Touch DNA and the Ability to Detect the Correct Source

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Abstract

Touch DNA refers to DNA recovered from an object that has been handled. The DNA recovery is due to a variety of factors including length of handling, individual differences in deposition of DNA (commonly referred to as "shedder status") and environmental factors the object is exposed to prior to DNA collection. On average, 6 picograms (pg) of nuclear DNA are recovered from a single shed epithelial cell that has not entered the advanced stages of apoptosis or programmed cell death. Using this calculation, one can estimate the number of cells that may have been collected from the surface of the object. In addition, our study reports on the average minimum length of time a smooth object needs to be handled with pressure for DNA transfer to occur (30 seconds), the range of DNA collected from a single thumbprint (6-16 pg), and contamination rates (5 – 35 alleles) observed in a standard DNA laboratory environment with 34 cycle low copy number testing and discusses inherent error rates in source attribution for DNA profiles.

Keywords: DNA, touch DNA, low copy number DNA, contamination rates, source attribution error

Introduction

Touch DNA is a popular category of standard forensic DNA testing and can be from either high copy number (HCN) or low copy number (LCN) DNA samples. High copy number DNA samples are typically classified as those containing greater than 100 picograms while low copy number DNA samples have 100 picograms of DNA or less. Since the average human cell contains 6 picograms per cell, any forensic sample that claims a value of less than a single cell is highly subjective. Most United States forensic science laboratories use only standard DNA testing due to the greater complexity of DNA mixtures recovered when enhanced PCR cycles are used in the detection of LCN DNA samples particularly on non-intimate handled items. The reluctance to implement LCN DNA test methods is due to a variety of issues including difficulty in recovering and interpreting trace DNA mixtures, as well as extremely high contamination rates leading to high source attribution error and a percentage of false inclusions (1-8).

In standard DNA testing, if the positive and negative controls fail to perform correctly, the data is not usable in court. This is the backbone of proper quality control in forensic testing and allows the court system to have confidence in the results being presented in DNA reports and at trial. The inherent problem with low copy number DNA testing is that along with increased sensitivity of detection, contamination rates are also substantially increased making mixture assessment very complicated. Contamination can be attributed to the analyst handling the sample (9) to extraneous DNA on laboratory plastics (10) and from crime scene personnel (11). A Commission was formed in 2006 to evaluate the scientific process of LCN testing and mixture interpretations and the forensic community has still not embraced the technology fully almost a decade later (1). Some conservative estimates of contamination rates for low copy number DNA testing are 8-11% per sample compared to the 0% allowed in standard DNA tests; in some cases, the contamination rates may exceed the estimated 11% error (12). If random DNA fragments are incorporated in the test result, a percentage of the population can be incorrectly included (false inclusion) in the sample when they never actually touched the object. This is called coincidental matching. This false inclusion rate has been estimated to be as high as 1 in 300 individuals in three person non-deducible DNA mixtures in one study (12). In deducible mixtures, where an analyst is asked to deduce the major component or source of DNA of the mixture, the error rate is high at an estimated 1 in 80 individuals in three person deducible mixtures for one study (12) or 10-13% error for general mixtures in a second blind study (3). The most recent study describes the use of a forensic statistical tool (FST) which is software that weights different alleles based on their expected frequency in different populations. The estimated false inclusion rates from this study are presented in Table 1 (13). The FST only has the ability to classify potential sources or exclude an individual as being a potential source to a mixture; inclusion is not able to be concluded with 100% scientific accuracy(12-13). To date, the underlying science of the associated population databases and weights given to potential contaminant alleles with the FST is the subject of debate in a series of on-going Frye hearings.

