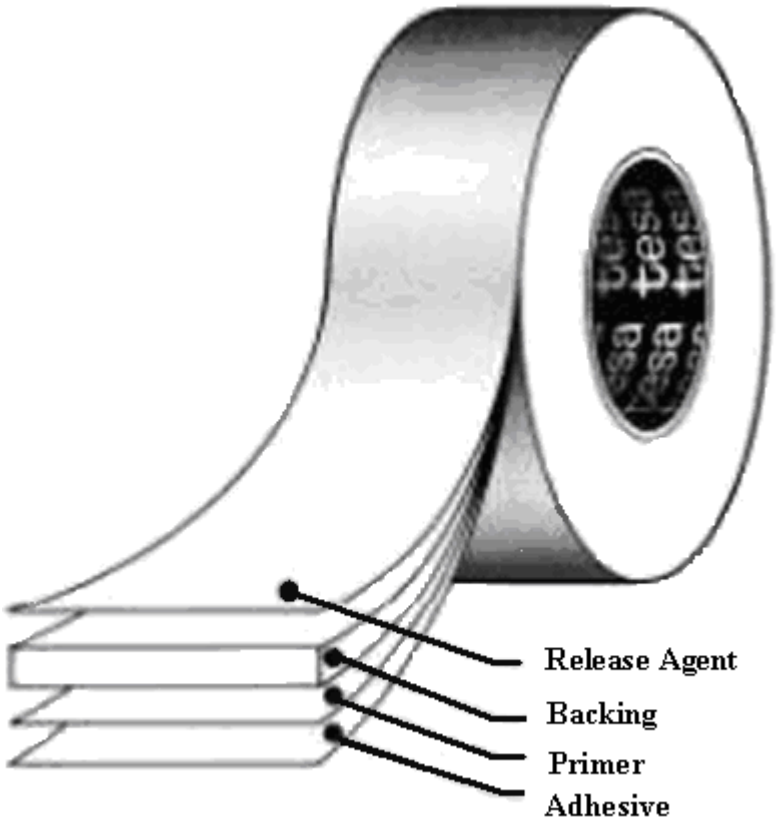
Bright and Petricevic (2004) also investigated collecting cells from the skin surface to generate a DNA profile. First, they swabbed the hands and feet of volunteers to determine whether an organic or chelating resin extraction was more suited to the skin swabs, and which area of the foot would yield the most complete DNA profile. They then correlated the areas of high yield on the foot to those on a shoe insole. Their method demonstrated that a profile can be generated from simple swabs of the skin, that an organic extraction is more effective than a chelating resin on this type of sample (66.6% recovery vs. 0% recovery), and that the top of the foot yielded more DNA than the sole.

Li and Harris (2003) applied tape to different regions of the body and compared the number of alleles they were able to detect from each region. They generated full profiles from lifts taken behind the ear, while the number of alleles detected decreased respectively in samples collected between the fingers, the back of the neck, the inside of the elbow, and the ankle. Such studies show the potential of using tape to collect reference samples from the skin, and that the area of the body being sampled plays a key role in determining how complete the subsequent DNA profile will be.

Tape construction:

When developing a method for retrieving DNA from tape, it is important to consider the composition of the tape itself, since interactions between the components of the tape and the chemicals used in the DNA isolation process, or even their interaction with the DNA itself, may impact subsequent DNA analysis. Compounding the problem is the fact that tape construction can be complex (Figure 1), involving many different layers, each of which could affect the reaction differently.

Figure 1. Common Construction of Adhesive Tapes



The layers shown in this diagram are those that are most commonly included in the construction of adhesive tapes (Image modified from Tesa Tape North America, http://www.tesatape.com/company/research/faq/faqs,40254,1.html).

The two layers found in all types of tape are the backing and the adhesive. The backing can be made from many different materials, including fabric, paper, cellulose acetate, or polyethylene. Additions may also be made to the backing of tapes that are meant to have some shielding properties, such as from magnetic fields or electrical interference (Dillingham, 2002). The adhesive, however, is probably the most complex layer. While the simplest versions are made of elastomers (chain-like molecules able to retain their shape after being stretched, such as rubber) and resin tackifiers (resinous material that makes the adhesive sticky), a combination of fillers, stabilizers and extenders may also be included (Smith, 2007). The exact formulation for the adhesive on each type of tape is generally proprietary, but all are complicated and vary depending on their intended purpose. Other layers may also be present, such as a release coat that allows the tape to be unwound from the roll, a primer coat that binds the adhesive to the backing, and a fabric reinforcement layer (Smith, 2007).

The type of tape determines the number of layers and their respective components. Packing tape, for example, has a backing made of polyester film and an adhesive made of acrylic or silicone (Nitto Denko, Product Information Sheet). Masking tape, on the other hand, has a crepe paper backing and uses a rubber based adhesive (3M, Product Information Sheet *b*). Duct tape is slightly more complex, and includes a third layer of fabric reinforcement, called the scrim, which is made of fibers woven together in a loose checkerboard pattern (Smith, 2007).

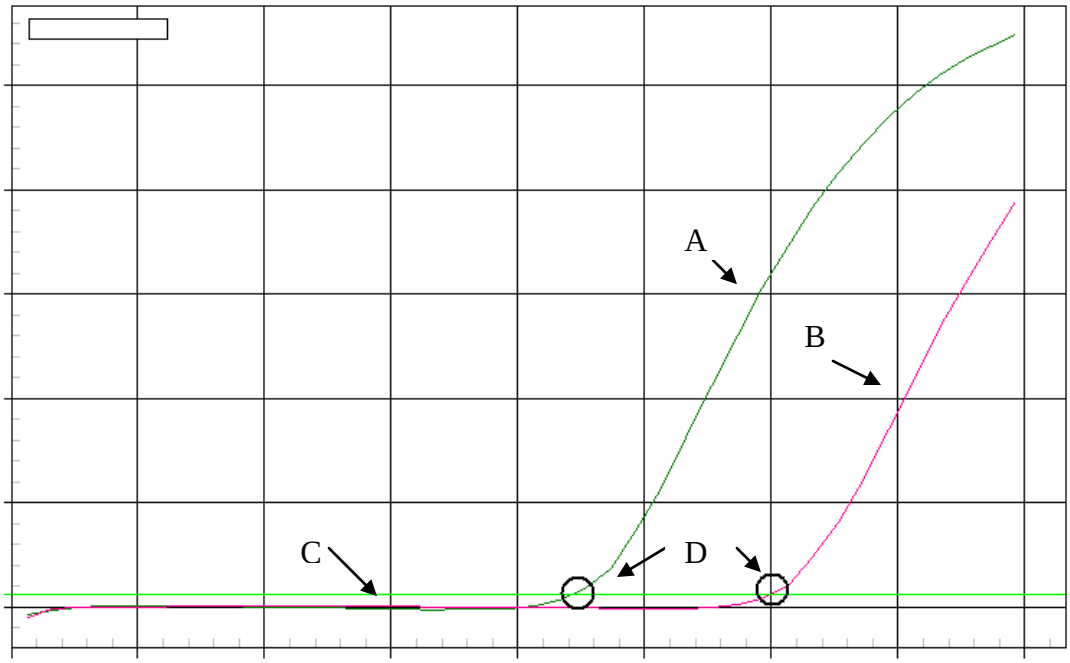
The variability among tape components means that all types of tape might not be well suited to the collection of cells or various DNA isolation techniques. Certain adhesives may be more difficult to free cells from, while the backing or fabric reinforcement layers of other tapes could break down during the extraction process and interfere with downstream DNA analysis.

There is a need, then, to determine which tapes are most suited to the DNA recovery process and which isolation procedures are most effective on them.

Quantifying DNA yields:

One method of evaluating the efficacy of a DNA recovery technique is by estimating the amount of DNA it collects. A sensitive and accurate method of DNA quantification is needed to identify the most successful recovery method. While multiple DNA quantification techniques exist, including UV spectrometry and SYBR-green staining, quantitative (Real-Time) PCR (qPCR) is commonly used in crime laboratories because it is automated, precise, and allows for high throughput (DNA Initiative, 2011). qPCR employs cycles of DNA replication, in which a specific region of the DNA is copied, producing new DNA strands. These new strands are then used as a template in the next cycle of replication, causing the amount of DNA to increase exponentially. A fluorescent probe binds to the newly replicated strands and the amount of DNA present is quantified by measuring the level of fluorescence detected during each cycle (Figure 2 lines A&B). When a section of DNA is duplicated, the strength of the overall fluorescent signal should increase at a rate proportional to the number of copies of DNA. The growth of that signal is detected and graphed as the reaction takes place.

Figure 2. Real Time PCR Graph



Graph showing the increase in fluorescent signal over time for two DNA extracts during qPCR A&B) Signal from extracts C) Threshold value D) Point where the signal from the extracts crosses the threshold value. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.

A threshold value is set (Figure 2 line C), and the number of cycles required for the fluorescent signal in each DNA extract to reach this value is recorded (Figure 2 point D). The DNA concentration of an unknown extract can then be estimated by comparing it to extracts of known concentration. For instance, if the known (Figure 2 line A) reached the threshold value after 22 cycles, and the unknown extract (Figure 2 line B) reached it after 30 cycles, then the known extract (A) contained more DNA because it took less time to reach the same signal level as the unknown (B). By including multiple known extracts in the run, a formula can be created to calculate the DNA concentration in any solution based on the number of cycles it took to reach the threshold.

