DNA profiles from fingermarks

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Criminal investigations would be considerably improved if DNA profiles could be routinely generated from single fingermarks. Here we report a direct DNA profiling method that was able to generate interpretable profiles from 71% of 170 fingermarks. The data are based on fingermarks from all 5 digits of 34 individuals. DNA was obtained from the fingermarks using a swab moistened with Triton-X, and the fibers were added directly to one of two commercial DNA profiling kits. All profiles were obtained without increasing the number of amplification cycles; therefore, our method is ideally suited for adoption by the forensic science community. We indicate the use of the technique in a criminal case in which a DNA profile was generated from a fingermark on tape that was wrapped around a drug seizure. Our direct DNA profiling approach is rapid and able to generate profiles from touched items when current forensic practices have little chance of success.

Fingermarks are essential forensic evidence in numerous criminal investigations. Generating a DNA profile from a fingermark for the purpose of human identification would be beneficial in resolving a broad spectrum of criminal investigations, ranging from theft to crimes of violence. DNA retrieved from fingermarks deposited by touch (referred to as "touch" DNA) is often degraded (1) and limited in quantity (2) and may contain elements that co-extract with the DNA (3), which can hinder subsequent amplification. Although forensic genetics has seen substantial improvements in DNA profiling sensitivity (4–6), typically the use of less than 100 pg of DNA template (equating to ~16 human somatic cells) can result in poor-quality profiles (7,8). This limit of sensitivity still precludes many items that have been touched at a crime scene from generating a usable DNA profile, despite their potential importance in a criminal investigation; these samples can include triggers, steering wheels, bullet cartridges, and handles of knives. In many criminal investigations, the ability to retrieve the maximum amount of DNA from touch DNA samples is of paramount importance in resolving the case.

The first DNA profile generated from a fingerprint was reported more than a decade ago (9) and revolutionized forensic science. Despite this advance in the field, research has found an extremely low success rate (5%–6%) when using the standard methodology in generating touch DNA profiles (10), highlighting the need for an improved methodology. The standard workflow for touch DNA samples includes an extraction step before amplification. However, many current extraction processes are thought to result in a loss of DNA (11,12); hence a touch DNA swab initially containing less than 100 pg of DNA may result in insufficient template at the PCR step to generate an informative profile. Many touch DNA samples are therefore either not submitted for DNA typing or fail to generate any data without further enhancement. Previous attempts to generate DNA profiles from fingermarks used a low copy number (LCN) methodology (9,13,14). However, any enhancement of the amplification process from limited and low-level DNA has the inherent risk of introducing stochastic events such as allelic drop-out, allelic drop-in, or an increase in stutter heights or allelic imbalance for a heterozygote. Many factors affect the deposition of DNA by touch at crime scenes, with previous reports classifying individuals as "shedders" or "nonshedders" (15–19) depending on the profiling success from fingermarks 15 minutes after handwashing. The issue of whether or not a person is a shedder and how this may or may not affect transfer of DNA is a concern in forensic science (16,20–22); however, in reality, there is no knowledge of a person's shedder status during a criminal act.

Here we report a procedure that eliminates the need for LCN technology by omitting the extraction process, therefore minimizing the opportunity for sample mix-up or introduction of extraneous DNA into the reaction vessel. If DNA exists as a free molecule, then it should bind readily to positively charged fibers (23). Furthermore, the use of a surfactant such as Triton-X is thought to assist in the binding of DNA to a swab (24) and hence, these two features were used to capture DNA from touched substrates. By placing the fibers that made contact with the swabbed surface directly into the PCR tube, there is no potential loss of DNA during an extraction process. Direct PCR has been used successfully by our laboratory in a forensic context to generate informative profiles from single hairs (25–27), and a similar approach to direct PCR is used here to generate DNA profiles from individual fingermarks using two different commercially available kits, Profiler Plus, which is designed to amplify 9 short terminal repeat (STR) loci plus the sex determining marker amelogenin (making a total of 20 alleles), and NGM SElect,

METHOD SUMMARY

A novel DNA profiling method using a swab moistened with Triton-X to obtain sample DNA from fingermarks followed by direct polymerase chain reaction (PCR) amplification to generate a forensic profile is described.

which targets 16 STR loci plus amelogenin (making a total of 34 alleles).