This article will focus on basic concepts of legal interest and the origin of source attribution errors in interpreting complex mixtures and touch DNA samples (1, 2, 9, 14). Common legal questions include how long would an individual have had to be in contact with an object to have DNA transferred? How much DNA would be transferred to the object over time? What factors affect the ability to conclusively identify the source of the DNA? Touch DNA data from research studies is relevant to all forensic science laboratories that process samples from property crimes and crime scenes that contain key objects such as knives, firearms, wallets, cell phones, containers containing drugs, money, and hats and masks as some typical examples. The underlying science of touch DNA recovered from criminal casework is directly related to the basic biology and genetics of normal skin regeneration and programmed cell death (apoptosis) and lends an understanding to the inherent variability in DNA recovery from handled items (9).

Materials and Methods

A. Contact Time Study

*Sample Preparation.*Glass slides were selected as the substrate for thumb print contact and the thumb was selected because of the largest surface area. Unwashed hands were used in this project so as to limit the number of variables involved. The thumb prints from 8 volunteers were applied to clean glass slides for 10, 30, and 60 seconds to represent a realistic time frame that a person may contact a surface in the commission of a crime.

Two outer time points at 5 minutes and 10 minutes were also obtained from 5 of the 8 volunteers. Two replicate sets of specimens were retrieved for each time of contact.

Morphological Analysis. Each of the slides was allowed to dry on a slide warmer for 5 minutes. After which they were stained with nuclear fast red for approximately 10 minutes and examined using a compound microscope. The number of nucleated cells were counted and recorded along with other observable cellular components. Blank slides and blank swabs were used as negative controls and were subjected to the same procedures for contamination detection. Positive buccal swabs were also submitted to the same procedures to ensure that the reagents worked accordingly.

*Recovery of Fingerprint and DNA Extraction.*The slides were first swabbed with the tip of a wet swab dampened with DNase-free water, followed by a dry swab to recover residual moisture. Both swabs were air-dried for approximately 60 minutes and the tips of the swabs removed and immerse in 300 µL of 10% Chelex® resin (Bio-Rad laboratories, Hercules, CA), 20 µL of Proteinase K (10mg/mL) and were added to 1.5 mL Eppendorf tubes and incubated at 56°C for 1.5 hours with vortexing every 30 minutes. The tubes were subsequently placed in a 100°C water bath for 10 minutes, vortexed for 10 seconds and centrifuged for 5 minutes. Following the Chelex® extraction, the Microcon-30® device (Amicon, Inc., Beverley, MA) was used to concentrate some of the samples up to 20 µL.

Quantitation of DNA. DNA quantitation was with the Quantifiler® Human DNA Quantification Kit (Applied Biosystems, Inc.; Foster City, CA) according to the manufacturers guidelines using a 7500 Real-Time PCR System (Applied Biosystems, Inc.; Foster City, CA).

PCR Amplification and Electrophoresis. The samples from the thumb prints were amplified with the AmpFℓSTR® Identifiler kit on a GeneAmp System 9700 thermal cycler, following the manufacturers' protocol. PCR amplification was performed in a final volume of 25 µL, composed of 15 µL of a master mix which consisted of AmpFℓSTR PCR Reaction Mix, AmpliTaq Gold DNA Polymerase, AmpFℓSTR Identifiler Primer Set and 10 µL of sample in the following conditions: 1 cycle at 95°C for 11 minutes; 34 cycles at 95°C for 1 minute, at 59°C for 1 minute, at 72°C for 1 minute; a last cycle of extension at 60°C for 60 minutes; and a final hold step at 4°C for ∞. Ten microliters of a PCR template from DNA cell line 9947A was used as a positive control during PCR amplification as well as 10 µL of TE buffer as a negative control. Capillary electrophoresis was carried out on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems, Inc.; Foster City, CA).

B. Contamination Study

Sample Preparation. Positive buccal swabs were collected from two donors and DNA extracted using the manufacturer's protocol for the QIAamp DNA Investigator kit (Qiagen Corp.; Valencia, CA) for the purpose of generating nondegraded control DNA for a titration study to establish the effects of 28 cycle and 34 cycle PCR amplification with and without post-PCR purification using Microcons (Merk Millipore; Darmstadt, Germany).