Goals of this study:

An optimized procedure for collecting and recovering DNA using tape lifts would not only allow efficient generation of a profile from a piece of evidence, but would also make better use of lifts already routinely taken at crime scenes. While some crime laboratories may currently utilize tape lifts to collect DNA, there has been little research in determining the best method to do so. The first goal of the research presented here was to determine the types of tape that are most suited to the collection of cells, and the best method of recovering DNA from them. DNA yields were compared from multiple types of tape used to lift a constant amount of dried blood from a surface, as well as from a variety of recovery techniques, including swabbing with different swabbing solutions, soaking in chloroform, and soaking in digestion buffer. The second goal was to determine where the most complete DNA profile can be recovered from on the body, and whether tape lifts or swabbing the skin is more effective. The results of this study are meant to contribute to the development of alternative and less invasive methods for collecting reference samples from individuals.

METHODS

The original portion of this study—optimizing the tape lift procedure—was divided into four parts. First, eight types of tape were swabbed using six different swabbing solutions to determine which was most successful at loosening cells from the tape. Second, the most effective swabbing solution was used on each type of tape to determine which tape yielded the most DNA. Third, the swabbing procedure was compared to soaking the tape in chloroform, to see if dissolving the adhesive directly and skipping the swabbing step was useful, and finally, the swabbing procedure was compared to soaking the tape in digestion buffer, again to skip the swabbing step.

In the subsequent portion of the study, the use of tape lifts to collect cells from skin was investigated. Lifts were taken from various areas of the body and the number of alleles detected was compared among them. The same body regions were also swabbed to determine if swabbing is more effective than tape lifts in retrieving skin cells.

Figure 3. Tapes Included in Study

Types of tape compared in this study. Back, left to right: elastic, duct, packing, masking, and electrical tapes. Front, left to right: lifting, Scotch®, and surgical tapes. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.

Optimization of the tape lift procedure:

Table 1. Types of Tape Tested

Type of Tape	Manufacturer
P-904 Permacel Packing Tape	Nitto Denko (Teaneck, NJ)
Tartan™ Utility Masking Tape #5142	3M (St. Paul, MN)
Scotch® Vinyl Electrical Tape Super 88	3M (St. Paul, MN)
3M™ Transpore™ surgical tape	3M (St. Paul, MN)
Scotch® Magic™ Tape 810	3M (St. Paul, MN)
Staples® brand duct tape	Staples, Inc. (Framingham, MA)
Elastikon Elastic Tape	Johnson & Johnson (New Brunswick, NJ)
LPC lifting tape # 1-1405	Lightning Powder Company, Inc (Jacksonville, FL)

The 8 tapes included in this study, as well as the manufacturer and their location.

Table 2. Swabbing Solutions Investigated

Swabbing Solution	Type of Solution	Manufacturer
Goo Gone® Spray Gel	Adhesive Remover	Magic American Products, Inc. (Bedford Heights, OH)
Un-du®	Adhesive Remover	Un-du Products, Inc. (St. Louis Park, MN)
Un-du® (VOC Compliant)	Adhesive Remover	Un-du Products, Inc. (St. Louis Park, MN)
Adhesive Remover	Adhesive Remover	Manco, Inc. (Avon, OH)
Sterile Water	Traditional Swabbing Agent	N/A
Digestion Buffer (20 mM TRIS, 15 mM EDTA, 1% SDS, pH 7.5)	Traditional Swabbing Agent	N/A

The types of swabbing solutions used in this study, as well as the manufacturers and locations.

Swabbing solution comparisons:

Blood from a single donor was used as a DNA source for the optimization portion of the study owing to an assumed consistent DNA concentration (Park et al., 2008). All consumables were autoclaved and UV irradiated for 5 min on each side (approximately 7.5 J/cm^2). Blood was spotted onto a sterile Petri dish and dried overnight (one 5 µL spot for each swabbing solution tested). A section of tape, approximately 2 inches long, was formed into a circle and used to lift the blood spot from the Petri dish by applying it repeatedly until all of the blood was removed or the tape no longer appeared to be lifting the spot. Only one side of the tape circle (approximately 1 inch) was pressed to the Petri dish. A Micro CleanFoam® swab (ITW Texwipe, South Mahwah, NJ) was saturated with one of the swabbing solutions and used to swab the tape until all visible blood was removed.

A second swab, moistened with digestion buffer, was used on the area of the Petri dish from which the lift was taken to determine if any blood was left behind. Each swab was placed in a 1.5 mL centrifuge tube with 300 µL digestion buffer and 2 µL proteinase K (pro K; 20 mg/mL). A positive control was created by adding 5 µL of blood directly to the digestion buffer/pro K mixture. A reagent blank was also generated containing only digestion buffer and pro K. Tubes were incubated at 55°C overnight. The swab was then removed from the tube, a pipette used to draw off any remaining solution, and the extract replaced in the tube. Four-hundred microliters of phenol was added and the tube was vortexed briefly and centrifuged for 3 min at full speed. The aqueous layer was transferred to a new tube with 400 µL chloroform, vortexed, and centrifuged for another 3 min at full speed. The aqueous layer was placed on a Microcon® YM-30 filter (Millipore, Billerica, MA) and centrifuged for 10 min at 14,000 X g.

The flow through was discarded and the extract washed using 300 µL low TE (10 mM TRIS, 0.1 mM EDTA) and centrifuged for 10 min at 14,000 X g. The wash step was repeated once. Finally, 30 µL of low TE was added. The filter was inverted in a new tube and centrifuged for 1 min at 2,000 X g. Two replicates were produced for each swabbing solution. The lifting, swabbing and extraction procedures were repeated for each type of tape.

DNA yields were quantified using a BioRad iQ™5 real-time PCR detection system (BioRad, Hercules, CA) and a Quantifiler® Human DNA Quantification kit (Applied Biosystems, Foster City, CA). The reaction consisted of 7.5 µL reaction mix, 6.3 µL of primer mix, and 1.2 µL of DNA. The Quantifiler® standard was diluted as directed by the manufacturer, ranging from 50 ng/ µL to 0.023 ng/ µL. The cycling procedure involved a 10 min polymerase activation step at 95°C, a 15 sec denaturing step at 95°C, and a 1 min dual annealing and extension step at 60°C. The last two steps were repeated for 40 cycles.

The same procedures were used for lifting tape, elastic tape and all subsequent DNA extractions and quantifications in this study; however, Amicon Ultra 30K Millipore™ filters (Millipore) were utilized in place of the Microcon YM-30 filters, which had been discontinued. The centrifugation portion of the wash steps was reduced to 5 min, and no TE was added following the second spin. The final centrifuge step was reduced to 1000 X g and the time was increased to 2 min.

Types of tapes comparisons:

The effectiveness of each tape type in lifting cells was compared using an approximately 1 inch long section to lift a blood spot (again by forming an approximately 2 inch long piece into

a circle). The tapes were swabbed with a foam swab saturated with digestion buffer and processed as above. A positive control and reagent blank were generated as previously described, and substrate controls were created by UV pre-treating a piece of each tape and placing a 1 inch long section in digestion buffer and pro K.

Tapes soaked in chloroform:

Duplicate blood lifts were taken using each type of tape. One was swabbed with digestion buffer while the portion of the other containing the blood stain was cut out and submerged in 400 µL chloroform to dissolve the adhesive. Layered on top of the chloroform was 300 µL of digestion buffer containing 2 µL of pro K. A positive control for the chloroform method was created by depositing 5 µL of blood directly into the chloroform before the other solutions were added, while reagent blanks were produced by combining chloroform, digestion buffer, and pro K as above. Substrate controls were generated by submerging a piece of each tape in chloroform following UV pretreatment, then adding digestion buffer and pro K.

The top of the tube was covered with parafilm and the tube rocked gently on its side overnight at room temperature. The tape was removed from the tube and discarded, except in the case of elastic, lifting, and Scotch® tapes, which had formed a gel. Tubes were centrifuged and the aqueous layer was removed and placed in a clean tube. Centrifugation also served to concentrate the gel at the bottom of the tube so the aqueous layer could be accessed. Experiments were run in triplicate.

Tapes soaked in digestion buffer:

Duplicate lifts were performed with four tapes (Scotch®, packing, surgical and duct). One piece was swabbed with digestion buffer, and the area containing the blood on the other was cut out and placed in 400 µL digestion buffer and 2 µL pro K. A positive control and reagent blank were generated as in the swabbing solution experiments. Three replicates were created using each recovery method and processed as above.

Collection of reference samples using tape lifts:

Preliminary comparison of tape lifts from skin:

Two tapes (lifting and surgical) were used to collect cells from four areas of the body on four participants: two male, two female. Approval for the use of human subjects was granted by the Michigan State University Committee on Research Involving Human Subjects (IRB#: 10-410). Tapes were used on one side of the body (lifting tape on the left side, surgical tape on the right side, or vice versa). Lifts were taken behind the ear, between the fingers (finger webbing), the fingertips, and the ankle between the ankle bone (lateral malleolus) and the Achilles tendon.

One side of a loosely rolled piece of tape (approximately 2 inches long) was applied to one of the body regions five times. For the fingertips and finger webbing, the tape lift was cumulative, meaning one piece of tape was applied to each region between the fingers or to each fingertip five times. A buccal swab from the volunteers was also collected. The area of the tape applied to the skin was cut out and placed in a 1.5 mL centrifuge tube with 400 µL digestion buffer and 2 µL pro K. A positive control was created by adding 5 µL of an epithelial cell solution (created from two buccal swabs following protocol outlined by Bille and colleagues

(2009*b*)) to digestion buffer and pro K, and a reagent blank produced using only the last two solutions. Each tube was incubated at 55°C overnight and processed as above.