Materials and methods

Contamination controls for low-template DNA

All laboratory steps preceding DNA amplification were carried out in a dedicated fume hood separate from post-PCR activity. Strict decontamination procedures were followed, including cleaning of equipment and work areas with 3% sodium hypochlorite and 70% isopropanol before and after use. Nontemplate controls (PCR blanks) were included in each experiment to monitor potential contamination from human DNA sources and cross-contamination from other samples. Before use, sterile and DNA-free plastic slides (Rinzl plastic; ProSci Tech, Kirwan, QLD, Australia) were cleaned with 70% isopropanol and Milli-Q ultrapure water (Merck Millipore, Victoria, Australia) and left to dry in a sterile fume hood before ultraviolet exposure for 15 min. As an additional negative control, slides were swabbed before individuals deposited fingermarks/DNA. The STR DNA profiles of both staff members (J.E.L.T. and A.L.) involved in the handling of the samples were genotyped. All buccal reference swabs provided by donors were processed after fingermark deposition and analysis.

DNA reference swab collection

Cotton buccal swabs (Copan Industries, Victoria, Australia) taken from the inner lining of the cheeks of 34 volunteers were collected. Ethical approval was obtained from Southern Adelaide Clinical Human Research Ethics Committee before starting. DNA was extracted following the DNA IQ Kit (Promega Corporation, Victoria, Australia) manufacturer's recommendations.

DNA deposition 15 minutes after handwashing

Thirty-four volunteers washed their hands with warm water (no soap or detergent was used) before depositing DNA. Fifteen minutes after handwashing, each volunteer deposited a fingermark using four fingers and thumb from his or her dominant hand onto sterile and DNA-free plastic slides. Contact was for 15 s using medium pressure (to ensure consistency) such that a fingermark was created. All slides were stored at 4°C before processing. All marks made by touch in this experiment were clearly visible, and these fingermarks were removed using a double-swab technique.

Targeted swabbing

A small portion of fibers (~2 mm^2) was cut from the tip of a sterile nylon FLOQswab (Copan Industries) using a new sterile scalpel blade. Fibers were pre-moistened with 2 µL of 0.1% Triton X (Sigma, Victoria, Australia) (preheated to 50°C) before swabbing. Sterile forceps were used to gather the 2-mm^2 portion of swab and apply pressure over the mark. Glue fragments of the swab held the fibers neatly in place. Swabbing consisted of 2 sets of 10 strokes horizontally (left to right direction) and 2 sets of 8 strokes vertically (top to bottom) over the mark using heavy pressure to ensure consistency. The procedure was repeated using a second dry swab. Sterile forceps were used to place swab fibers (4 mm^2) from both swabbing events into the same 0.2-mL PCR tube.

DNA amplification using Profiler Plus

STR typing was performed using the AmpFLSTR Profiler Plus kit (Life Technologies, Victoria, Australia) following the manufacturer's recommendations but at half the volume. The final reaction consisted of 10 µL Profiler Plus reaction mix, 5 µL Profiler Plus primer mix, 1 µL (5 U) AmpliTaq Gold (Life Technologies), and DNA template (4 mm^2 swabbed fibers for samples or 1 ng of eluted DNA extract from a buccal swab). Additional PCR facilitators were added to the PCR due to previous studies suggesting an increase in performance. Purified molecular biology grade BSA (0.1 µg) (New England Biolabs, New South Wales, Australia) and DMSO (dimethyl sulfoxide) (5%) (Expand Long Range dNTPACK; Roche, Victoria, Australia) were added to the PCR tube, and cycling was performed on a 9700 GeneAmp thermal cycler (Life Technologies). Cycling consisted of an initial denaturation at 95°C for 11 min followed by 29 cycles of 94°C for 1 min, 59°C for 1 min, 72°C for 1 min, and then a final extension at 60°C for 45 min followed by a hold at 4°C. PCR products were analyzed on a 3130*x*l Genetic Analyzer (Life Technologies) in a 14-µL final volume that consisted of 1.5 µL PCR product (as recommended by the Profiler Plus kit manufacturer), 12 µL HiDi formamide (Life Technologies), and 0.5 µL ROX-500 Size Standard (Life Technologies).

