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Optimization of the collection and analysis of touch DNA traces

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INTRODUCTION

Touched surfaces at crime scenes are frequently swabbed in order to recover genetic material. These trace samples typically contain less DNA than the minimum requirement for PCR amplification used in our laboratory (200 pg). Touch DNA traces may significantly contribute to the outcome of an investigation, and it is important to address if other genetic information can be retrieved.

The aim of this work was to optimize a strategy for detection, collection, and analysis of touch DNA traces. Nylon 4N6FLOWSwabs™ (Copan, Italy) and cotton swabs (Puritan, USA) were compared by collecting touch DNA traces from glass and gun shell casings. Shed cells were visualized using Diamond™ nucleic acid dye (Promega, USA) and a digital fluorescent microscope. Various collection and extraction methods were tested. Collected samples were amplified with the AmpFlSTR® NGM SElect™ kit and with the Precision ID mtDNA Whole Genome Panel (Thermo Fisher Scientific, USA).

MATERIAL AND METHODS

Visualization of genetic material

Diamond™ Nucleic Acid Dye (DD) was diluted 20x in 75% ethanol according to [1]. 3µL of the DD dilution was pipetted onto the samples; samples were placed in a flow hood until dry (Fig. 1). Samples were visualized with a Dino-Lite digital microscope (AnMo Electronics Corporation, Taiwan) – DD has an excitation wavelength of 494nm and emission at 558nm.

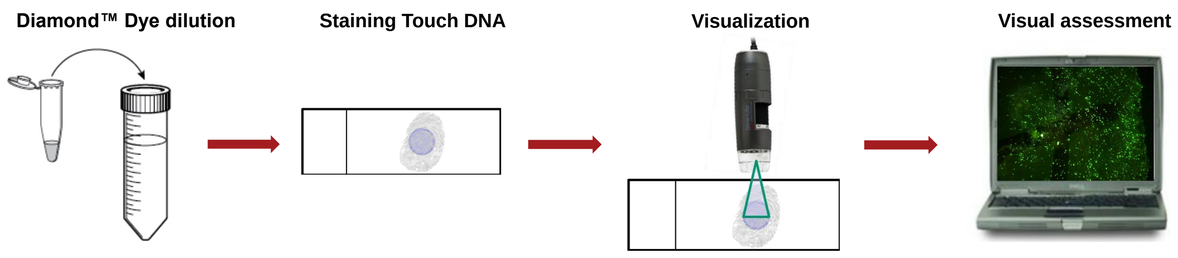

Figure 1. Visualization of touch DNA samples with Diamond™ Dye and digital microscope.

Collection of samples, DNA extraction and quantification

Figure 2. Collection procedure of touch DNA using swabs. Chelex-extracts and OneTouch™ lysates were quantified using the Quantifiler Trio DNA Quantification Kit (Thermo Fisher Scientific).

STR and mtDNA typing

Samples were amplified with the AmpFlSTR® NGM SElect™ PCR Amplification Kit (Thermo Fisher Scientific), using 10µL eluent or 500pg template DNA. CE was performed with ABI 3500xL Genetic Analyzer (Thermo Fisher Scientific).
Samples were amplified with the Precision ID mtDNA whole genome panel (Thermo Fisher Scientific) according to [2]. Libraries were quantified with the qPCR Ion Library TaqMan™ Quantitation kit (Thermo Fisher Scientific) in duplicates and pooled to 35pM. Sequencing was performed on the Ion S5™ System (Thermo Fisher Scientific) using Ion 530™ Chips (Thermo Fisher Scientific).
Buccal swab reference samples from all volunteers were included in both CE and MPS analyses for comparison.

Data analysis

Electropherograms were analysed in GeneMapper® ID-X Software v.1.4 (Thermo Fisher Scientific). The thresholds for allele calling were 75 RFU.
Sequencing data were analysed with the MitoVariantCaller plugin in Converge™ (Thermo Fisher Scientific). The criteria were: variant frequency ≥ 90%, minimum variant coverage on either strand ≥ 10 reads, total allele coverage ≥ 20 reads, maximum strand bias = 0.6. Reads with low quality or with signs of degradation were filtered and excluded from the analysis. Known nuclear mitochondrial DNA segments (NUMTs) were identified and removed.

CONCLUSIONS

- Recovery of touch DNA from glass and gun shells could be optimized using DD to visualize touched areas.
- Recovery of DNA was most effective using the OneTouch™ Touch DNA kit or using moistened Puritan cotton swabs (full and partial STR profiles were obtained).
- mtDNA analysis provided additional information in samples where STR genotyping was not successful or partial profiles were obtained.

RESULTS

- Shed cells were successfully visualized and collected with both cotton and nylon 4N6FLOQSwabs™ (Fig. 3). The nylon swabs appeared to collect shed cells from gun shell casing inscriptions more efficiently when visualizing DD fluorescence before and after collection.
- DNA yield for each method is presented on Table 1. The yield was higher for cotton than for nylon swabs. OneTouch processed samples had the highest yield.
- Results of STR genotyping from 41 samples collected from gun shell casings are presented in Table 2. No STR profile was obtained in 16 samples. Full profiles (17 STRs) were obtained in all samples processed with the OneTouch™ protocol. Direct PCR, chelex-extraction, and mechanical rupture had similar performance. Stochastic effects were observed in most of the samples (Table 3 and Fig. 4).
- A total of 68 samples were typed with the Precision ID mtDNA whole genome panel (Thermo Fisher Scientific). Full profiles were obtained for 65% of the samples (Fig. 5). Only one sample did not result in any mtDNA sequences. Partial profiles were obtained for the remaining samples.

