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Research article

Development of a novel miniSTR multiplex assay for typing degraded DNA samples

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Abstract

A multiplex PCR was developed for the analysis of the sex-determining gene Amelogenin, four conventional STR (short tandem repeat; THO1, D18S51, D21S11 and FGA) loci with a reduced amplicon size and four miniSTR loci (D1S1677, D2S441, D10S1248 and D22S1045). A concordance study in a population of 198 Belgians revealed no differences for the conventional STR loci while a sensitivity study showed a reproducible DNA profile with as low as 30 pg of input DNA.

Keywords: miniSTR; Multiplex PCR; Population genetics; Belgian

1. Introduction

The analysis of DNA samples in forensic casework has until recently relied on the use of commercial multiplex STR (short tandem repeat) systems in a length range of 100–400 base pairs (bp). The application of such systems results in a full DNA profile in the majority of high-quality DNA samples. However, the increased demand to analyze limited DNA samples from evidential skin contact (currently 50% of the workload) or skeletal remains increases the number of samples where only a partial DNA profile is obtained due to low-quantity DNA and/or DNA degradation. It has been demonstrated that the sensitivity for degraded DNA samples can be increased by reducing the size of the STR amplicons (miniSTRs) through relocation of the primers closer to the repeat region [1–3].

The objectives of our study were to develop a miniSTR mutiplex assay that should partially complement the existing commercial multiplex STR systems and at the same time include several miniSTRs that have been recommended as additional loci for the European Standard Set of Loci [4–5].

2. Materials and methods

DNA amplification was done in a reaction volume of 25 μl using Qiagen® Multiplex PCR Kit (Qiagen, Hilden, Germany), 5 μl of optimized primer-mix (Table 1; forward primers were fluorescently labelled) and 5 μl of DNA (0.5–1 ng) under standard PCR conditions of 15 min at 95 °C, 30 cycles of 1 min at 94 °C, 1 min at 55 °C and 1 min at 72 °C (“9600” ramp speed), and a final extension of 45 min at 60 °C on a GeneAmp 9700 PCR System (Applied Biosystems, Foster City, CA). PCR products (1 μl) were analyzed on an ABI PRISM 3130 XL Genetic Analyzer (Applied Biosystems) with a 37 cm capillary and using POP-6™ as separation medium. Fragment size determination and genotyping were done with GeneMapper v3.2 (Applied Biosystems) and GS-500[LIZ] as the internal size standard. Allelic ladders were developed for the novel miniSTR loci (D1, D2, D10 and D22) by pooling the amplification products for different alleles from a population study and re-amplification. Allelic ladders for the other loci were obtained by following the procedure described by Coble [6]. Allele frequencies, match probability and power of exclusion were calculated with PowerStats V1.2 (Promega, Madison, WI).

3. Results and discussion

The following criteria were used for the selection of STR loci for the miniSTR mutiplex: inclusion of miniSTR loci

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Table 1
Selected loci included in the miniSTR multiplex assay

Locus	Size range (bp)	Match probability	Power of exclusion	Fluorescent label	Primer sequences
AMEL	126–132	–	–	6-FAM	[7]
D1S1667	78–117p	0.122	0.624	PET	[1]
D2S441	75–110	0.124	0.472	6-FAM	[1]
D10S1248	80–135	0.103	0.439	VIC	[1]
D18S51	110–190	0.035	0.732	NED	[2]
D21S11	145–220	0.043	0.672	VIC	[3]
D22S1045	74–110	0.127	0.532	NED	[1]
THO1	145–190	0.091	0.692	6-FAM	[8]
FGA	118–270	0.038	0.773	PET	[2]
Combined		1.03×10^{-9}	0.99968		

Match probability and power of exclusion were calculated from the data of a population study of 198 Caucasians of Belgian descent.

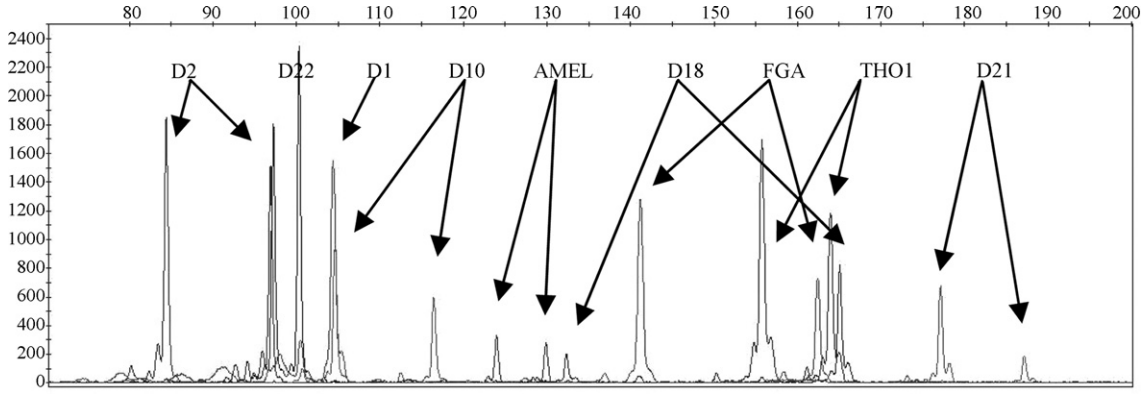

Fig. 1. Result of miniSTR amplification of 30 pg DNA with 34 cycles.

recommended as novel loci for the European Standard Set of Loci (Table 1; D2, D10 and D22); observed locus dropout of at least 40% in casework samples with partial profiles and a matching probability of less than 0.05; included in the European Standard Set of Loci (Table 1; D21, D18 and FGA). In addition, AMEL and THO1 were also included while D1S1677 was selected for its size less than 120 bp. The primers were chosen from literature so that the length of the amplicons was less than 200 bp for the most frequent observed alleles.

The optimized PCR protocol was applied to a DNA panel of 198 Belgian Caucasians previously typed with PowerPlex® 16 [9]. Full concordance was found for the common loci THO1, D18S51, D21S11 and FGA indicating that the redesigned primer sets do not lead to allele dropout or size shifts due to the presence of a primer-binding site mutation or an insertion/deletion in the flanking site [10,11]. Different alleles for D1, D2, D10 and D22 were selected from the population study and sequenced in order to identify the number of repeats. These selected alleles were then used for the construction of allelic ladders for the miniSTR loci. No deviation from Hardy–Weinberg equilibrium was observed. The combined match probability and power of exclusion shows the discriminatory value of this novel multiplex in forensic casework and paternity determinations.

A sensitivity study on a serial dilution of DNA samples under the standard conditions of 30 cycles showed the occurrence of allele dropout or locus dropout when the DNA input was less than 100 pg of DNA. By increasing the number of cycles to 34, a reproducible DNA profile was obtained when the amount of DNA was decreased to 30 pg of DNA (Fig. 1). This sensitivity was two- to four-fold higher in comparison to the commercial STR multiplexes (e.g. SGM Plus or PowerPlex® 16). The forensic usefulness was demonstrated in an identification case of skeletal remains that showed DNA degradation. Application of the developed miniSTR multiplex made it possible to type those STR (D18, D21 and FGA) that showed locus dropout with SGM Plus. These alleles were confirmed by comparison with the DNA profiles of putative relatives. Further validation studies concerning DNA degradation are in progress and will be reported elsewhere.

Conflict of interest

None.

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