DNA amplification using NGM SElect

STR typing was performed using the AmpFLSTR NGM SElect PCR Amplification Kit (Life Technologies) following the manufacturer's recommendations but with additional AmpliTaq Gold. The final reaction consisted of 10 µL NGM SElect reaction mix, 5 µL NGM primer mix, 1 µL (5U) AmpliTaq Gold, and DNA template (4 mm^2 swabbed fibers for samples or 1 ng of eluted DNA extract from a buccal swab). Cycling was performed on a 9700 GeneAmp thermal cycler and consisted of an initial denaturation at 95°C for 11 min followed by 30 cycles of 94°C for 20 s, 59°C for 3 min, and then a final extension at 60°C for 10 min. PCR products were analyzed on a 3130*x*l Genetic Analyzer in an 11.5-µL final volume that consisted of 1.5 µL PCR product, 9.5 µL HiDi Formamide, and 0.5 µL of GeneScan 600 LIZ Size Standard v2.0 (Life Technologies).

DNA deposition immediately after handwashing

Two individuals who consistently generated informative DNA profiles from fingermarks deposited 15 min after handwashing (using direct PCR) were asked to provide additional prints immediately after handwashing. Volunteers deposited a fingermark using 4 fingers and thumb of their dominant hand (as detailed above) to create 10 fingermarks. Sterile nylon FLOQswabs were used to swab the 10 fingermarks (in the same manner as detailed above), and the DNA was amplified using the Profiler Plus kit following the manufacturer's recommendations (as detailed above).

DNA extraction from a fingermark 15 minutes after handwashing

Two individuals who consistently generated informative DNA profiles from fingermarks

deposited 15 min after handwashing (using direct PCR) were asked to provide additional prints for an extraction experiment. Volunteers deposited a fingermark using 4 fingers and thumb of their dominant hand (as detailed earlier) to create 10 fingermarks. Sterile nylon FLOQswabs were used to swab the 10 fingermarks (in the same manner as detailed earlier, except that the whole swab was used for an extraction). Extraction followed the DNA IQ Kit manufacturer's (Promega Corporation) recommendations. PCR amplification followed the Profiler Plus recommendations (as detailed earlier).

Data analysis

All DNA profiles were analyzed using GeneMapper ID software (v3.2.1) (Life Technologies), and a peak detection threshold of 30 relative fluorescence units (RFU) (3 standard deviations above the baseline) was used to assign alleles. A wildcard designation was used for potential homozygotes with peak heights less than 150 RFU to account for potential allelic drop-out (e.g., "11, F" instead of "11,11"). A profile was considered to be full when all alleles were detected above the threshold RFU and matched the reference profile of the donor. Allelic drop-out was reported as alleles with peak heights less than 30 RFU. Additional alleles were reported if peak heights were greater than 30 RFU (3 standard deviations above the baseline) and did not match the donor providing the sample. An informative DNA profile (one considered uploadable to the Australian DNA databases) was defined as having at least 12 alleles (plus Amelogenin) that matched the reference DNA profile. The profiling success (%) was measured by dividing the number of alleles successfully called by the total number of expected alleles and multiplying this value by 100.

Results and discussion

DNA profiles were generated from swab fibers collected from 170 fingermarks created by 34 individuals. Each fingermark was created after a period of only 15 minutes after handwashing. From the total sample size (170 fingermarks donated by each of the 5 digits of 34 donors), 122 DNA profiles (71%) were recorded with 12 or more alleles (sufficient for uploading to the Australian DNA Database), with only 4 samples (<2%) failing to yield any DNA (see Tables 1 and 2). The success rate was 66% to 74%, depending on the STR DNA profiling kit used, and was obtained without the need for increased PCR cycle number.

Five of the 10 thumbprints produced full DNA profiles using Profiler Plus with a further 3 producing 17 or more alleles such that only a single thumb print produced a very partial profile with 7 of the possible 20 alleles obtained. Similar results were obtained for the index and middle fingers (Table 1).

