International Congress Series 1239 (2003) 865–868

Development of two new multiplex systems (M1: D3S1358, D8S1179, D7S820, D16S539, Penta E and M2: D5S818, D13S317, D10S516, Penta D) for routine and forensic casework

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Abstract

Two multiplexes comprising nine STRs (M1: D3S1358, D8S1179, D7S820, D16S539, Penta E and M2: D5S818, D13S317, D10S516, Penta D) were developed. Investigations about sensitivity, mixtures, stains, etc., were made and Caucasian population data were obtained.

Keywords: Multiplex PCR; PowerPlex16®; Penta D; Penta E; D10S516

1. Introduction

In recent years, multiplex PCR-based DNA profiling has become a routine method at forensic laboratories across the world. A number of different multiplex systems have been developed in order to improve sensitivity and throughput of DNA analysis, thus substantially reducing the costs per profiling task. When the PowerPlex16® primer sequences had been published, we developed a pentaplex (M1) and a quadruplex (M2) comprising non-overlapping STRs that are based upon some of these PowerPlex16™ primers combined with the highly polymorphic D10S516 [1] and D8S1179 [2]. The implementation of two pentanucleotide repeats Penta D and Penta E for the monochrome ALFexpress® was of specific interest.

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0531-5131/03
PII: S0531-5131(02)00547-2

2. Material and methods

All blood samples were taken from unrelated Caucasian West Germans (Blood bank of the University of Cologne, Germany). DNA was extracted by a simple salting out procedure. PCR was carried out in two multiplex reactions (M1 and M2). All primer sequences were kindly provided by Rita Weisspfennig (Promega) except D10S516 according to Ref. [1] and D8S1179 according to Ref. [2]. For both multiplex systems, PCR conditions were optimised as follows: 0.5–10 ng DNA, 1.5 U Taq DNA Polymerase (Promega), $MgCl_2$ 2 mM, dNTPs 200 μM each, Reaction Buffer 3 μl, in a total volume of 25 μl. Primer concentrations: D5S818 0.1 μM, D13S317 0.15 μM, D10S516 0.5 μM, Penta D 0.8 μM, D3S1358 0.1 μM, D8S1179 0.7 μM, D7S820 0.16 μM, D16S359 0.2 μM and Penta E 0.4 μM.

Thermal cycling protocol for both multiplexes (GeneAmp® 2400 Thermal cycler): Predenaturation 95 °C 2 min, 32 cycles: 94 °C 35 s, 60.5 °C 35 s, 72 °C 45 s, final extension: 72 °C for 8 min. *Sensitivity tests*: Different dilutions from 1 ng to 50 pg DNA quantified by photometer followed by Gibco ACES™ 2.0 human DNA Quantitation System were used. *Mixtures and stains (blood, cigarettes, oral swabs, bone)*: DNA samples from German DNA profiling group (GEDNAP) blind trial 20 and 21 were tested. Bone sample derived from routine casework. *FailSafe™ PCR PreMix Selection Kit* (*EPICENTRE®, distributed by BIOzym Diagnostik GmbH*): PCR conditions were according to manufacturers manual, primer concentrations and thermal cycling protocol as above, all 12 premixes supplied in the kit were tested with the same DNA template (10 ng/assay). Cy5-labeled PCR products were separated and analysed with the ALFexpress® system (Amersham Pharmacia Biotech). Amplified DNA was mixed with internal Cy5-labelled 106 bp amelogenin fragment. External lane standards by Pharmacia (100–500 bp) and/or a mix of K562-alleles were used for adjustment. Allelic ladders have been standardized with known allele values according to Refs. [3–5] and with reference DNA from M. Gené (Santiago de Compostela, Spain). Most common alleles were composed to build the ladders. *Statistics*: Software DNA view (C.H. Brenner, Berkeley, CA).