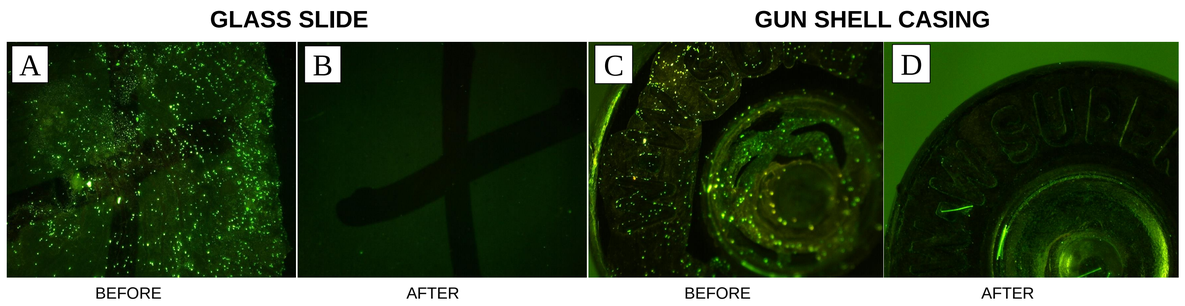

Figure 3. Touch DNA on glass slides (A) and gun shells casings (C) before and after collection using cotton (B) or nylon swabs (D).

Table 1. Quantification results of 25 Chelex-extracted samples from cotton swabs, nylon 4N6FLOQSwabs™ and the OneTouch™ protocol.

	RANGE (pg/µL)*	MEDIAN (pg/µL)*
Cotton	5.0 - 168.0	16.85
4N6FLOQSwabs™	0.3 - 116.5	1.65
OneTouch	15 - 676.5	19.2

*Elution volume: cotton and 4N6FLOQSwabs™: 30µL; OneTouch protocol: 100µL

Table 2. Results of STR analyses of touch DNA deposited on 41 gun shell casings.

	DIRECT PCR	CHELEX	MECHANICAL RUPTURE	ONETOUCH™
No profile	4	5	7	0
Partial profile	7	5	5	0
Full profile	1	1	0	6

Table 3. Number of stochastic effects and correctly typed loci in 68 samples (41 gun shell casings and 27 glass slides).

	DIRECT PCR	CHELEX	MECHANICAL RUPTURE	ONETOUCH™
Locus drop-out	9.3	9.2	10.5	0
Allele drop-out	3.7	1.3	3.2	0
Allele drop-in	1.3	1.4	1.3	4.7
Correctly typed loci	4.8	5.8	4.3	12.8

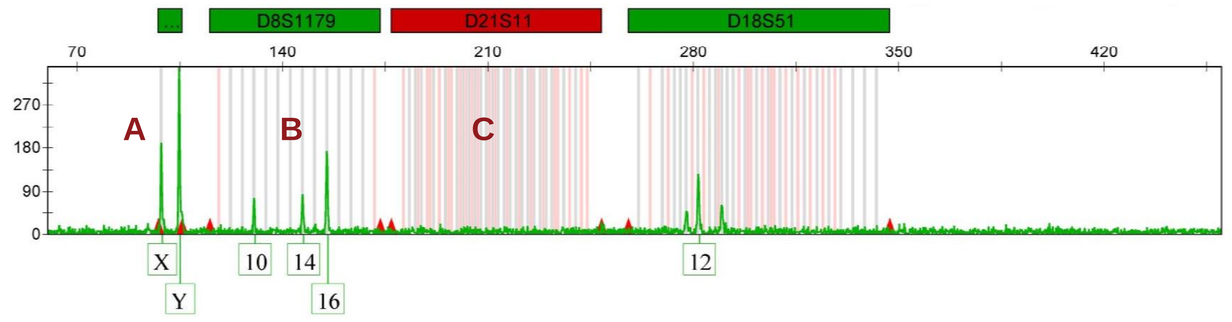

Figure 4. Example of an electropherogram of a sample collected with a cotton swab, followed by direct PCR. A) Heterozygote imbalance; B) allele drop-in; C) locus drop-out.

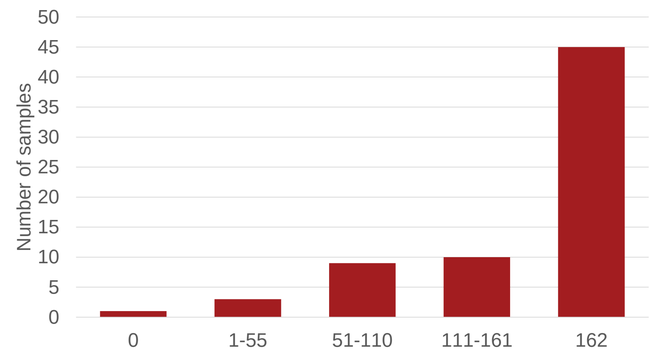

Figure 5. The number of amplified fragments in 68 samples. A total of 162 fragments were amplified with the Precision ID mtDNA whole genome panel.

REFERENCES

1. Kanokwongnuwut P, *et al* (2018) Forensic Sci. Int. Genet. 36: 20-25.
2. Pereira, V. *et al* (2018) Electrophoresis. 39: 2766-2775.