DNAs from two lifting tape and two surgical tape lifts from each participant, representing all body regions tested, were amplified using an Identifiler® Human DNA Amplification Kit (Applied Biosystems, Foster City, CA). Reactions contained 4 µL AmpFℓSTR® PCR Reaction Mix, 1 µL AmpFℓSTR® Identifiler® Primer Set, and 0.5 µL (2.5 units) Amplitaq Gold® DNA Polymerase. One nanogram of DNA was added to the reaction (or a maximum of 4.5 µL of DNA) and the total volume brought to 10 µL with the addition of water when necessary. A positive control was created as above, with 4.5 µL AmpFℓSTR® Control DNA 007. The cycling protocol included an initial incubation at 95°C for 11 min, 30 cycles of denaturing at 94°C for 1 min, annealing at 59°C for 1 min, and extension at 72°C for one min, and a final hold at 60°C for 1 h.

Capillary electrophoresis was performed using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems). The reactions contained 25 µL of formamide, 0.5 µL of GeneScan 500 LIZ Size Standard, and 1.5 µL of DNA or allelic ladder. One drop of mineral oil was added to each tube and the DNAs were separated using performance optimized polymer 4 and 1X running buffer containing EDTA. Parameters for electrophoresis included a run temperature of 60°C, injection time of 5 sec at 15 kV, and a run time of 28 min at 15 kV. Data were analyzed using GeneMapper ID v3.2.1, with panel idenfiler_v1, analysis method "Identifiler Ken", size standard CE_G5-HID_GS500, and matrix DS-33 Matrix 7-12-10. Based on negative preliminary results, the rest of the lifting tape extracts were not analyzed.

Tape specific PCR inhibition:

Four volunteers had one of each of the eight types of tape applied ten times behind one ear, with a second type applied behind the other ear. The area of the tape applied to the skin was cut out, the DNA recovered using the digestion buffer soaking method, and the extract analyzed using the protocol outlined above. The process was repeated with another set of tape lifts, except that the tape was swabbed using digestion buffer. Positive controls and reagent blanks were produced as in the previous experiment.

Determination of optimal body areas for DNA recovery:

One side of a loosely rolled piece of surgical tape (approximately 2 inches long) was applied repeatedly to one of six areas on four participants: behind the ear, the finger webbing, the fingertips, both the fingertips and the finger webbing, the inside of the wrist, and the back of the hand. Combination fingertip and finger webbing lifts were collected from the non-dominant hand from half of the individuals, and the dominant hand from the other half. All other lifts were taken on the opposite side of the body as the finger combination.

The tapes were subjected to the digestion buffer soaking method, positive controls and reagent blanks generated, and the DNA extracted, quantified, and amplified as above. Capillary electrophoresis was performed on an AB 3500 Genetic Analyzer (Applied Biosystems). An appropriate amount of mastermix, equivalent to 8.985 μL formamide and 0.015 μL GeneScan 500 LIZ Size Standard per sample, was produced; 9 μL mastermix was combined with 1 μL of DNA or allelic ladder. Parameters for electrophoresis included a temperature of 60°C, an injection time of 10 sec at 16 kV, and a run time of 22.2 min at 19.5 kV. Data were analyzed

using GeneMapper® ID Software v4.1, with panel Identifiler_v2, analysis method HID Analysis Method G5 POP7, and size standard GS500.

Tape lifts and swabbing skin comparisons:

Participants were asked to draw a letter and number from an envelope and write them down together on a form out of sight of the analyst. This letter was written on the tube containing his/her buccal swab, and the number on the tubes containing tape lifts or skin swabs.

Three areas of the body from 20 volunteers were compared: behind the ear, the finger combination, and the back of the hand. Regions were swabbed with digestion buffer on one side of the body and sampled using surgical tape on the other side. Half of the participants were swabbed on their dominant side, while the other half were swabbed on their non-dominant side. Tape lifts were taken from the side of the body that was not swabbed. The fingertips and finger webbing were swabbed cumulatively, with approximately 2 sec spent on each fingertip or webbing. Tape lifts taken from this area were cumulative, with each area pressed to the tape 5 times. A buccal swab was collected from every participant.

The tape lifts were subjected to the digestion buffer soaking method detailed above. The swab heads were cut off and submerged in 200 μL digestion buffer and 2 μL of pro K, and incubated overnight at 55°C. The swab heads were placed in a spin basket and centrifuged at 5000 rpm (2655 X g) for 1 min; the eluate was combined with the solution in the original tube. Positive controls and reagent blanks were created, and the DNAs extracted and quantified as in the previous experiment. STRs were tested as described above for the AB 3500.

Analysis of buccal swabs:

The head of each buccal swab was cut off, placed in a new 1.5 mL tube, and stored in a freezer at -20°C. Buccal swabs were processed after all the experimental lifts and swabs, using the protocol outlined for the skin swabs; however, 400 μL digestion buffer was used and the DNAs were diluted to 0.5 ng/ μL.

Statistical Analysis:

Comparison of DNA quantities in optimization experiments:

Subgroups of data were too small to test for normality, so nonparametric tests were used to compare the effectiveness of swabbing solutions, tapes, and recovery methods (soaking or swabbing). Comparisons of DNA yield among swabbing solutions and among tapes were completed using an SPSS 17 software package (IBM, Somers, NY) and a Kruskal-Wallis test ($\alpha = 0.05$). Swabbing solutions were also compared by combining the data from all tapes into one set. A Kolmogorov-Smirnov test was used to test for normality, and the difference in quantity among solutions evaluated using a one-way ANOVA and a Tukey HSD test ($\alpha = 0.05$). DNA quantities were compared between soaking and swabbing methods using a Mann-Whitney U test ($\alpha = 0.05$) for each tape.

Comparison of DNA quantities and correct allele percentages from swabbed and tape lifted body regions:

DNA quantities were grouped by body region and recovery method (Swabs behind the ear, tape lifts behind the ear, etc) and tested for normality using a Kolmogorov-Smirnov test ($\alpha = 0.05$). The effect the body region, recovery method, or hand dominance had on the DNA yields

were determined using SPSS 17 and 21 total Kruskal-Wallis tests ($\alpha = 0.05$) comparing the DNA quantities as indicated in Table 3. Combinations of quantities for each test were generated by holding all variables constant except the one being tested. If the variable consisted of more than two subgroups, as was the case for body region, and the result of the Kruskal-Wallis test was significant, pair-wise comparisons were performed using Mann-Whitney U tests ($\alpha = 0.05$). The critical p-values used in both tests were manually adjusted with a Bonferroni correction, which involves dividing the target p-value (0.05) by the number of paired comparisons to be made, as described by Scholfield (2010).

STR profiles generated from the sets of swabs and tape lifts taken from behind the ear, the finger combination, and the back of the hand, were compared to the buccal swab profiles to determine if allelic drop-in or drop-out had occurred. The percentage of correct alleles detected (correct allele calls) in each DNA extract was calculated by counting the number of correct allele calls and dividing it by the total correct allele calls possible (32, assuming heterozygosity at all loci; Table 3). A single allele call from a homozygous locus was counted twice. If the profile from the skin was homozygous at a locus where the buccal profile was heterozygous, the single allele call was counted once. The percentages of correct alleles were then compared using the method described above for the DNA quantities from these lifts and swabs.

Table 3. Comparison of DNA quantities and detected allele percentages among body regions, recovery methods, and the side of body sampled

Variable	Data Compared		
Body region, regardless of dominance	AS	BS	CS
	AT	BT	CT
Body region, accounting for dominance	AS DOM	BS DOM	CS DOM
	AT DOM	BT DOM	CT DOM
	AS NON	BS NON	CS NON
	AT NON	BT NON	CT NON
Recovery method, regardless of dominance	AS	AT	
	BS	BT	
	CS	CT	

Variable	Data Compared	
Recovery method, accounting for dominance	AS DOM	AT DOM
	AS NON	AT NON
	BS DOM	BT DOM
	BS NON	BT NON
	CS DOM	CT DOM
	CS NON	CT NON
Dominance	AS DOM	AS NON
	AT DOM	AT NON
	BS DOM	BS NON
	BT DOM	BT NON
	CT DOM	CT NON
	CS DOM	CS NON

Each row under the heading "Data Compared" indicates a separate Kruskal-Wallis test was used to compare data from those extracts. These combinations were determined by holding all variables except the one being tested constant. For example, row 1 (left) indicates a comparison between AS, BS and CS extracts (the method was held constant) to determine if there was a significant difference in DNA quantity recovered among body regions, regardless of dominance. The same combination of extracts was used to compare detected allele percentages among body regions. A = behind the ear, B = finger combination, C = back of hand, S = swab, T = tape, DOM = dominant side sample, and NON = non-dominant side sample.

RESULTS

Optimization of the Tape Lift Procedure:

DNA yields from tape swabbed with various solutions:

There was marked variability in the swabbing solutions' behavior on the tapes. Digestion buffer and water were easiest to use because the blood was soluble in them, whereas the adhesive removers caused the blood to flake off the tape, making it difficult to collect with a swab. The Un-du products were the most difficult solutions to work with because they evaporated quickly, allowing the blood flakes to re-adhere to the tape. Another difficulty arose when the packing tape was swabbed with any of the adhesive removers: the adhesive completely detached from the backing, often trapping blood flakes inside.

The amount of DNA recovered from the tapes ranged from 0 to >1 ng/µL (Table 4), though the yields seemed to increase as more tapes were tested over a series of weeks (two types per week). There was no significant difference in DNA recovery among the swabbing solutions when tapes were tested separately (Table 5). The combined DNA yields for each swabbing solution were normally distributed (Appendix B). Digestion buffer and water were equally effective, and both gave significantly higher yields than the adhesive removers (Table 6).