Quantitation of DNA. DNA quantitation was performed with the Quantifiler® Human DNA Quantification Kit (Applied Biosystems, Inc.; Foster City, CA) according to the manufacturer guidelines using a 7500 Real-Time PCR System (Applied Biosystems, Inc.; Foster City, CA). A titration series consisting of DNA concentration ranges (range 1: 81-100.5ng, range 2: 47.1-68ng, range 3: 20.9-25.85, range 4: 9.3-16.75, range 5: 2.24-6.45ng, range 6: undetermined – 1.14ng) was created and tested in duplicate.

PCR Amplification and Electrophoresis. The samples from the extracted DNA were amplified with the AmpFℓSTR® Identifiler Plus kit on a GeneAmp System 9700 thermal cycler at 25 microliter reaction

volumes following the manufacturers' protocol (28 cycles) or with the addition of PCR cycles to a total of 34. For various parts of the study, PCR product was subjected to Microcon filtration to remove competing residual PCR primers. Ten microliters of a PCR template from DNA cell line 9947A was used as a positive control during PCR amplification as well as 10 µL of TE buffer as a negative control. Capillary electrophoresis was carried out on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems, Inc.; Foster City, CA).

Results

A. Contact Time Study

In general, the cell counts from the thumb prints on the glass substrates did not show much variability either from donor to donor or from the variation of time of contact. From observation, the slides showed mostly single nucleated keratinocytes scattered on the glass slide and sheets of corneocytes that had a pink hue to the cytoplasm after staining with nuclear fast red, a DNA-specific stain. Corneocytes are keratinocytes that are in the process of loss of cellular organelles and a nucleus before they are shed off the surface of the skin in the normal skin regeneration cycle (14). The pink color in the cytoplasm is likely due to the nuclear contents that have dissipated into the cytoplasm after the nucleus has undergone apoptosis. There was no difference in the type of cells observed from 10 seconds to 10 minutesin duration of touch in this study although the number of anucleated cells counted was greater for the left hand than the right hand (2-3 fold higher). For the majority of the thumb prints that were stained, only a single nucleated keratinocyte was observed from the volunteers (Table 2) and the average amount of DNA recovered from a single thumbprint was in the range of 6 – 16 picograms or 1-3 cell equivalents. The primary source of DNA appears to be from non-nucleated transitional corneocytes undergoing the mid stages of programmed cell death (Figure 1). Partial DNA profiles were obtained after genotyping with the Identifiler kit but the resultant DNA profiles in all samples were not complete and the number of alleles that were detected was variable from thumbprint to thumbprint (14). The amount of DNA recovered from individual thumbprints is consistent with the cell counts and microscopic evaluation regarding touch DNA deposition (Figure 2).

B. Contamination Study

Overall, for the comparison of 28 and 34 cycle PCR products, the results were similar at higher levels of DNA template (100 pg or more). While increased PCR cycles enhanced sensitivity of detection and more replicate alleles were noted, baseline noise was also increased. The optimal situation for clear allele calls and minimal baseline noise was a 28 cycle method with a post-PCR clean up procedure using Microcon filtration devices (Figure 3). As expected, as the DNA concentration decreased, the ability to detect the correct profile also decreased. The purpose of the post-PCR clean up procedure was to reduce the presence of residual PCR primers that compete with the desired DNA fragments for injection into the capillary electrophoresis instrument. Our data is in agreement with published data from a previous study by Smith et al. (15).

The detection of non-donor alleles or contamination was higher in the 34 cycle and 34 cycle post-purified samples as anticipated due to the increased sensitivity of the method (1, 10 – 12). No contaminant alleles were detected in the standard 28 cycle amplified samples. Five replicate non-donor alleles were detected in

the 28 cycle post-purified sample and all of the additional alleles could be contributed to the analyst. Contaminant or non-donor alleles when sourced for the 34 cycle and 34 cycle post-purified amplifications were primarily of unknown origin and were approximately 6-fold higher (35 alleles) than that observed in the 28 cycle post-purified samples (9).