3. Results and discussion

Population studies: See Table 1. Matching probability of all nine loci is about 1 in 7.18×10^{12}; paternity exclusion is 1 in 9.55×10^{5}. *Sensitivity tests*: With both multiplex systems DNA amounts down to 100 pg were reliably amplified. First allele dropouts appeared at 50 pg DNA. *Mixtures and stains*: Forensic casework material as well as stains and mixtures that derived from blind trials 20 and 21 were tested successfully. Mixtures were tested effectively up to a proportion of 4:1. In some cases, the increase of PCR cycles may help to amplify lower DNA amounts, but this depends on the quality of DNA regarding possible inhibitors or degradation. *FailSafe™ PCR PreMix Selection Kit* (*EPICENTRE®*): In our tests, nearly every PreMix showed fine results. This kit might be an alternative for more difficult primer/template combinations or to avoid contamination by using less pipetting steps and solutions. *Problems in course of investigations*: Both multiplex systems deliver reasonable results concerning amplification and typing of DNA.

Table 1
Allele and frequency distribution of M1 and M2 loci

Allele	D3S1358	D8S1179	D7S820	D16S539	Penta E	D5S818	D13S317	D10S516	Penta D
	$n=196$	$n=194$	$n=188$	$n=187$	$n=192$	$n=194$	$n=194$	$n=187$	$n=187$
5					0.076				
7			0.035		0.156	0.003	0.003		0.003
8		0.034	0.138	0.04	0.005	0.008	0.101		0.016
9		0.01	0.173	0.128	0.021	0.057	0.08		0.209
10		0.095	0.271	0.072	0.083	0.062	0.057		0.123
11		0.088	0.186	0.294	0.102	0.343	0.304		0.115
12		0.157	0.157	0.273	0.161	0.381	0.299		0.27
13	0.018	0.32	0.032	0.163	0.104	0.124	0.106		0.187
14	0.135	0.173	0.08	0.029	0.063	0.021	0.052		0.056
15	0.253	0.09			0.049	0.003			0.016
16	0.207	0.028			0.073				0.003
17	0.209	0.003			0.039				0.003
18	0.166	0.003			0.031				
19	0.013				0.016				
20					0.018				
21					0.003				
25								0.005	
27								0.01	
28								0.08	
29								0.22	
29.3								0.06	
30								0.23	
31								0.17	
32								0.02	
33								0.04	
34								0.02	
35								0.04	
36								0.06	
37								0.04	
38								0.01	
45								0.005	
47								0.01	
48								0.01	
OH	0.83	0.80	0.82	0.72	0.89	0.68	0.76	0.86	0.80
EH	0.80	0.82	0.81	0.79	0.90	0.71	0.79	0.85	0.82
CE	0.61	0.65	0.63	0.59	0.80	0.48	0.59	0.71	0.64
PD	0.932	0.945	0.941	0.924	0.982	0.872	0.924	0.962	0.942
HWE	$p=0.09$	$p=0.23$	$p=0.92$	$p=0.09$	$p=0.85$	$p=0.31$	$p=0.67$	$p=0.28$	$p=0.13$

O/EH: observed/expected heterozygosity, CE: chance of exclusion, PD: power of discrimination

In the beginning different signal intensities especially for D8, Penta D and Penta E were approximated with quite high primer concentrations whereas D3- and D5-primer amounts had to be decreased because of their high signal intensity which lead to stutters that could be mistaken as alleles. Another problem concerned the running behaviour of D13S317 alleles and ladders. Although PowerPlex16™ primer sequences were used, the fragments (PCR products and D13-ladder) appeared about 8 bp too long. AlleleLink 1.02 software

corrected this when K562 allele 8 was defined as external standard. Using the 200 bp external standard by Amersham Pharmacia Biotech the allelic D13-ladder was found by the software only when the alleles were redefined with the higher lengths (+8 bp). The alleles were typed correctly but were still too long. The only explanation we could find was a different running behaviour in our system due to secondary structures as the primers were published correctly. Nevertheless, this problem had no influence on typing results, but we plan to verify the allele sequences of our D13-PCR products.

In all, these two new multiplexes build a reliable easy to use and low cost alternative to commonly available kits.

Acknowledgements

The author would like to thank Dr. M. Gené for sending DNA reference material and for gently support.

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