Table 4. Average DNA recovery (ng/µL) using each swabbing solution

Tape	Adhesive Remover	Goo Gone	Un-du	Un-du VOC	Digestion Buffer	Water	PC	RB
Scotch®	0.120	0.000	0.090	0.040	0.240	0.140	0.520	0.000
Packing	0.191	0.080	0.100	0.134	0.355	0.548	1.117	0.000
Duct	0.220	0.203	0.130	0.003	1.442	0.277	0.570	0.000
Masking	0.933	0.374	0.669	0.088	1.096	0.908	2.667	0.001
Electrical	0.210	0.240	0.290	0.250	0.890	1.070	1.230	0.000
Surgical	0.290	0.130	0.210	0.170	1.810	1.030	1.150	0.000
Lifting	0.540	0.500	0.420	0.350	0.740	0.460	0.630	0.000
Elastic	0.030	0.030	0.040	0.070	0.940	1.040	1.310	0.000
Average	0.317	0.195	0.244	0.138	0.939	0.684	1.149	0.000

All quantities are the average of two trials. The bottom row displays the average for each swabbing solution across all tapes. PC = Positive control, RB = Reagent Blank

Table 5. Comparison of DNA yields from six swabbing solutions on each tape

Type of tape	Significance
Scotch®	0.705
Packing	0.096
Duct	0.158
Masking	0.790
Electrical	0.176
Surgical	0.072
Lifting	0.097
Elastic	0.139

There was no significant difference in DNA recovery among swabbing solutions when each tape was considered individually. P-values from nonparametric Kruskal-Wallis tests are shown.

Table 6. Comparison of all DNA quantities recovered using each swabbing solution

Solution 1	Solution 2	Significance
DB	AR	<0.001
	GG	<0.001
	Un	<0.001
	V	<0.001
	W	0.328
W	AR	0.027
	GG	0.001
	Un	0.004
	V	<0.001
	DB	0.328

Water and digestion buffer gave significantly higher yields than the adhesive removers, and were not significantly different from each other. P-values from a Tukey HSD test on the combined DNA yield data from all 8 tapes are shown. DB = digestion buffer, AR = adhesive remover, GG = Goo Gone®, Un = Un-du®, V = VOC compliant Un-du®, W = Water

DNA yields from various tapes:

A side by side tape comparison was completed using a set of blood lifts created on the same day, since the lifts from the previous experiment were generated over a series of weeks and thus the DNA yields less consistent among tapes (Table 4). Digestion buffer appeared to remove cells equally well on all types based on the visible amount of blood left after swabbing. This was supported by the recovered DNA quantities (Table 7), where there was no significant difference among tapes ($p = 0.477$, Kruskal-Wallis). Similarly, the substrate controls for each yielded 0 ng/µL of DNA when soaked in digestion buffer.

There was, however, variation in the ease of use of the tapes. The adhesives of duct, elastic, and electrical tapes transferred to the analyst's gloves, the Petri dish, the swab, and later, to the tube it was placed in. Packing and duct tapes were challenging to remove from the Petri dish because they had a stronger adhesive, while electrical tape's adhesive turned the swabbing

solution black, making it difficult to determine how much of the stain had been collected. In contrast, the area containing the blood on transparent tapes (Scotch®, packing, surgical, and lifting tapes) was easy to identify and isolate.

Only a very light, thin ring, outlining where the spot had been, generally remained after blood was tape lifted from a Petri dish. DNA collected from these rings (Table 7) showed there was no significant difference in the amount of cells left behind by the different types of tape ($p = 0.136$, Kruskal-Wallis).

Table 7. Average quantity of recovered and residual DNA from each tape

Sample	Recovered Quantity (ng/µL)	Residual Quantity (ng/µL)
Packing	1.63	0.011
Lifting	1.48	0.022
Surgical	1.44	0.013
Masking	1.29	0.073
Scotch®	1.50	0.080
Electrical	1.68	0.040
Duct	1.60	0.136
Elastic	0.96	0.055
PC	1.66	
RB	0.00	

Quantities in column 1 were recovered from tapes using digestion buffer, while those in column 2 represent blood rings left on a Petri dish after tape lifting. There was little variation in the amount of DNA recovered from the tapes, or in the amount left behind by them. PC = Positive control, RB = Reagent Blank

DNA yields from tapes soaked in chloroform:

Soaking the tape in chloroform appeared to remove the adhesive from the tape, although in the cases of elastic, lifting, and Scotch® tapes, the backing also dissolved, forming a gel. The

electrical tape turned the chloroform layer black, but the pigment did not travel to the aqueous layer. Chloroform soaked substrate controls yielded 0 ng/µL of DNA, and swabbing yielded consistently more DNA than the chloroform method (Table 8). There was no significant difference in DNA recovery among types of tape when they were soaked in chloroform ($p = 0.293$, Kruskal-Wallis), nor when they were swabbed with digestion buffer ($p = 0.376$, Kruskal-Wallis). However, the swabbing method yielded significantly more DNA than the chloroform method for all tapes tested (Table 8).

Table 8. Average DNA quantity (ng/µL) recovered with chloroform and swabbing methods

Tape	Chloroform	Swab	Significance
Surgical	0.22	1.21	0.025
Masking	0.25	1.03	0.025
Scotch®	0.30	1.33	0.025
Packing	0.09	1.06	0.022
Duct	0.23	1.13	0.025
Elastic	0.10	1.05	0.025
Lifting	0.27	1.03	0.025
Electrical	0.25	0.81	0.023
PC	0.77	2.15	
RB	0.00	0.00	

Columns 2 and 3 display averages of 3 trials. P-values from one-tailed Mann-Whitney U tests, in column 4, indicated that swabbing yielded significantly more DNA than soaking in chloroform for all 8 tapes. PC = Positive control, RB = Reagent blank

DNA yields from tapes soaked in digestion buffer:

Soaking the tapes in digestion buffer had no visible affect on them, except that the lifting and Scotch® tapes became opaque after incubation overnight. The elastic, electrical, and duct tapes left adhesive residue on the sides of the tube. Similar to swabbing, soaking the electrical tape turned the solution black. The pigment, however, remained in the organic layer.

Both techniques yielded similar amounts of DNA (Table 9), with no statistical difference in DNA recovery among types of tape when soaked in digestion buffer ($p = 0.976$, Kruskal-Wallis), nor when swabbed with digestion buffer ($p = 0.794$, Kruskal-Wallis). There was also no difference in DNA recovery between the soaking and swabbing methods for any of the tapes (Table 9).

Table 9. Average amount of DNA recovered using the soaking and swabbing methods

Tape	Swab	Soak	Significance
Scotch®	1.99	1.64	0.414
Packing	1.50	1.51	0.413
Surgical	1.39	1.68	0.138
Duct	1.53	1.55	0.414
PC	1.90		
RB	0.00		

Quantities in columns 2 and 3 are averages of 3 trials. P-values from one-tailed Mann-Whitney U tests (column 4) show there was no significant difference in the amount of DNA recovered using either method on any of the tapes. PC = positive control, RB = Reagent Blank

Collection of reference samples from skin using tape lifts:

Preliminary comparison of tape lifts from skin:

Two translucent tapes (surgical and lifting) were used. The amount of visible cellular debris transferred to the tape varied more from individual to individual than by body region. For the majority of people, however, the most material seemed to be lifted from the ankle. There was no visible difference in the amount lifted between the two types of tape.

There was no indication of inhibition in either type of tape at the qPCR stage. DNA quantities from these lifts can be found in Appendix A, Table A1. All surgical tape lifts gave a full or partial STR profile, while none taken with lifting tape yielded a profile, indicating PCR inhibitors were present in the lifting tape extracts. Full profiles were recovered from three of the

surgical tape lifts taken from behind the ear, while the fourth was partial, with 56% of the alleles detected (Table A2). One of the lifts taken from the finger webbing gave a full profile while the other three gave partial profiles, with an average allele recovery of 57%. Only partial profiles were obtained from the fingertips and ankle, with allele recovery percentages of 89% and 26% respectively. Allelic drop-in, consisting of approximately one extra peak per affected locus, was detected in one lift taken from behind the ear (three loci) and two lifts taken from the finger webbing (one locus). Both the 007 positive control and the reagent blank behaved as expected (100% and 0% of alleles detected respectively).

Tape specific PCR inhibition:

Comparison of all eight tapes when soaked indicated that masking tape lifts were inhibited at the qPCR stage, and so were not carried on to STR analysis. STR profiles were generated from lifts taken with all other tapes except lifting and Scotch®, indicating inhibitors were present in these two. When tapes were swabbed, there was no indication of inhibitors (at either PCR stage) in the DNAs extracted from the lifting or masking tapes, but were still detected at the STR level in the DNA from the Scotch® tape lifts. A full profile was generated from the 007 positive control, and no alleles were detected in the reagent blank.

Determination of optimal body areas for DNA recovery:

The amount of cellular debris visibly transferred to the tape varied more by the individual it was collected from than the body region. The most skin debris appeared to be transferred from behind the ear or the finger webbing, though it was less obvious than in the previous ankle lifts. DNA quantities from these lifts are displayed in Table A3.

All tape lifts from behind the ear, as well as the 007 positive control, gave full profiles (Table 10). The allele percentages from the rest of the body regions decreased in the following order: back of the hand, fingertips, finger combination, finger webbing, and the inside of the wrist. The number of alleles detected following each lift can be found in Table A4. The reagent blank yielded no alleles. Lifts taken from the non-dominant hand gave consistently higher yields than the corresponding dominant hand lifts, though the yields were very similar in fingertip lifts from either side. Allelic drop-in of one allele per affected locus was present in three profiles from the fingertips and two from the inside of the wrist, involving at most three loci.

