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The development of two new STR multiplex systems

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Abstract. Here we show two new amplification systems, which were realised for the amplification of 80 highly polymorphic loci, in combination with an Infrared Automated DNA Sequencer LI-COR 4200 (LI-COR, Nebraska, USA). The two amplification systems were indicated as MU5 and MU6. The amplification system MU5 contained D12S391, F13A01, SE33 and Penta D loci, the amplification system MU6 the loci D2S1338, D19S433, F13B and Penta E. Both systems were performed using forward IRDye™ 800 labelled and reverse unlabelled primers, in combination with the QIAGEN Multiplex PCR kit (Qiagen, Germany). The extension of the set of STRs in our laboratory is useful to further improve our ability to resolve complex kinship cases (e.g. deficiency cases, incest, paternity tests with mutations). Moreover, these new multiplex systems can also be used for manual typing, because the STRs do not overlap in size.

Keywords: Short tandem repeat; Multiplex systems; Infrared sequencer

1. Introduction

In our laboratory, we extensively use an Infrared Automatic DNA Sequencer LI-COR 4200 (LICOR, Nebraska, USA) for forensic analyses (paternity tests and stains analysis). The Infrared technology is efficient and robust, although it has become less frequent in forensic genetic laboratories. Amplification of the STR loci for forensic purposes is routinely performed using UV, automated DNA sequencers and commercially available kits. Validation studies, publications and the experience of many laboratories are now available and in our opinion there is no reason for changing the technology. Moreover, we verified that the results obtained using the Infrared technology are comparable with the use of Ultraviolet technology and commercially available kits [1]. Our protocol is based on the loci of the FBI CODIS system (LI-COR 4200) [2]. However, new STRs loci introduced by commercial kits are becoming important in the forensic community. Penta D and Penta E are included in PowerPlex ® 16 System (Promega). D2S1338 and D19S433 are included in AmpFlSTR SGM Plus ™ and AmpFlSTR Identifiler™ (Applied Biosystems). Moreover, SE33 (ACPB22) is used in the German DNA database. We performed two new amplifications systems to analyse all these STR markers, in combination with D12S391, F13B and F13A01 markers.

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2. Materials and methods

DNA samples from donors and K562 cell lines DNA were used to test the efficiency of these multiplex systems. Then, we used the two amplification systems to analyse three paraffin-embedded tissues, in comparison with blood of the donors.

Both amplification systems were performed using forward IRDye™ 800 labelled and reverse unlabelled primers, in combination with the QIAGEN Multiplex PCR Kit (Qiagen, Germany) [3]. PCR was performed in 12.5 μl, using 5.8 μl of 2× QIAGEN Multiplex PCR Master Mix and 5.8 μl of a mix of primers. Final concentration for D12S391, Penta D, D2S1338, D19S433, F13B and Penta E was 0.2 μM. For F13A01 it was 0.3 μM and for SE33 0.1 μM. In each PCR mix we used 0.9 μl of undosed DNA. For both systems, the amplification condition was the following: 95 °C for 15 min, then 94 °C for 30 s, 60 °C for 90 s, 72 °C for 90 s. 30 cycles of PCR were performed, with a final extension period of 10 min a 72 °C. Gel electrophoresis was performed in a LI-COR® model 4200 monolaser automated fluorescent DNA sequencer, like described [2].

3. Results

The two amplifications systems produced defined autoradiogram-like patterns in the virtual image of the computer. The QIAGEN Multiplex PCR kit is specifically developed for multiplex PCR and provides an easy-to-use master-mix format. We verified that this eliminates the need for optimisation, making the development of multiplex PCR assays both simple and fast. However, we found that it is possible to obtain a balanced intensity between the microsatellites, using different concentration of the primers. We used a previously published sequence for all primers, except for F13A01 and F13B (Table 1). Efficient primer annealing and extension, irrespective of the primer sequence, is achieved by Factor MP in QIAGEN Multiplex PCR Master Mix. Factor MP increases the local

Table 1
Characteristics of the loci include in MU5 and MU6 amplification systems

	Locus	Chromosomal location	Primer sequence	Allele size (bp)	GenBank accession
MU5	D12S391	12	A-AACAGGATCAATGGATGCAT B-AGCCTCCATATCACTTGAGC	129–173	G08921
	F13A01	6p24–p25	A-GAGGTTGCACTCCAGCCTTT B-AGCCCCAAGGAAGATGAGAAAC	179–235	This work
	SE33	6	A-AATCTGGGCGACAAGAGTGA B-ACATCTCCCCTACCGCTATA	233–333	V00481
	Penta D	21q	A-GAAGGTCGAAGCTGAAGTG B-ATTAGAATTCTTTAATCTGGACACAAG	376–449	AP001752
MU6	D2S1338	2q35–37.1	A-CCAGTGGATTTGGAAACAGA B-ACCTAGCATGGTACCTGCAG	160–212	G08202
	D19S433	19q12–13.1	A-CCTGGGCAACAGAATAAGAT B-TAGGTTTTTAAGGAACAGGTGG	178–212	G08036
	F13B	1q31–q32.1	A-TGAGGTGGTGTACTACCATA B-GTGGTACACGCCTGTAATC	265–289	This work
	Penta E	15q	A-ATTACCAACATGAAAGGGTACCAATA B-TGGGTTATTAATTGAGAAAACTCCTTACAATTT	379–474	AC027004

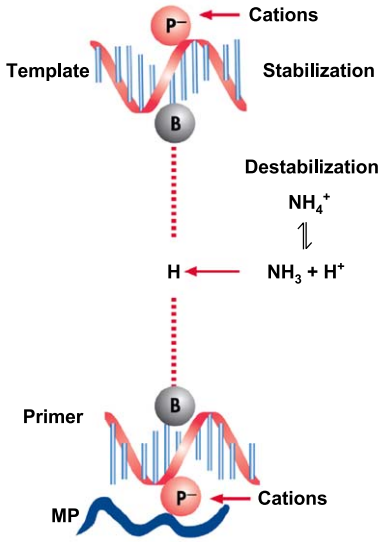

Fig. 1. Mg^{2+} and other salts bind to phosphate groups (P^-) on the DNA backbone, which stabilizes the annealing of the primers to the template. NH_4^+ can interact with the hydrogen bonds between the bases, destabilizing principally the weak hydrogen bonds at mismatched bases (B) of non specifically bound primers. Factor MP (MP) enabling efficient extension of all primers in the reaction (kindly admitted from QIAGEN®).

concentration of primers at the template DNA and stabilises specifically bound primers (Fig. 1). The analysis of paraffin-embedded tissue gave comparable results in comparison with blood of donors. Moreover, we used the QIAGEN Multiplex PCR Master Mix for the amplification of the four amplification systems for CODIS [1]. Preliminary results suggest the usefulness of this new amplification format also for these amplification systems.

The use of these systems, together with the previously described multiplex systems (MU1:AME, vWA, FGA and D16S359; MU2:D3S1358, TPOX, TH01 and CSF1PO; MU3:D8S1179, D21S11 and D18S51; MU4: D7S820, D5S818 and D13S317), enable us to analyse 21 polymorphic loci and amelogenin for gender determination. The extension of the set of STRs is useful to further improve our ability to resolve complex kinship cases (e.g. deficiency cases, incest, paternity tests with mutations). Moreover, these new multiplex systems can also be used for manual typing and for the automatic sequencers that employ just one fluorochrome, because the STRs do not overlap in size.

References

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