Discussion

From this study, a single thumbprint on a glass slide applied with pressure for 30 seconds is sufficient to generate a DNA profile that may consist of a single allele or upwards of a fifty percent complete DNA profile. As a general qualitative observation, the smaller DNA fragments were more successfully amplified which is as expected due to environmental factors and DNase enzymes from the skin that cause degradation. The recovery of some DNA allows touch DNA to be used for exclusionary purposes, however, the ability to easily transfer corneocytes in a single touch or as multiple transfer events makes it challenging to interpret complex DNA mixtures that consist of more than two individuals. In theory, many individuals may have handled common items, such as kitchen knives, firearms, baseball hats, automotive door handles, etc. and the continuous deposition of DNA along with degradation and removal of adherent skin cells through use of an object reinforces the concept that the interpretation of the true number of contributors and the subsequent correct assignment of detected alleles to a single individual with complete accuracy a challenging forensic issue (1, 2, 14).

The estimated error rates for source attribution vary for two reasons and are dependent on (a) the prior assumption of contributors to the sample (b) contamination rates and (c) the major contributor assessment when deducing profiles from a mixture due to variable peak height of the detected DNA fragments by inherent differences in PCR amplification efficiency. These factors make it challenging to establish which alleles are from the commission of the crime, from previous object handling or from contamination during collection or processing. One can, however, effectively collect DNA and ascertain if an individual may have come in contact with the item but DNA test results and trial testimony should clearly reflect the appropriate weight of the evidence by including the possibility of contamination, secondary transfer of skin cells or contact prior to the incident, and coincidental match rates for the courts. In addition, some complex DNA mixtures simply are not able to be deconvoluted into major and minor contributor fractions with scientific accuracy and are effectively used for simple comparisons for the possibility of a potential DNA donors to the sample.

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Table 1. Estimated False Inclusion Rates for Complex 3-Person DNA Mixtures Using Likelihood Ratios (LR) and FST

LR value	Deducible mixture	Non-deducible mixture
0.001	1 in 80	1 in 300
0.01	1 in 170	1 in 610
0	1 in 410	1 in 1300
1	1 in 1200	1 in 3100
10	1 in 4400	1 in 7600
100	1 in 13,000	1 in 40,000
1000	1 in 31,000	1 in 60,000

Table 2. Cell Counts from Thumb Prints Supplied at 30 Seconds of Pressure on a Glass Slide

Subject	Nucleated Cells	Stripped Nuclei	Other Observations	Total
1	0	0	Corneocytes	0
2	0	0	Corneocytes	0
3	1	0	Corneocytes	1
4	1	1	Corneocytes	2
5	1	0	Corneocytes	1
6	0	0	Corneocytes	0
7	0	0	Corneocytes	0
8	0	0	Corneocytes	0