Table 10. Average allele recovery from each body region

	Behind the Ear	**Finger Webbing**	**Fingertips**	**Finger Combination**	**Inside of the wrist**	**Back of the hand**
Overall	100.00%	63.28%	89.84%	71.09%	59.38%	96.09%
Dominant side samples	100.00%	31.25%	89.06%	46.88%	46.88%	92.19%
Non-dominant side samples	100.00%	95.31%	90.63%	95.31%	71.88%	100.00%

The lifts from behind the ear were taken from the same side of the body as all other lifts except the finger combination. Row 1 displays average percentages of detected alleles from four lifts, when data from both body sides are combined. Rows 2 and 3 present the average of two lifts, holding the side of the body constant.

Tape lifts and swabbing comparisons on skin:

DNA quantities from the swabs and tape lifts of all 20 participants can be found in Table A5. None of the DNA quantity subgroups (swabs behind the ear, swabs of the back of the hand,

etc) were normally distributed (Table B2). Swabbing yielded significantly more DNA than tape lifts on the back of the hand and the finger combination, when data from both body sides were combined (Bonferroni corrected critical p-value of 0.017; Table 11A). However, significantly more DNA was recovered by swabbing all three body regions on the dominant side of the body, while the recovery techniques were equally effective on the non-dominant side of the body (Bonferroni corrected critical p-value of 0.0083; Table 11B).

Table 11. Comparison of swabbing and tape lifts

A.

Sample	Quantity P-value	Allele P-value
Behind Ear	0.117	0.543
Finger Combination	<0.001*	0.484
Back of Hand	<0.001*	0.101

B.

Sample	Quantity P-value	Allele P-value
A Dominant	0.007*	0.147
A Non-dominant	0.326	0.292
B Dominant	0.001*	0.147
B Non-dominant	0.045	0.343
C Dominant	0.001*	0.69
C Non-dominant	0.104	0.048

Column 2 (both tables) indicates comparisons of DNA quantity and column 3 represents comparisons of allele percentage, using Kruskal-Wallis tests. The Bonferroni corrected critical p-value for Table A was 0.017, and for Table B was 0.0083. Table A represents combined data from both sides of the body. In Table B, A = Behind the ear, B = Finger Combination, C = Back of the Hand. * = significant difference in DNA quantity between recovery methods.

Each subgroup of detected allele percentages had a non-normal distribution ($p < 0.001$) when data were organized by body area and recovery method (Swabs behind the ear, tape behind the ear, etc). Full profiles were almost always generated using either recovery method (Table 12), and there was no significant difference in the percentage of correct alleles detected between swabs and tape lifts, on either body side and when data were combined (Bonferroni corrected

critical p-value of 0.0083 and 0.017 respectively; Table 13A&B). However, the peak heights were considerably higher in the electropherograms of swab extracts, generally ranging from 2,000–10,000 relative fluorescent units (RFUs), as compared to peak heights of 50–500 RFUs in the profiles generated from tape lifts. The 007 positive controls and reagent blanks (one replicate for each run on the AB 3500) behaved as expected.

Table 12. Average percentage of alleles recovered using a swabbing or tape lift technique

	Swabs			Tape Lifts		
	Behind the Ear	**Finger Combination**	**Back of the Hand**	**Behind the Ear**	**Finger Combination**	**Back of the Hand**
Overall	99.68%	94.41%	95.69%	98.75%	99.70%	86.85%
Dominant side samples	100.00%	89.37%	92.12%	97.49%	100.00%	87.43%
Non-dominant side samples	99.33%	100.00%	99.67%	100.00%	99.40%	86.27%

Averages from three body regions are presented for each method (20 replicates). One side of the body was swabbed, the other tape lifted, alternating between individuals. Averages from each side are also shown (10 replicates each).

Finger combination swabs recovered significantly more DNA than from the back of the hand when data from each body side were combined (Bonferroni corrected critical p-value of 0.025; Table 13A). There was no significant difference, however, in the amount of DNA recovered among swabs of the dominant side body regions, while non-dominant side swabs of the back of the hand yielded significantly less DNA than the other two regions (Bonferroni corrected critical p-value of 0.0125; Table 13B).

Tape lifts from the finger regions yielded significantly more DNA than those from the back of the hand when data from both sides of the body were combined (Table 13A). However, the dominant side lifts from both behind the ear and the finger regions yielded significantly more DNA than those from the back of the hand; there was no significant difference on the non-dominant side (Table 13B).

Table 13. Comparison of DNA quantity recovered among body regions

A.

Sample	Kruskal-Wallis	Mann-Whitney U Test
Swabbed	0.008*	
A&B		0.091
A&C		0.031
B&C		0.001*
Tape	<.001*	
A&B		0.209
A&C		<.001*
B&C		<.001*

B.

Sample	Kruskal-Wallis	Mann-Whitney U Test
Dominant Side Swabs	0.271	
Non-dominant Side Swabs	0.007*	
A&B		0.013
A&C		0.002*
B&C		0.021
Dominant Side Tape	0.001*	
A&B		0.5
A&C		<.001*
B&C		0.003*
Non-dominant side tape	0.014	

P-values from Kruskal-Wallis and one-sided Mann-Whitney U tests. The Bonferroni corrected critical p-value for Table A is 0.025, and for Table B is 0.0125. When the Kruskal-Wallis test indicated a significant difference, a one-tailed Mann-Whitney U test was used for each pair-wise comparison of subgroups. Table A represents combined data from both body sides, whereas Table B represents data from each body side. A = Behind the ear, B = Finger Combination, and C = Back of the Hand. * = significant difference in DNA quantity.

There was no significant difference in percentage of correct alleles detected among swabbed body regions, or in tape lifts taken from behind the ear and the finger areas, on either

side of the body or when the data from both sides were combined (Bonferroni corrected critical p-value of 0.0125 and 0.025 respectively; Table 14A and 14B). However, lifts from these regions generated significantly higher percentages of correct alleles than lifts taken from the back of the hand in the combined data set (Table 14A). The only significant difference between tape lifted body regions in the body side subgroups was between the back of the non-dominant hand and behind the non-dominant side ear, in which the hand yielded fewer alleles (Table 14B). All other regions were equally effective on both sides of the body.

Table 14. Comparison of percentage of alleles detected from multiple body areas

A.

Sample	Kruskal-Wallis	Mann-Whitney U Test
Swabbed	0.332	
Tape	0.002*	
A&B		0.258
A&C		0.005*
B&C		0.002*

B.

Sample	Kruskal-Wallis	Mann-Whitney U Test
Dominant Side Swabs	0.213	
Non-dominant Side Swabs	0.549	
Dominant Side Tape	0.071	
Non-dominant side tape	0.011*	
A&B		0.159
A&C		0.007*
B&C		0.020

P-values generated by Kruskal-Wallis and Mann-Whitney U tests. The Bonferroni corrected critical p-value for Table A is 0.025, and for Table B is 0.0125. When the Kruskal-Wallis test indicated a significant difference, a one-tailed Mann-Whitney U test was used for each pair-wise comparison. Table A represents combined body side data, whereas Table B represents separate body sides. A = Behind the ear, B = Finger Combination, and C = Back of the Hand. * = significant difference in percentage of detected alleles

There was no significant difference in the DNA yield or the percentage of detected alleles in dominant side and non-dominant side lifts or swabs (Bonferroni corrected critical p-value of

0.0083; Table 15) when the body area and recovery method were held constant (ex. Swabs behind the dominant side ear compared to swabs behind the non-dominant side ear, etc).

Table 15. Comparison of dominant and non-dominant side lifts or swabs

Sample	Quantity p-value	Allele p-value
A Swab	0.034	0.292
B Swab	0.564	0.168
C Swab	0.248	0.252
A Tape	0.112	0.147
B Tape	0.545	0.317
C Tape	0.544	0.901

There was no significant difference between dominant and non-dominant side DNA yields or detected allele percentages. No trend in which side of the body yielded more DNA (Table A5) or gave the higher percentage of correct alleles (Table 12) was observed. P-values generated by Kruskal-Wallis tests are shown. Column 2 indicates comparisons of DNA quantity and column 3 indicates comparison of allele percentage. The Bonferroni corrected critical p-value for this set of comparisons was 0.0083. A = Behind the Ear, B = Finger combination, and C = Back of the hand.

Allelic drop-in was detected in two (of 20) swabs from the back of the hand and tape lifts behind the ear, one finger combination tape lift, and five tape lifts from the back of the hand, in both the dominant and non-dominant side. Drop-in generally affected one–five loci, each of which usually contained one extra allele. One tape lift from the back of the hand showed drop-in at nine loci, with two extra alleles per locus.

DISCUSSION

DNA evidence is a powerful forensic tool, so the most sensitive and effective methods for DNA recovery should be utilized. Recovery techniques commonly employed by crime labs include swabbing and soaking the material in question, but the use of tape lifts has recently begun to draw attention due to potential advantages. For instance, tape lifts may be more suited to some substrates than swabbing or soaking, may avoid PCR inhibitors, and tape lifts routinely taken at crime scenes can also be a source of DNA after examination for other types of evidence.

Tape may also be used in the commission of crime. In the process of applying the tape, the handler often touches the adhesive side, leaving skin cells behind, the DNA from which can potentially be used for identification. Whether the tape itself is evidence, or a tool to recover cells, an optimized procedure for processing tape is crucial to generating the most complete STR profile possible. Currently, there is no consensus on the most effective type of tape for DNA recovery or the best processing method, so a primary goal of this study was to optimize the tape lift procedure.

