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Optimization of the collection and analysis of touch DNA traces

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ABSTRACT

The aim of this work was to optimize a strategy for detection, collection, and analysis of touch DNA traces. 4N6FLOWSwabs™ and cotton swabs were compared by collecting touch DNA traces from glass slides and gun shell casings. Shed cells were visualized using Diamond™ nucleic acid dye and a digital fluorescent microscope. Different collection and extraction methods were tested. Collected samples were amplified with the AmpFlSTR® NGMSElect™ kit and the Precision ID mtDNA Whole Genome Panel.

1. Introduction

Surfaces at crime scenes touched by the offender(s) or victim(s) are frequently swabbed in order to recover genetic material. These traces have often very low amounts of DNA, less than the minimum required for PCR amplification at our laboratory (200 pg).

Analysing touch DNA data is complicated because of the occurrence of stochastic effects such as stutters, allele imbalance, drop-ins and drop-outs [1]. Mitochondrial DNA can be an alternative since it is present in higher numbers, and the amplification does not produce stutters [2,3].

In this study, the sample collection efficiency of nylon swabs was compared to cotton swabs. Touch DNA traces were collected from various surfaces. Several collection and extraction methods were tested. The samples were amplified with the AmpFlSTR® NGMSElect™ kit (Thermo Fisher Scientific, USA) and with the Precision ID mtDNA Whole Genome Panel (Thermo Fisher Scientific).

2. Material and methods

2.1. Samples

A total of 68 touch DNA traces were used in this study (41 from gun shell casings and 27 from glass slides). Buccal swab reference samples from all volunteers were included for comparison.

2.2. Visualization of genetic material

Diamond™ Nucleic Acid Dye (DD, Promega, USA) was diluted 20x in 75% ethanol [3]. Three µL of the DD dilution were pipetted onto the samples and dried. Stained DNA was visualized with a Dino-Lite digital microscope (AnMo Electronics Corporation, Taiwan) (DD has an excitation wavelength of 494 nm and emission at 558 nm).

2.3. Collection and processing of DNA

Nylon 4N6FLOQSwabs™ (Copan S.p.A., Italy) and Puritan® cotton swabs (Puritan, USA) were moistened with 20 µL of water. Surface were swabbed using the moistened side first, then the dry side. Swab heads were broken off into spin baskets for direct PCR and mechanical rupture, or placed directly into Eppendorf tubes for chelex extraction. Twenty µL of water were added to the spin baskets. Samples were vortexed for 15 s at maximum speed and centrifuged for 2 min at 13,000 rpm to collect the eluent in a collection tube.

Chelex extraction was performed using 1 mL of 20% chelex. Mechanical rupturing of the cells was performed using one 3 mm stainless steel bead in the eluent tube and the TissueLyser II instrument (Qiagen, Germany). Homogenization time was 35 s at 30 Hz.

Samples collected with the OneTouch™ Touch DNA kit (Independent Forensics, USA) were processed according to the protocol provided by the manufacturer until, and including, the purification step. Chelex extracts and OneTouch™ lysates were quantified using the Quantifiler Trio DNA Quantification kit (Thermo Fisher Scientific).

2.4. Genetic and data analyses

Samples were amplified with the AmpFlSTR® NGMSElect™ PCR Amplification Kit, using 10 µL eluent or 500 pg template DNA, according to the manufacturer's instructions. Capillary electrophoresis was performed with ABI3500xL Genetic Analyzer (Thermo Fisher Scientific) and profiles were analysed in GeneMapper® ID-X Software v.1.4 (Thermo Fisher Scientific). The threshold for allele calling was 75

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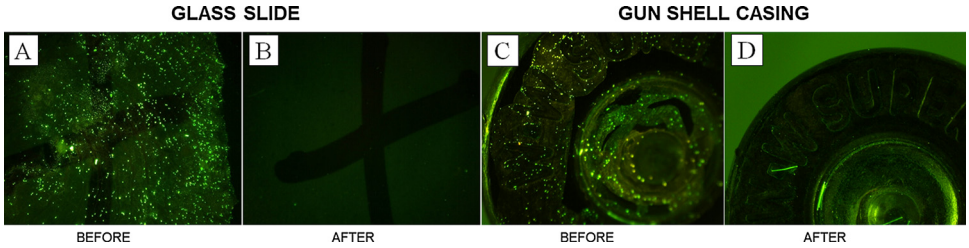

Fig. 1. Touch DNA on glass slides (A) and gun shells casings (C) before and after collection using cotton (B) or nylon swabs (D).

Table 1
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

RFUs. Samples were also investigated with the Precision ID mtDNA whole genome panel [4]. Libraries were quantified in duplicates with the qPCR Ion Library TaqMan™ Quantitation kit (Thermo Fisher Scientific) and pooled to 35 pM. Sequencing was performed on the Ion S5™ System using Ion 530™ Chips (Thermo Fisher Scientific). Sequencing data were analysed with the MitoVariantCaller plugin in Converge™ (Thermo Fisher Scientific). The following criteria were used: 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. Reads with known nuclear mitochondrial DNA segments (NUMTs) were identified and removed.

3. Results

Shed cells were successfully visualized using DD and easily observed using the digital microscope (Fig. 1). 4N6FLOQSwabs™appeared to collect shed cells from gun shell casing inscriptions more efficiently than cotton swabs when visualizing DD fluorescence before and after collection.

DNA yield was evaluated after chelex extraction and the OneTouch™ protocol. The yield was higher for samples collected with Puritan® swabs (range (r): 5.0–168.0 pg/μL, median (m): 16.85 pg/μL) than for nylon swabs (r: 0.3–116.5 pg/μL, m: 1.65 pg/μL). OneTouch™ processed samples had the highest yield (r: 15–676.5 pg/μL, m: 19.2 pg/μL). All samples reached the minimum requirement for STR amplification (200 pg in up to 15 μL) and the elution volumes were larger than for the other methods.

Out of 41 touch DNA samples collected from gun shell casings, 16 samples resulted in no STR profiles. Full profiles (17 STRs) were obtained for the 6 OneTouch™ processed samples. Direct PCR, chelex extraction, and mechanical rupture had similar performance (data not shown). Stochastic effects were observed in most samples (Table 1). Locus drop-outs were most frequent for samples processed with mechanical rupture, followed by direct PCR and chelex extracted samples (Table 1). Drop-in frequencies were similar for the three methods. OneTouch processed samples had no locus drop-outs, but the drop-in rate was high (average: 4.7).

Full mtDNA profiles (162 fragments) were obtained for 65% of the samples. Only one sample could not be sequenced. Partial profiles (111–161 fragments) were obtained for 15% of the samples.

4. Conclusions

Recovery of touch DNA could be optimized using DD to visualize touched areas. In this study, the collection of DNA was most effective using the OneTouch™ Touch DNA kit or using moistened Puritan® swabs. mtDNA analysis provided additional information in samples where STR genotyping was not successful or partial STR profiles were obtained.

Declaration of Competing Interest

None.

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