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A triplex-PCR for SE33, D12S391 and D8S1132 and a singleplex-PCR for D6S389 in a single run

G. Dorner *, E.M. Dauber, S. Wenda, B. Glock, W.R. Mayr

Division of Blood Group Serology, Medical University of Vienna, Austria

Abstract. In this study, we investigated the loci SE33, D12S391 and D8S1132 in a triplex-PCR and the locus D6S389 in a singleplex-PCR, which were analyzed simultaneously in a single run, within a sample of 342 unrelated Austrian Caucasoid individuals. © 2005 Elsevier B.V. All rights reserved.

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1. Introduction

A triplex-PCR was developed for the highly polymorphic STR loci SE33, D12S391 and D8S1132 [1–3]. All loci were labelled with different dyes as they have partially overlapping size ranges. The STR locus D6S389 [4,5] had to be amplified separately, as a multiplex-PCR without changing the primer sequences of the other loci was not possible. One of the D6S389 primers was labelled with a fourth dye and therefore both PCR products could be analyzed simultaneously.

2. Materials and methods

DNA of 342 unrelated Austrian Caucasoid individuals was extracted by the salting out procedure. The conditions for the triplex-PCR were chosen as follows (reaction volume 15 μl): 1–2 ng DNA, 200 μM dNTPs, 1.5 μl 10× PCR Puffer AmpliTaq Gold, 0.6 U AmpliTaq Gold® DNA Polymerase (Applied Biosystems, Foster City, USA), 0.4 μM (SE33 6FAM label) and 0.2 μM (D12S391 VIC, D8S1132 NED label) of each primer [1–3]. All forward primers were fluorescent labelled. Thermal cycling was carried out on an GeneAmp 9700 PCR-System (Applied Biosystems, Foster City, USA) in 9600 mode: 95

* Corresponding author. Klinische Abteilung für Blutgruppenserologie, A-1090 Wien, Währinger Gürtel 18-20, Austria. Tel.: +43 1 40400 5320; fax: +43 1 40400 5321.

E-mail address: gudrun.dorner@meduniwien.ac.at (G. Dorner).

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Table 1
Statistic parameters

Parameters	SE33	D12S391	D8S1132	D6S389
Observed heterozygosity	0.953	0.898	0.857	0.924
χ^2	86.86	25.91	39.20	48.28
df	78	28	28	36
p	0.234	0.573	0.065	0.078
Polymorphism information content	0.940	0.870	0.840	0.890
Matching probability	0.009	0.026	0.039	0.023
Power of discrimination	0.991	0.974	0.961	0.977
Typical paternity index	10.69	4.89	3.49	6.58
Power of exclusion	0.905	0.791	0.708	0.845

°C 11 min for 1 cycle; 94 °C 1 min, 61 °C 1 min, 72 °C 1 min 30 s for 28 cycles; 72 °C 45 min for 1 cycle. D6S389 was analyzed as described elsewhere [5], but the forward primer was PET labelled.

Both PCR products (triplex and singleplex) were analyzed simultaneously on an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, USA) in the same run. Alleles were assigned by comparison with an allelic ladder mix, which contained reamplified commercially available ladders (Serac, Bad Homburg, Germany) and a sequenced allelic ladder for D6S389 [5]. The Genotyper® software (Applied Biosystems, Foster City, USA) was used to assign allelic designations automatically.

3. Results and discussion

39, 28, 15 and 12 different alleles have been found at the SE33, the D6S389, the D12S391 and the D8S1132 locus, respectively, ranging in size between 233–329 bp (SE33), 261–335 bp (D6S389), 209–253bp (D12S391) and 130–174 bp (D8S1132). Our data were in concordance with former results, obtained after singleplex PCR and native polyacrylamide gel electrophoresis (SE33, $n=146$; D8S1132, $n=342$) or denaturing fragment analysis on an ALF DNA Sequencer (D12S391, $n=146$). Statistic parameters are given in Table 1. No deviation from Hardy–Weinberg equilibrium was observed. Some rare and new SE33 alleles have been found and sequenced (Table 2).

Table 2
Sequence polymorphism of SE33

Allele name	5′-Flanking region				Central region					3′-Flanking region				
	AAAG	AG	AAAG	AG	AAAG	AAAAAG	AAAG	AAAAAG	AAAG	G	AAGG	AAAG	AG	bp
14.2	2	1	0	0	18	0	0	0	0	1	0	3	1	243
15.2	2	1	0	0	19	0	0	0	0	1	0	3	1	247
15.3[a]	2	1	0	0	16	0	0	0	0	1	0	3	1	248
24.2	2	1	3	1	9	1	14	0	0	1	1	2	1	283
31.1	2	1	3	del G	13	1	17	0	0	1	1	2	1	310
33.2	2	1	3	1	15	1	17	0	0	1	1	2	1	319
34.2	1	1	3	1	11	1	23	0	0	1	1	2	1	323
35	2	1	3	1	11	1	10	1	11	1	1	2	1	325

[a] Insertion of G (AAAG)3 in the 3′-flanking region.

4. Conclusion

The combination of these four loci turned out to be highly informative and therefore suitable for identity and paternity testing. In a further study, these four markers were used to establish a "screening test" for paternity cases [6].

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