The first step towards optimization involved determining which solution yielded the most DNA when used to swab tape. The highest yields from tape lifts of dried blood were obtained using digestion buffer or water rather than the adhesive removers. The traditional solutions were also easier to use because the blood was soluble in them and was absorbed by the swab head. In contrast, the blood flakes created by the adhesive removers were much more difficult to collect with a swab. While some forensic laboratories currently utilize the Un-du solutions to swab tape, this study suggested these solutions were less efficient because they evaporated quickly, allowing the tape to become sticky again. An effective recovery method should both rehydrate and loosen cells. The Un-du products not only failed to rehydrate the blood, but they only

loosened cells for a short period of time. This is most likely why significantly higher DNA yields were obtained from tapes swabbed with digestion buffer or water than those swabbed with Un-du products.

The relative success of water and digestion buffer in this study is based on blood lifts, but a similar trend has been reported for lifts of skin cells. A parallel study in the Forensic Biology Laboratory at Michigan State University, using the same protocol as this one but with an epithelial cell solution as the DNA source, showed digestion buffer yielded significantly more DNA than the adhesive removers, and that water yielded significantly more than the VOC compliant Un-du (I Folland personal communication). Similarly, Petrovick and colleagues (2011) reported that a detergent solution gave the highest DNA yield, followed by water and then Un-du, when used as a swabbing solution to retrieve epithelial cells from handled electrical tape. However, the type of detergent used may also play a role. Thomasma and Foran (2009) found that a 1% SDS solution gave higher DNA yields than other laboratory and commercial detergent solutions when used to swab fingerprints on glass slides. Based on the results of these studies, an optimized tape lift procedure involving swabbing should utilize digestion buffer/1% SDS to wet the swabs.

There was no significant difference in DNA recovery among tapes, indicating similar amounts of DNA can be collected from various types. Some, however, required more applications to lift the blood spot than others. Packing, Scotch®, lifting, and surgical tapes generally lifted the majority of the stain in one application. All other tapes took many more applications to remove the entire blood spot. This difference may be partially explained by the tape backing. In this study, tapes were rolled into a circle before lifting; the rolling process, however, was complicated in tapes with flexible cloth backings or scrim (elastic and duct tapes),

because they tended to fold on and stick to themselves after very few manipulations by the analyst. Tapes with synthetic backings, like packing tape (polyester film; Nitto Denko, Product Data Sheet) and lifting or Scotch® tapes (acetate; Miller, personal communication; 3M, Product Information Sheet *a*), were easier to roll, allowing a smooth tape surface to be applied to the Petri dish and thus required fewer applications.

When choosing a tape to use for recovering cells, one should consider both ease of use and the visibility of the stain. Tapes with strong or large amounts of adhesive (duct, elastic, electrical and packing tapes) were difficult to work with. These were a challenge to remove from the Petri dish (as well as the analyst's gloves), and left adhesive residue on all materials they contacted. Further, tapes that were easy to tear (masking, Scotch®, and surgical tape) were preferable. Not only did using scissors slow the set up process because it required handling more materials, but some of the tapes were also difficult to cut. The tape stuck to the blades of the scissors and worsened with the buildup of adhesive residue following multiple cuts. Finally, transparent tapes (Scotch®, surgical, lifting and packing tapes) were beneficial because the area containing cells was obvious, allowing less tape to be soaked or swabbed, and potential contamination reduced.

The visibility of the stain or cellular residue should also be considered when a lifting tape is chosen. For example, residue that is difficult to see, such as in touch samples, should be collected with a tape that requires the fewest number of applications, since the collector cannot tell when cells have been lifted. Conversely, visible residue should be lifted with a tape through which it can be seen easily, such as packing, lifting, or Scotch® tape, because it allows cells to be concentrated in one place (the same tape surface can be applied repeatedly). In contrast,

electrical and other dark tapes are not suitable for lifting a visible stain because the area containing cells is nearly impossible to locate on the tape.

The ideal tape for DNA recovery should be transparent, easy to tear, and have a moderately strong adhesive so that the majority of cells are collected in one lift, but should still be easily removable from a surface. It should not leave residual adhesive, and have a supportive backing that makes the tape easy to roll, such as acetate or a polyester film. Of the tapes tested in this study, lifting and surgical tapes came closest to fulfilling these criteria.

Analysis of both the level of DNA left behind and the recovered DNA quantities resulted in the same conclusion: all tapes tested collected the majority of the cells, indicating that a stronger adhesive might not necessarily correlate with higher cellular recovery. In fact, it was hypothesized that cells would be harder to recover from strong adhesives. Digestion buffer, however, collected the majority of the cells from the tapes regardless of the adhesive.

Although swabbing with digestion buffer proved to be an effective method for recovering cells, the technique required more materials and manipulations than a soaking method. Foran and colleagues (1997) successfully retrieved DNA from hair embedded in glue traps by dissolving the glue in chloroform. In this method, the glue is soluble in the organic layer and the DNA in the aqueous layer. The technique was modified for tape lifts in this study, but resulted in significantly lower DNA yields than swabbing with digestion buffer. It is possible that breakdown of the tapes, especially in the cases of elastic, lifting and Scotch® tapes (which formed a gel), trapped the DNA and created a physical barrier to the aqueous layer. Another potential problem with the chloroform method is that the chloroform will cause SDS to precipitate out of the digestion buffer if subjected to forceful mixing methods such as vortexing.

To combat this, the tubes were rocked gently overnight. However, the presence of the tape may have prevented thorough mixing, causing less DNA to enter the aqueous layer.

Soaking the tape in digestion buffer also bypassed the swabbing step, and could potentially yield more DNA than swabbing because some residual solution was always left on the tape by the swab; soaking allows the entire tape surface containing cells to be placed in the digestion solution. Despite these advantages, there was no significant difference in DNA quantities between recovery methods, indicating that the techniques were equally effective at recovering cells from tape, and the residual swab solution contained only a minor portion of the DNA.

A potential pitfall of the soaking technique is that the underside of the tape, which was not exposed to UV pretreatment, is submerged in the digestion buffer, increasing the contamination potential. It also introduces all layers, and thus all components of the tape, to the solution. Interactions between the digestion buffer/ pro K and the tape could create or liberate compounds that inhibit later DNA analysis. In this study, three tapes were found to contain inhibitors: lifting, Scotch®, and masking. The lifting and Scotch® tapes became opaque after soaking overnight, indicating a reaction with the acetate backing of these tapes (A Miller, Personal Communication; 3M, Product Information Sheet *a*), while the masking tape's crepe paper backing appeared unchanged (3M, Product Information Sheet *b*). Either the backing is not the source of the inhibitor, or a different inhibitor is present in the paper and acetate backed tapes.

Other potential sources of the inhibitor(s) are the adhesive and the primer coat. The adhesive appeared to dissolve when the tape was soaked; however, there are no obvious similarities between the adhesives of the inhibitor-containing tapes. The masking tape has a

rubber based adhesive while the Scotch® tape has an acrylic adhesive (3M, Product Information Sheet *b*; 3M, Product Information Sheet *a*). Furthermore, the tapes that did not contain inhibitors also included rubber and acrylic based adhesives. Swabs come into contact with this layer as well, and yet no inhibition was detected when two of the three tapes were swabbed. Based on this information it seems unlikely that the adhesive is the source of an inhibitor.

Differences in the swabbing and soaking methods may provide some insight into the inhibitor's identity. The same solution was used in both, except that pro K was added when the tapes were soaked. This might suggest an adverse interaction between a component of the tape and the proteinase. The only other differences in exposure to the solution were the amount of time and that all layers—not just the adhesive—came into contact with it. It is reasonable, then, that the liberation of the inhibitor is time dependent, or that the inhibitor is located in a layer that only contacts the solution when soaked.

The inhibitor is likely a different compound in each of the three tapes, since the stage at which it was detected varied, and was affected by the recovery method used on the tape. For instance, when the tapes were soaked, inhibition was detected in the masking tape extracts at the qPCR level, while it was not detected in the Scotch® and lifting tape extracts until the STR stage. This may indicate a higher concentration of inhibitor in the masking tape extracts, since more DNA was added to the reaction for STR analysis than for quantification (up to 4 µl vs. 1 µl), or two inhibitors that behaved differently. There was some variation in the PCR inhibition seen in the lifting and Scotch® tape extracts as well: swabbed lifting tape extracts did not contain inhibitors whereas swabbed Scotch® tape extracts did. Identification of an inhibitor would be simpler if there was a common component in all three inhibitor-containing tapes which

did not exist in the other tapes. Identification of such a component would require detailed information on each tape's composition, which is often complicated and proprietary, so without further chemical analysis it is impossible to pinpoint a specific source of an inhibitor. It is likely, however, that soaking the tape altered the salt concentration or increased the concentration of chelating agents in the solution, preventing the polymerase from functioning in the PCR reaction.

Surgical tape was selected for all subsequent lifts because it was translucent, easy to tear, had a moderately strong adhesive that did not leave much residue behind, and did not contain PCR inhibitors. It was also specifically designed to be used on the skin, which was ideal for addressing the second goal of this study: to determine which areas of the body yield the most complete DNA profile, and whether tapes or swabs are a more effective means of collecting a reference sample from skin.

The experiments conducted in this study were under optimal conditions, where blood was lifted from a smooth, hard surface that would not interfere with lifting. This allowed the tapes' ability to lift cells to be compared without the added variable of interference by the substrate. It is unlikely, however, that real-world crime scene objects will be as suited to lifting as a Petri dish. A rough surface, for instance, would be less suited to tape lifts, as the tape would have to be pressed into all depressions and crevices in the material to maximize DNA yields. Swabbing or soaking, on the other hand, could more easily reach all areas of an irregular surface. Tape lifts would also be less successful on evidence where the DNA source may have soaked into the substrate (such as a blood stain on clothing), since the technique only collects surface debris and swabbing or soaking solutions will draw out embedded material.