Full DNA profiles were observed from 38% (19 of 50) of the fingermarks using Profiler Plus. Of these 50 fingermarks using all 10 donors and all 5 digits, 66% (33 of 50) generated 12 or more alleles with a further 28% (14/50) of the profiles generating at least 1 allele. A total of 696 alleles from a possible 1000 were observed.

Twelve of the 24 thumbprints analyzed using the 17 loci NGM SElect kit produced 25 or more alleles (characterized as full DNA profiles when using other STR typing kits) (Table 2). Only 4 of the 24 donors gave poor results, with fewer than 12 alleles for each of the 5 digits, and only 1 of the 120 fingermarks failed to yield DNA.

Of a possible 4080 alleles from all 5 digits and all 24 donors, 2266 matching alleles were produced. By contrast, all 10 swabs from which DNA was extracted by standard forensic processes failed to result in any DNA profile, as expected.

Table 1. DNA profiles obtained from 10 donors showing success rates using the Profiler Plus STR kit (of a possible 20 total STR alleles, 10 total loci).

	Finger used for depositing DNA					
	Thumb total alleles (out of 20)	Index total alleles (out of 20)	Middle total alleles (out of 20)	Ring total alleles (out of 20)	Pinky total alleles (out of 20)	Informative profiles (out of 5 digits)‡
Donor 1 ♂	20	20	20	20	20	5 (100%)
Donor 2 ♂	20	3	0†	20	20	3 (60%)
Donor 3 ♂	20	12	19	18	11	4 (80%)
Donor 4 ♂	12	20	20	0†	0†	3 (60%)
Donor 5 ♂	17	15	8	1	6	2 (40%)
Donor 6 ♀	20	10	17	6	20	3 (60%)
Donor 7 ♀	19	20	20	13	4	4 (80%)
Donor 8 ♀	7	9	8	2	10	0 (0%)
Donor 9 ♀	20	20	20	19	20	5 (100%)
Donor 10 ♀	17	5	17	16	15	4 (80%)

The first column designates the donor and the sex of the individual. Columns 2 to 6 report the number of alleles recorded from each donor. A maximum of 20 alleles indicates a full DNA profile. Numbers less than 20 indicate a partial or failed DNA profile. Three DNA profiles resulted in no alleles (indicated by †).
‡Informative profiles indicate the total number of profiles obtained from each individual that can be considered uploadable to the Australian DNA database (≥12 alleles) and are displayed in parentheses as the percentage profiling success for that individual.

Negative controls were performed using the same swabs and slides as well as the same solutions to detect any alleles from these sources, and no alleles were detected from all 34 negative controls. Any alleles detected in the fingermark DNA profiles, either matching the donor or otherwise, were most likely attributable to transfer to the slide at the time of deposition of the fingermark.

Examples of the DNA profiles obtained by direct PCR using either the Profiler Plus kit (Figure 1) or NGM SElect kit (Figure 2) illustrate that many profiles exhibited no increase in stutter products, a stochastic effect that may be observed in low-template DNA typing. Allelic drop-out did occur in samples that did not generate full DNA profiles, and additional alleles were recorded as unknown alleles that did not match the donor. Single-source DNA profiles were generated from 40% of the samples amplified using the Profiler Plus kit and 36% of the samples amplified using the NGM SElect kit. The surface touched by the individuals had been cleaned before use, and negative controls from areas on which the fingermark was placed failed to yield DNA profiles. The substrate on which the fingermark was deposited was open to the environment for approximately 2 minutes during sample collection, allowing potential deposition of DNA from other sources. Additionally, donors were active during the 15 minutes after handwashing such that DNA from other persons may have been deposited onto the surface of the finger by secondary or tertiary transfer, such as holding a shared item. This low-level secondary transfer has been observed under laboratory conditions (17–19, 21) and would be expected in real life when examining a substrate at a crime scene.