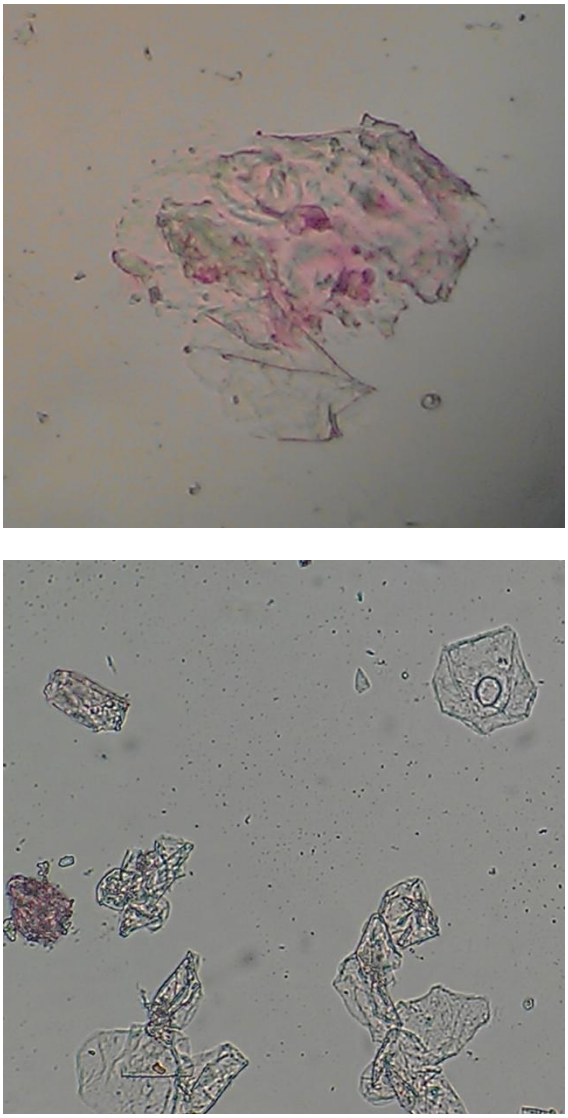

Figure 1. Shed epithelial cells from a thumbprint with 30 seconds of pressure on a glass slide stained with nuclear fast red, a DNA-specific stain. Olympus compound microscope, 400X total magnification.

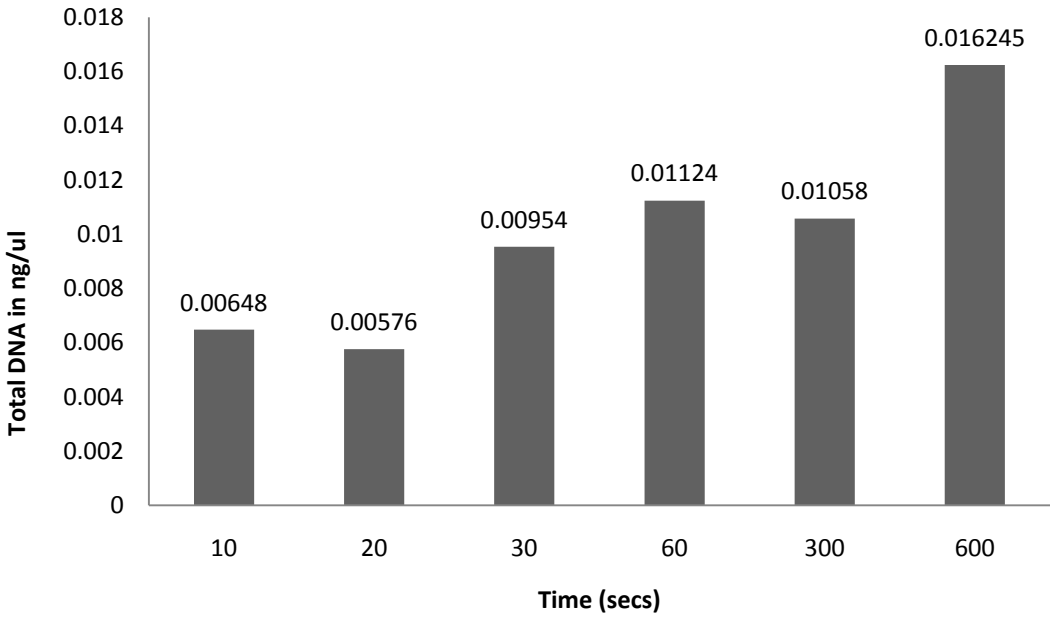

Figure 2. Total DNA in ng/µl obtained from the all subjects at the specified duration of touch. Average recovery from a single thumbprint is 6 – 16 pg of DNA per thumbprint across the time course.

Figure 3. Average Percent Correct Alleles per PCR Treatment and DNA Concentration Range