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The use of mini-STRs on degraded DNA samples

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Abstract. Forensic laboratories – more frequently than in the recent past – are beginning to study degraded and/or compromised DNA. These samples usually contain small amounts of DNA (LCN DNA). With these exhibits, even relying on the most efficient extraction system, or amplifying with increased number of PCR cycles, the STRs profiles are often incomplete or exposed to stochastic effects. In this paper we refer to the use of a mini-pentaplex (FGA, D21S11, CSFPO, D7S820 and TH01) and a mini-quadruplex (Penta D, D2S1338, Amelogenin, and D18S51) used to analyse casework samples which gave negative or very partial results, when analysed by the commercially available kits. The preliminary results obtained from this study are promising and in this report we demonstrate that we can obtain profiles with relatively lower quantity of DNA (approximately 50 pg or less) in a reliable manner. © 2005 Published by Elsevier B.V.

Keywords: Mini-STR; Degraded DNA; DNA typing

1. Introduction

Whilst typing degraded DNA and DNA from a few residual cells collected at crime scene is not uncommon to get partial STR profiles with the commercially available kits, the main problem is encountered in amplification in the higher molecular mass loci. We have set up a mini-pentaplex (FGA, D21S11, CSF1PO, D7S820 and TH01) and a mini-quadruplex (Penta D, D2S1338, Amelogenin and D18S51) with some of these heavier STRs present in commercial kits as AmpF*l*STR® Identifiler™ and Powerplex 16. We surveyed international literature for the primer sets related to short amplicons and employed the primers described by Butler et al. [1]. Both mini-multiplexes were tested on a range of samples that gave negative or partial typing with commercial kits. We were able to obtain remarkably clean data. This encourages us to continue these studies, because there are crime stains that are not detectable by the quantification procedures due to low DNA quantity.

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Table 1
Pentaplex loci and labelled primers

STR Locus	F-Primers	R-Primers
FGA	5′ 6-FAM	No
D7S820	5′ NED	Tail 5′-GTTTCTT
D21S11	5′ VIC	No
CSF1PO	5′ VIC	No
TH01	5′ PET	Tail 5′-GTTTCTT

2. Materials and methods

The three samples considered in this paper were collected on different crime scenes and consisted in a cigarette butt, a glass and a razor blade. The external paper of the cigarette butt (sample 2) was cut, saliva on glass (sample 1) and tissue residue on a razor blade (sample 3), were collected using an OralSwab (Whatman) before the extraction procedure. All this stuff was then subject to the lysis incubation step and subsequently extracted using "QIAamp 96 DNA Swab BioRobot Test" silica columns in association with BioRobot 9604 (QIAGEN), according to the manufacturer's protocol [2]. Final elution was done with 50 μl of DD water. DNA quantification was conducted performing a Dot-Blot analysis with Quantiblot kit (Applied Biosystems). Despite the fact that quantification step had not shown a sufficient quantity of DNA, we decided to perform the PCR anyway, using the "Identifiler" kit (ABD), according to the original protocol.

On the same extracted material we also tested a new casting of primers, designed according to Butler et al. [1] with four different dyes (FAM, NED, VIC and PET) pulled together (Tables 1 and 2). PCR conditions suggested by the authors were observed, with the exception of the final volume (12.5 μl). Both multiplexes were used to amplify the Identifiler and PowerPlex diluted ladders, in order to obtain affordable mini-STRs ladders to use in each run made.

PCR fragments were separated with capillary electrophoresis and the size call allele was done by GeneMapper v.3.2 (Applied Biosystems).

3. Results and discussion

The main idea for this study was to move primers forward along the genomic sequence, as near as possible to the repeat unit core, in order to obtain amplicons under the 250 bp sizes instead of those heavier in the commercial multiplex kits. The base pair reductions gained for the same alleles of the different loci with respect to the Identifiler and PowerPlex 16.2 kits are shown in Table 3. Height peaks of the multiplex tested are reported in Table 4, as proportional average in RFU for each genotype, together with the stochastic effects or the negative results, was obtained.

Table 2
Quadruplex loci and labelled primers

aSTR Locus	F-Primers	F-Primers
Penta D	5′ 6-FAM	No
D2S1338	5′ NED	No
D18S51	5′ PET	No
Amelogenin	5′ PET	No

Table 3
Comparison between commercials and mini-STR loci characteristics

STR loci	Allele range	Allele spread (bp)	Identifiler (unpublished)	PowerPlex	Mini-STRs	Size reductions
FGA	12.2–51.2	156	200–356	308–464	125–281	75–183
D7S820	5–15	40	251–291	211–251	136–176	115–75
D21S11	24–38.2	58	184–242	203–261	153–211	31–50
CSF1PO	6–16	40	304–344	321–361	89–129	215–232
TH01	3–14	44	159–203	152–196	55–99	104–97
Penta D	2.2–17	73	–	376–449	94–167	282
D2S1338	15–28	52	307–359	–	90–142	217
D18S51	7–27	80	263–343	286–366	113–193	150–173

Table 4
Present work example profiles

Loci	Sample 1—Glass			Sample 2—Cigarette butt			Sample 3—Razor blade		
	Identifiler PowerPlex	Quadruplex	Pentaplex	Identifiler PowerPlex	Quadruplex	Pentaplex	Identifiler PowerPlex	Quadruplex	Pentaplex
CSF1PO	KO		2400	10*,°		1200	KO		8000
D18S51	100*	KO		20*,°	140		KO	500	
D2S1338	40*,°	700		30*	500		KO	8000	
D7S820	30*,°		400	30*		700	150*		1200
TPOX	120*			80*			150*		
D13S317	200			80*			200		
D19S433	200			50*			1400		
D3S1358	350			150*			2000		
D8S1179	180			100*			400		
VWA	100*			80*			300		
D16S539	100*			50*			300		
D21S11	80*		600	80*		400	100*,^		1600
D5S818	50*			50*			200		
FGA	60*,°		1400	30*,°		2500	80*,°		2400
TH01	160		3000	70*		1400	700		3500
AMEL	350	1300		70*	180		800	6000	
PentaD	KO	400		KO	160		KO	3000	

*Needs confirmation, °drop-out and ^extra peaks.

Looking at the RFU values, the differences appear very impressive even if no statistical gains could be argued within only three pieces of evidence. It is easy to note that the major rewards in terms of peak heights come from the STRs where the size reductions were more consistent.

Efficiency of primers for reduced STRs size is shown once again, even if other experiments for primer concentration, balancing or adjustments, able to introduce another STR in the quadruplex (Penta E), are still needed.

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