This study showed that all tapes tested were equally effective at lifting cells from the Petri dish. A substrate that interferes with lifting, then, would be problematic for all tapes. Some

types, however, may be easier to use on certain substrates than others. Tapes with stronger adhesives, for instance, may be more difficult to remove from flexible substrates, like cloth, in a smooth motion because the substrate will bend with the tape. These tapes may also pick up more dirt or fibers that could interfere with the DNA analysis process. The recommendations made previously in this section, however, discourage the use of tapes with strong adhesives. In general, tapes are expected to be equally effective on other substrates, though a comparison among types on a more difficult substrate would be helpful in confirming the theory.

The body areas chosen for this study were based on research by Li and Harris (2003) and Zamir and colleagues (2000). The former found that full profiles were obtained from behind the ear, and 65.5% of alleles were detected from lifts taken between the fingers. Zamir and colleagues (2000) successfully obtained STR profiles from fingerprints left on tape, which indicates the fingertips themselves may be a potential source of reference DNA. The inside of the wrist, back of the hand and ankle were included in this study because the hands and feet are often exposed on people in traditional Middle Eastern dress and might be a socially acceptable body region to sample. While Li and Harris (2003) reported recovering no alleles from tape lifts of the ankle, a different area, located between the ankle bone (lateral malleolus) and Achilles tendon, was chosen for this study. However, preliminary results indicated that this region was not a viable option for reference samples because at most, four out of the 32 STR alleles were detected. This may be because the skin in this region is thick, so surface cells are much older and more likely de-nucleated or the DNA degraded than in areas of thin skin. The fact that the DNA quantities recovered from the ankle were often an order of magnitude lower than those from behind the ear, despite the larger amount of skin debris visible on the ankle lifts, supports this

hypothesis. The inside of the wrist also yielded consistently lower allele percentages than other body regions and was not carried on to later portions of the study.

Once tape lifts and swabs were collected from a larger sample of individuals, the DNA quantities were grouped the same way for statistical analysis as the allele percentages (Table 3). The same trends, however, were not seen in both data sets. For instance, swabbing yielded significantly more DNA than tape lifts on all dominant side body areas, and on two of the three areas when body side data were combined, but there was no significant difference in the percentage of detected alleles using either method. The body region sampled also affected DNA quantities differently than the STR profiles. Significantly more DNA was recovered by swabbing the finger combination than the back of the hand, and yet there was no difference in the number of allele calls from these regions. Similarly, significantly more DNA was recovered from swabs of behind the non-dominant side ear than the back of the non-dominant hand, but there was no significant difference in the number of alleles recovered in these areas. And while both data sets show that tape lifts were significantly less effective on the back of the hand than the other two regions tested, and that the trend was limited to one side of the body, the side varied. Dominant side DNA quantities were significantly different, as opposed to non-dominant side allele percentages. Such inconsistencies indicate that the differences in DNA quantity did not impact the completeness of the STR profile. While one body area may have yielded significantly less DNA than another, that lower amount was still enough to yield a nearly full profile.

All profiles generated from skin lifts and swabs were consistent with their corresponding buccal swab, indicating all three body regions and both recovery methods yielded an accurate profile. No region or method, however, was as successful as the buccal swabs, which yielded full profiles for all 20 individuals. This does not necessarily mean that reference samples from the

skin should be dismissed. A full profile was obtained from all but one swab of behind the ear (missing a single locus), and no allelic drop-in was seen. Considering that a single locus loss could be due to a random event, swabbing behind the ear may be a suitable alternative to buccal swabs. However, access to this area may be problematic for those whose religion or culture requires them to cover their head or hair.

The other combinations of body regions and recovery methods generally yielded 95% of alleles or higher, which correlates to the loss of at most, two alleles. Using only 14 of the 16 loci to calculate the probability of two people sharing that profile (assuming each of the dropped alleles came from a different locus), the probability still has the potential to be many orders of magnitude higher than the population of the Earth. It should also be noted that the combined DNA index system (CODIS, the DNA database used by US police agencies to match profiles from evidence to convicted offenders), uses only 13 loci. It is possible, then, that the body regions sampled here may yield enough alleles for a full CODIS profile. Although these recovery methods are not as successful as a buccal swab, the profile they produce is certainly complete enough to make exclusion possible.

Yields from the finger combination regions and the back of the hand may be improved by taking multiple swabs from the combination of finger areas described here, or swabbing both hands and combining the extracts. Additional swabs would mean a higher final concentration of DNA and the potential recovery of dropped alleles, which could result in full profiles, as seen with buccal swabs. Multiple swabs of the back of the hands may also be an option, although allelic drop-in was present in the profiles from this region, and if the extra alleles are actually the result of contamination, multiple swabs could compound the problem.

The number of correct alleles detected using both swabbing and tape lifting methods was similar, but due to higher peak heights and less drop-in, swabbing is preferable to tape lifts. The level of allelic drop-in in profiles from tape lifted regions indicates that it is not a suitable alternative to buccal swabs. While some of the extra alleles may be contamination introduced by soaking the tape, 70% of the profiles with allelic drop-in had very small peak heights (averaging 50–100 RFU), demonstrating that the extra alleles could be a stochastic result caused by small DNA quantities and not contamination. Reference samples may be taken from the hands using the swabbing method, but more research should be conducted to determine if minor adjustments to the procedure could produce full profiles 100% of the time.

Conclusion:

Tape lifts are an effective method for recovering cells, though their use may need to be limited to certain types of substrates or samples. This study focused on recovering cells from a smooth, firm surface, such as a Petri dish or a hairless region of the body. The tapes tested were equally effective on this type of substrate, but should be tested on other, less ideal surfaces to verify that one is not more suitable than another under harsher circumstances.

Tapes may serve as both a tool to collect cells, and as evidence containing them. This study should impact the way tape evidence is approached, as certain tapes were shown to introduce PCR inhibitors to the DNA extracts. In general, tape evidence should be swabbed rather than soaked, as it is unlikely that the tape in question will be the same type and brand as was used in this study, resulting in an unknown inhibitor-containing status.

An optimized tape lift procedure (when used as a tool to recover cells) should begin with deciding which tape is easiest to use and most suited to the task at hand. Once the type of tape is

selected, the recovery method can be chosen. Soaking the tape in digestion buffer requires fewer materials and manipulations than the swabbing method, but should only be used on tapes shown not to contain inhibitors. If the swabbing method is chosen, digestion buffer/1% SDS or water are the best choice of solution since they were easier to use and gave higher DNA yields than the adhesive removers.

Reference samples from the skin should be collected by swabbing behind the ear, although in some cultures, it may not be socially acceptable to sample this area. The hand, wrist and ankle regions tested are not viable DNA sources for reference samples, since they failed to consistently produce full profiles and so were less effective than buccal swabs. Regions of the hand, however, may yield enough alleles when swabbed to produce acceptably small match probabilities for some laboratories. Finally, tape lifts, as tested here, are not recommended for the collection of reference samples in general, as more allelic drop-in and considerably lower peak heights were observed.

APPENDICES

APPENDIX A

DNA QUANTITIES AND STR PROFILES

Table A1. DNA quantities from preliminary tape lifts of 4 body regions

Sample	ng/µL		Sample	ng/µL		Sample	ng/µL
15 AL	0.328		20 AL	0.125		PC	0.067
15 BL	0.069		20 BL	0.032		RB	0.000
15 CL	0.036		20 CL	0.031			
15 DL	0.017		20 DL	0.024			
15 AS	0.288		20 AS	0.187			
15 BS	0.037		20 BS	0.023			
15 CS	0.271		20 CS	0.092			
15 DS	0.007		20 DS	0.037			
17 AL	0.236		24 AL	0.413			
17 BL	0.147		24 BL	0.021			
17 CL	0.104		24 CL	0.047			
17 DL	0.039		24 DL	0.027			
17 AS	0.168		24 AS	0.169			
17 BS	0.105		24 BS	0.039			
17 CS	0.087		24 CS	0.035			
17 DS	0.030		24 DS	0.016			

Data represent the first preliminary set of body areas sampled using two types of tape. A = Behind the Ear, B = Finger Webbing, C = Fingertips, D = Ankle, L= Lifting Tape, S = Surgical Tape, 15–24 = Volunteer Number, PC = Positive Control, RB = Reagent Blank

Table A2. Number of alleles detected from preliminary tape lifts of 4 body regions

Volunteer	A	B	C	D
15	32	31	17	3
20	32	22	30	4
17	15	10	26	1
24	32	11	23	3
Average	27.75	18.50	24.00	2.75

The total number of detected alleles possible was 32. Data represent the first preliminary set of body regions sampled. The average number of alleles detected for each body region is also presented. A = Behind the Ear, B = Finger Webbing, C = Fingertips, D = Ankle, L= Lifting Tape, S = Surgical Tape, 15–24 = Volunteer Number

Table A3. DNA quantities from preliminary tape lifts of 6 body regions

Sample	ng/µL		Sample	ng/µL		Sample	ng/µL
1A	0.19		3A	0.12		PC	0.16
1B	0.03		3B	0.03		RB	0.00
1C	0.03		3C	0.08			
1D	0.16		3D	0.10			
1E	0.03		3E	0.03			
1F	0.22		3F	0.01			
2A	0.12		4A	0.47			
2B	0.15		4B	0.06			
2C	0.11		4C	0.02			
2D	0.12		4D	0.03			
2E	0.07		4E	0.01			
2F	0.07		4F	0.04			

Data represent the second preliminary set of body regions tested. A = Behind ear, B = Finger Webbing, C = Fingertips, D = Finger Combination, E = Inside of Wrist, F = Back of Hand, 1-4 = Volunteer Numbers, PC = Positive Control, RB = Reagent Blank