It remains unknown if the DNA from a fingermark is found within cellular material or as a free molecule. Regardless of the type of DNA present on the skin and available for transfer, it has been reported that the amount of DNA on the skin increases with time since handwashing (15). The 15 minute time period between handwashing and fingermark deposition was chosen deliberately as the minimum time point to provide consistency between this study and the original study into transfer of DNA to touched items (15). To confirm this, we asked two volunteers (who had consistently deposited sufficient DNA for informative profiles to be generated) to create fingermarks from each finger and thumb of the dominant hand in the usual way but immediately after handwashing. No alleles were detected in any of the resulting DNA profiles, indicating that rinsing in water had removed any DNA on the skin of the individuals tested. Our data indicate that sufficient DNA had accumulated on the surface of the skin to generate DNA profiles within 15 minutes after all detectable DNA had been removed. Based on the data from 15 minutes after handwashing, there is an approximately 66% to 74% chance of gaining a DNA profile with 12 or more alleles, with many full DNA profiles being observed.

Using our optimized technique, we were requested by the South Australia Police Service to generate a DNA profile from a smeared fingermark on tape that was wrapped around a drug seizure. The resulting NGM SElect profile contained 31 dominant alleles (of a possible 34). A further 10 low-level alleles were also recorded, indicating a low-level mixture of persons

Table 2. DNA profiles obtained from 14 donors showing the success rate using the NGM SElect STR kit (of a possible 34 total STR alleles, 17 total loci).

	Finger used for depositing DNA					
	Thumb total alleles (out of 34)	Index total alleles (out of 34)	Middle total alleles (out of 34)	Ring total alleles (out of 34)	Pinky total alleles (out of 34)	Informative profiles‡ (out of 5 digits)
Donor 11 ♂	33	34	34	32	32	5 (100%)
Donor 12 ♂	26	26	19	22	16	5 (100%)
Donor 13 ♂	18	4	8	14	24	3 (60%)
Donor 14 ♂	33	34	25	31	4	4 (80%)
Donor 15 ♂	17	16	9	1	16	3 (60%)
Donor 16 ♂	23	16	11	13	24	4 (80%)
Donor 17 ♂	3	3	2	1	1	0 (0%)
Donor 18 ♂	12	7	15	15	8	3 (60%)
Donor 19 ♂	25	28	28	24	26	5 (100%)
Donor 20 ♀	23	22	19	20	26	5 (100%)
Donor 21 ♀	34	34	31	34	32	5 (100%)
Donor 22 ♀	5	2	3	2	1	0 (0%)
Donor 23 ♀	20	23	21	22	19	5 (100%)
Donor 24 ♀	24	26	25	25	27	5 (100%)
Donor 25 ♀	14	18	19	4	5	3 (60%)
Donor 26 ♀	7	10	10	8	12	1 (20%)
Donor 27 ♀	32	32	17	23	20	5 (100%)
Donor 28 ♀	25	27	23	8	16	4 (80%)
Donor 29 ♀	27	21	22	15	17	5 (100%)
Donor 30 ♀	11	7	4	0†	7	0 (0%)
Donor 31 ♀	33	33	5	27	33	4 (80%)
Donor 32 ♀	26	28	29	26	30	5 (100%)
Donor 33 ♀	28	17	19	12	12	5 (100%)
Donor 34 ♀	28	16	17	27	26	5 (100%)

The first column designates the donor and the sex of the individual. Columns 2 to 6 report the number of alleles recorded from each donor. A maximum of 34 alleles indicates a full DNA profile. Numbers less than 34 indicate a partial or failed DNA profile. Only one DNA profile resulted in no alleles (indicated by †).
‡Informative profiles indicate the total number of profiles obtained from each individual that can be considered uploadable to the Australian DNA database (≥12 alleles) and are displayed in parentheses as the percentage profiling success for that individual.

depositing DNA onto the drug packaging (data not shown).

In this study, we demonstrate the ability to generate informative DNA profiles from latent fingermarks deposited by touch. Our method is rapid (23) because there is no need for a DNA extraction step, and there is a reduction in associated costs because there is no need to purchase DNA extraction kits. Most importantly for forensic science laboratories, by eliminating the need to increase PCR cycle number or concentrate the amplified products, the procedure is easily adapted into working practices, allowing laboratories accredited to ISO17025 to use the method. Still, future work is required to assess the limitations of direct PCR and to determine what effect chemical enhancement methods will have on the DNA template and resulting DNA profile.

