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Maximization of STR DNA typing success for touched objects

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Abstract. In order to produce database-eligible DNA profiles from touched objects, each testing procedure including sample recovery, extraction, amplification and separation was evaluated and optimized. The developed methodologies were tested on control samples as well on fingerprints deposited on a variety of substrates such as credit cards, keys, and pens. All samples were amplified in triplicate to confirm the presence of each allele and to detect drop-ins. Overall the modifications implemented produced reproducible results for DNA titrated to 20 pg. For DNA dilutions, 25 pg routinely resulted in full profiles, and 12.5 pg determined 76.9% of the database loci tested. Similarly, for the touched objects, 75.8% of the 20-pg to 100-pg samples yielded database-eligible profiles; the remaining samples either were mixtures or contained an insufficient number of allelic calls. Here, the three-amplification approach was crucial and produced more complete profiles with confidence in the allelic assignments. DNA amounts below 20 pg did show partial profiles with correct allelic determinations that could have been compared in a specific case but were often too incomplete for database entry.

Keywords: Contact DNA; Evidence collection; Increased PCR cycle numbers; 3100 modifications

1. Introduction

In order to test property crimes which often contain only contact DNA, current testing procedures were modified staying within the framework of the STR loci and primer sets accepted for US databases.

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2. Materials and methods

Different types of collection swabs were tested using fingerprints from volunteers or defined cell counts of purified human embryonic kidney (HEK) cells on a flat surface. 0.01% SDS was used as a wetting solution. All samples were extracted using a 30-minute incubation with 0.01% SDS and proteinase K at 56 °C, followed by incubation at 100 °C for 10 min. Concentration of the extract and removal of the SDS was accomplished through centrifugation with a Microcon® 100 column (Millipore, Bedford, MA) pre-treated with 1 ng of Poly A RNA [1]. Extracts were quantitated on a Rotorgene 3000 (Corbett Research, Melbourne, Australia) with quantitative real-time PCR based on the procedure of Nicklas and Buel [2], with the exception of the use of 28,000× SYBR Green I (Molecular Probes, Oregon), 0.525 mg/mL BSA, and a 25 μL reaction volume.

Samples were amplified in triplicate with AmpF*l*STR Identifiler™ (Applied Biosystems, Foster City, CA), or Powerplex® 16 (Promega, Madison, Wisconsin), as specified, and cycled 31 or 32 times, respectively, with a minimum of 6.25 pg of DNA per amplification [3]. The annealing times were doubled and the reaction volumes were halved for both kits. A total of 6 μL of amplified product was separated on the 3100 Genetic Prism® Analyzer (Applied Biosystems, Foster City, CA). Injection conditions were adjusted based on DNA input and three different conditions were used routinely: 100 pg at 1 kV for 22 s; 25–50 pg at 3 kV for 20 s; <25 pg at 6 kV for 30 s. Peak intensities were maximized by not using variable binning and by setting the baseline window to 251. Data was analyzed employing a minimum threshold of 75 RFU and a 10% general filter. Only alleles that occurred in two of three amplifications were assigned. Samples were deemed non-mixtures if they contained less than three repeating alleles.

3. Results and discussion

In order to improve cell removal from evidence and the subsequent DNA release from the swab, several collection devices were compared. Fingerprint samples swabbed with our specialized swab produced at least 2.5 times more alleles than those collected with fabrics commonly used in forensic laboratories such as cotton wipes and Dacron (data not shown). Various extraction methodologies were also evaluated; a single tube digestion with SDS followed by purification and concentration with a Microcon® column recovered more DNA than protocols with many manipulations [1]. The addition of carrier DNA or RNA to the Microcon® minimized DNA loss, which proved essential to samples with very low amounts of DNA [1].

A total of 109 touched objects, including 30 single fingerprint samples, were extracted. Most of these samples could be defined as low copy number (LCN) DNA samples, providing less than 100 pg for each of the three amplifications (data not shown). For example, less than a total of 40 pg were recovered from 57.8% of the samples, which included the majority of the single fingerprints. Single fingerprint samples, however, may not accurately reflect evidentiary touched objects which likely contain more than one fingerprint after direct contact. A few fingerprints, nevertheless, were among the 42.2% of the samples that yielded at least 40 pg. Since only 3.66% of the extracted samples contained more than 1000 pg, potentiating the generation of full profiles from amplification with standard procedures, optimization of amplification was warranted.

Database-compatible commercial kits were thus tested for sensitivity and specificity; results recommended implementation of Identifiler™ with a doubled annealing time, increased cycle number, and a reduced reaction volume [3]. To accommodate the occurrence of spurious alleles, samples were amplified in triplicate with only repeating alleles assigned. In this manner, with the use

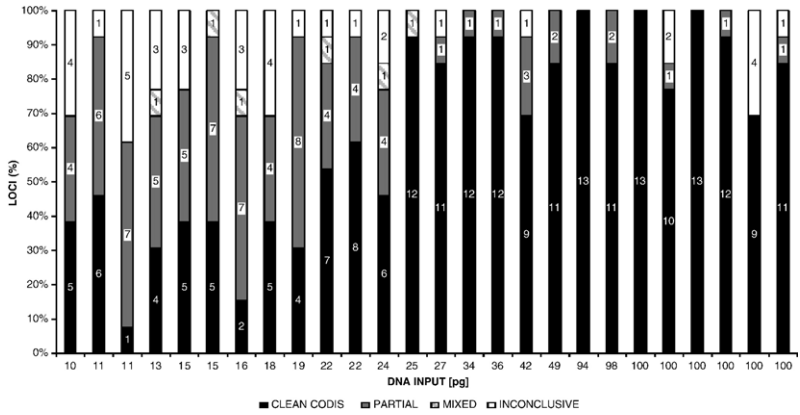

Fig. 1. Loci determination for clean, non-mixture samples. 10–100 pg of DNA recovered from touched objects was amplified in triplicate with Identifiler™ reagents for 31 cycles. Only alleles that occurred in two of three amplifications were assigned. Data are expressed as the percentage of loci from each sample that are clean, partial, mixed with at least three alleles, or inconclusive.

of a megaplex kit, all database loci were amplified simultaneously allowing confirmation of each allele [4]. A titration study of purified DNA revealed that, on average, 7 or 9 loci were generated from amplification of 6.25 pg and 12.5 pg of DNA, respectively, and DNA, 25 pg and above, produced full profiles (data not shown). No drop-ins were repeated with these studies of control DNA. For samples less than 25 pg, on average, more alleles were assigned in the composite profiles.

For the 39 amplified touched objects, 66.7% of resultant profiles could be attributed to one donor and a major component could be deduced from 17.9%. The remaining 15.4% either was a multicomponent mixture or contained too many inconclusive loci for interpretation. Employing the specialized swab and modified extraction, amplification and sample separation protocols, 75.8% of the samples amplified with 20–100 pg of DNA, produced database eligible profiles consisting of a minimum of six clean loci (Fig. 1). Although 90% of the samples amplified with 10–20 pg of DNA generated at least eight partially determined loci, only 10% of these samples qualified for the database. Many of these samples contained partial or inconclusive loci which were low-level mixtures and could not be fully interpreted. These results suggest that samples containing at least 20 pg of DNA may generate profiles sufficient for database entry, whereas profiles produced from amplification of 10–20 pg of DNA may be useful for direct comparison.

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