Table A4. Number of alleles detected in preliminary tape lifts of 6 body regions

Volunteer	A	B	C	D	E	F
1	32	10	27	4	25	32
2	32	32	30	30	30	32
3	32	10	30	26	5	27
4	32	29	28	31	16	32
Average	32.00	20.25	28.75	22.75	19.00	30.75

The total number of detected alleles possible was 32. Data represent the second preliminary set of body regions. The average number of alleles detected from each body region is also presented. A = Behind ear, B = Finger Webbing, C = Fingertips, D = Finger Combination, E = Inside of Wrist, F = Back of Hand, 1–4 = Volunteer Numbers

Table A5. DNA quantities from sets of swabs and tape lifts of the skin

1	**ng/µL**		**3**	**ng/µL**		**4**	**ng/µL**
AS	0.319		**AS**	0.088		**AS**	0.169
BS	0.360		**BS**	0.154		**BS**	0.158
CS	0.042		**CS**	0.051		**CS**	0.032
AT	0.045		**AT**	0.023		**AT**	0.076
BT	0.032		**BT**	0.163		**BT**	0.011
CT	0.013		**CT**	0.005		**CT**	0.011
5	**ng/µL**		**6**	**ng/µL**		**7**	**ng/µL**
AS	0.568		**AS**	0.068		**AS**	0.225
BS	0.203		**BS**	0.173		**BS**	0.133
CS	0.078		**CS**	0.147		**CS**	0.340
AT	0.203		**AT**	0.103		**AT**	0.311
BT	0.144		**BT**	0.102		**BT**	0.021
CT	0.062		**CT**	0.037		**CT**	0.014
8	**ng/µL**		**9**	**ng/µL**		**10**	**ng/µL**
AS	0.049		**AS**	0.338		**AS**	0.072
BS	0.192		**BS**	0.512		**BS**	0.321
CS	0.992		**CS**	0.050		**CS**	0.081
AT	0.179		**AT**	0.137		**AT**	0.055
BT	0.158		**BT**	0.108		**BT**	0.078
CT	0.116		**CT**	LOST		**CT**	0.014

Table A5. (cont'd)

11	ng/µL		12	ng/µL		13	ng/µL
AS	0.162		**AS**	0.590		**AS**	0.292
BS	0.163		**BS**	0.313		**BS**	1.267
CS	0.038		**CS**	0.716		**CS**	0.027
AT	0.026		**AT**	0.079		**AT**	0.067
BT	0.030		**BT**	0.046		**BT**	0.108
CT	0.043		**CT**	0.049		**CT**	0.007
14	**ng/µL**		**16**	**ng/µL**		**18**	**ng/µL**
AS	0.053		**AS**	LOST		**AS**	0.072
BS	0.083		**BS**	LOST		**BS**	0.160
CS	0.039		**CS**	0.088		**CS**	0.034
AT	0.019		**AT**	0.083		**AT**	0.042
BT	0.005		**BT**	0.031		**BT**	0.075
CT	0.007		**CT**	0.007		**CT**	0.057
21	**ng/µL**		**22**	**ng/µL**		**23**	**ng/µL**
AS	0.059		**AS**	0.057		**AS**	0.372
BS	0.093		**BS**	0.448		**BS**	0.311
CS	0.073		**CS**	0.000		**CS**	0.299
AT	0.071		**AT**	0.074		**AT**	0.780
BT	0.040		**BT**	0.043		**BT**	0.181
CT	0.013		**CT**	0.018		**CT**	0.028
25	**ng/µL**		**26**	**ng/µL**		**PC**	0.251
AS	0.185		**AS**	0.092		**RB**	0.000
BS	0.224		**BS**	0.120			
CS	0.044		**CS**	0.064			
AT	0.119		**AT**	0.031			
BT	0.099		**BT**	0.031			
CT	0.004		**CT**	0.015			

Data represent each swab and tape lift taken from 20 individuals. Quantities for PC and RB are the average of four. A = Behind the Ear, B = Finger Combination, C = Back of the Hand, S = Swab, T = Tape, 1–26 = Volunteer Number, PC = Positive Control, RB = Reagent Blank

Table A6. DNA quantities from buccal swabs

Sample	ng/µL
A	9.58
B	27.92
C	108.33
E	39.33
F	30.08
H	22.50
I	14.33
J	17.75
K	16.67
L	41.83

Sample	ng/µL
M	21.50
N	39.58
O	24.08
Q	29.08
R	79.08
T	6.78
V	14.67
W	48.67
X	21.33
Z	15.67

Data represent each buccal swab from the 20 participants in the portion of the study comparing swabs and tape lifts on the skin.

Table A7. Percentage of correct alleles detected in swabs and tape lifts from 20 individuals

	AS	BS	CS	AT	BT	CT
8	100	100	100	100	100	100
13**	100	100	100	100	100	100
14	100	25	65	100	100	100
18**	100	100	100	100	100	100
21**	100	100	100	100	100	100
4**	100	100	100	100	100	87.5
11	100	100	100	100	94	94
1	100	100	100	100	100	72*
22**	100	100	LOST	100	100	100
16**	LOST	LOST	100	100	100	81*
6**	100	100	100	100	100	100
7	100	100	100	100	100	90.6
3**	94	100	100	90.6*	100	72*
23	100	100	100	100	100	100
25	100	100	87.5*	100	100	62.5*
9	100	100	100	100	100	43.6*
10**	100	100	100	100	100	100
5	100	68.7	68.7	100	100	100
26**	100	100	97*	84.3*	100*	34
12	100	100	100	100	100	100

Percentages were calculated by dividing the total number of correct alleles detected by the total number possible (32). Homozygous alleles were counted twice. A = Behind the Ear, B = Finger Combination, C = Back of the Hand, S = Swab, T = Tape, 1–26 = Volunteer Number. * = allelic drop in. Volunteer numbers followed by ** mean the tape lifts were taken on the dominant side of the body, while volunteer numbers without the symbols indicate the swabs were taken on the dominant side of the body. Gray cells indicated the DNA extracts were lost before the quantification step.

APPENDIX B

STATISTICAL TABLES

Table B1. Results of statistical comparison of swabbing solutions

Tests of Normality

	Adhesive Remover	Kolmogorov-Smirnov		
		Statistic	df	Sig.
DNA Quantity	AR	.187	16	.139
	GG	.173	16	.200
	Un	.151	16	.200
	V	.157	16	.200
	DB	.106	16	.200
	W	.126	16	.200

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8.045	5	1.609	14.576	<.001
Within Groups	9.934	90	.110		
Total	17.979	95			

Multiple Comparisons

Tukey HSD

(I) Swabbing Solution	(J) Swabbing Solution	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
AR	GG	.1221875	.1174638	.903	-.219871	.464246
	Un	.0732500	.1174638	.989	-.268808	.415308
	V	.1893750	.1174638	.593	-.152683	.531433
	DB	-.6078125*	.1174638	<.001	-.949871	-.265754
	W	-.3680000*	.1174638	.027	-.710058	-.025942
GG	AR	-.1221875	.1174638	.903	-.464246	.219871
	Un	-.0489375	.1174638	.998	-.390996	.293121
	V	.0671875	.1174638	.993	-.274871	.409246
	DB	-.7300000*	.1174638	<.001	-1.07205	-.387942
	W	-.4901875*	.1174638	.001	-.832246	-.148129

Table B1. (cont'd)

(I) Swabbing Solution	(J) Swabbing Solution	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Un	AR	-0.07325	0.11746	0.989	-0.4153	0.26881
	GG	0.0489375	0.11746	0.998	-0.2931	0.391
	V	0.116125	0.11746	0.92	-0.2259	0.45818
	DB	-.6810625*	0.11746	<.001	-1.0231	-0.339
	W	-.4412500*	0.11746	0.004	-0.7833	-0.0992
V	AR	-0.189375	0.11746	0.593	-0.5314	0.15268
	GG	-0.0671875	0.11746	0.993	-0.4092	0.27487
	Un	-0.116125	0.11746	0.92	-0.4582	0.22593
	DB	-.7971875*	0.11746	<.001	-1.1392	-0.4551
	W	-.5573750*	0.11746	<.001	-0.8994	-0.2153
DB	AR	.6078125*	0.11746	<.001	0.26575	0.94987
	GG	.7300000*	0.11746	<.001	0.38794	1.07206
	Un	.6810625*	0.11746	<.001	0.339	1.02312
	V	.7971875*	0.11746	<.001	0.45513	1.13925
	W	0.2398125	0.11746	0.328	-0.1022	0.58187
W	AR	.3680000*	0.11746	0.027	0.02594	0.71006
	GG	.4901875*	0.11746	0.001	0.14813	0.83225
	Un	.4412500*	0.11746	0.004	0.09919	0.78331
	V	.5573750*	0.11746	<.001	0.21532	0.89943
	DB	-0.2398125	0.11746	0.328	-0.5819	0.10225

Results of Kolmogorov-Smirnov test for normality, and ANOVA and Tukey HSD tests (α = 0.05) comparing the amount of DNA recovered using each swabbing solution when the type of tape was not considered. V = VOC compliant Un-du, GG = Goo Gone, Un = Un-du, AR = Adhesive Remover, W = Water, DB = Digestion Buffer, * = Mean difference is significant at the 0.05 level. All subgroups are normally distributed. Water and digestion buffer were equally effective, and gave significantly higher yields than any of the adhesive removers,

Table B2. Normality test of DNA quantities from swabs and tape lifts of the skin

Sample	Kolmogorov-Smirnov
A Swabs	0.013
B Swabs	0.010
C Swabs	<0.001
A Tape	<0.001
B Tape	0.029
C Tape	0.001

Each row indicates a subgroup of the data in Table A5, none of which were normally distributed ($\alpha = 0.05$). A = Behind the Ear, B = Finger Combination, C = Back of the Hand

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