Author contributions

J.E.L.T. and A.L. developed the experimental design and coauthored the paper. J.E.L.T. performed the laboratory work.

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Competing interests

The authors declare no competing interests.

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Figure 1. STR DNA profile generated using the Profiler Plus kit. The full DNA profile displayed was generated from a fingermark deposited 15 minutes after handwashing using direct PCR at 29 cycles. STR: short terminal repeat.

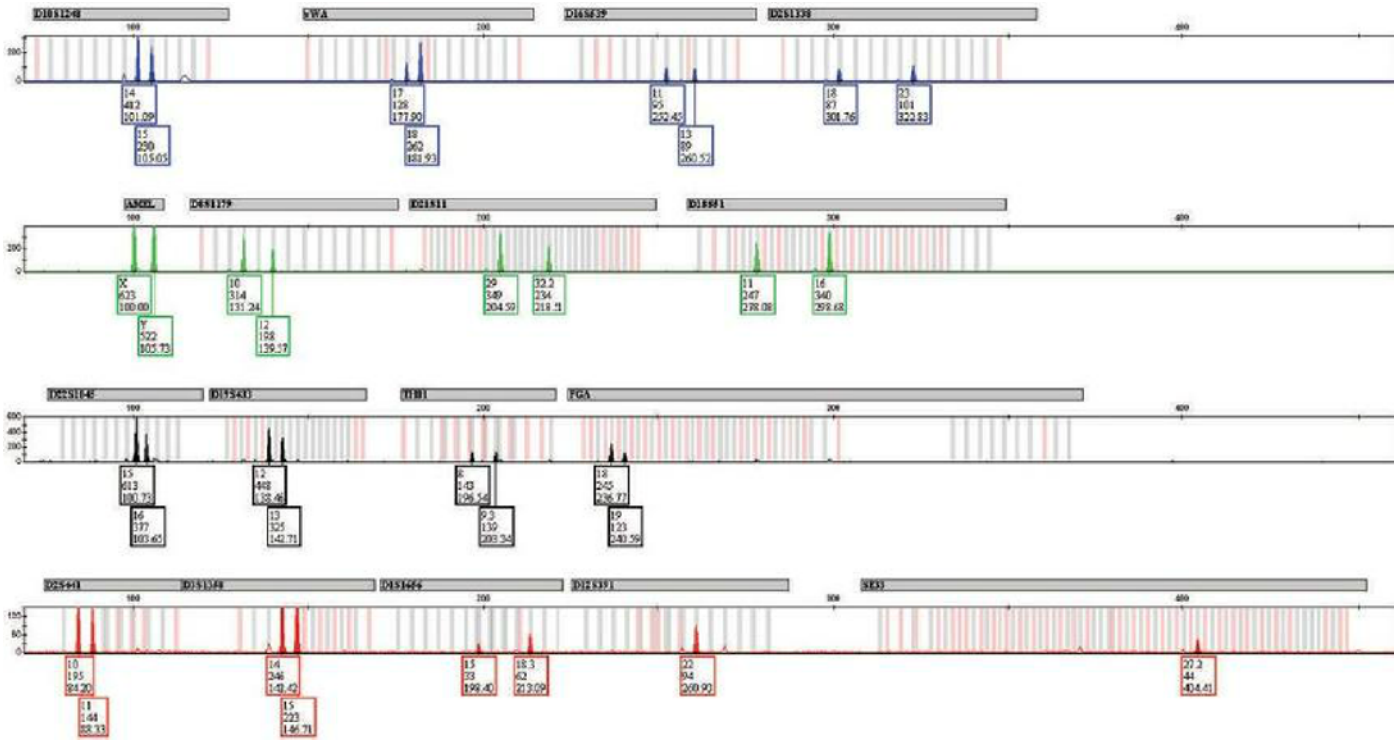

Figure 2. STR DNA profile generated using the NGM SElect kit. The DNA profile displayed was generated from a fingermark deposited 15 minutes after handwashing using direct PCR at 30 cycles. STR: short terminal repeat.

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