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Brief Communication

Improvement of a multiplex PCR system for DYS441, DYS442, DYS443, DYS444 and DYS445, and a population study in 340 Japanese males

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Abstract

A multiplex PCR system for five Y-STRs (DYS441, DYS442, DYS443, DYS444 and DYS445) has been improved to increase the probability of obtaining a DNA typing result from aged samples. Newly designed PCR primers for amplification of the DYS441 and DYS442 loci and optimization of PCR conditions enabled successful typing from blood and semen stains that had been stored for more than seven years at room temperature. Analysis of 340 Japanese males revealed 7, 5, 6, 5 and 4 alleles at the DYS441, DYS442, DYS443, DYS444 and DYS445 loci, respectively, yielding 122 haplotypes with a cumulative haplotype diversity of 0.97.

Keywords: Forensic science; DNA typing; DYS441–DYS445; Multiplex polymerase chain reaction; Short tandem repeat

1. Introduction

Y chromosomal STR system is highly helpful for forensic applications such as identification of male DNA in sexual assault cases and paternity testing, particularly in deficiency cases [1,2]. Recently, we identified five novel male-specific and polymorphic tetranucleotide STRs—DYS441, DYS442, DYS443, DYS444 and DYS445 [3,4], and developed a multiplex PCR system consisting of these five STRs that was applicable for forensic and anthropological analyses [5]. However, in certain situations such as cases involving old stains or highly decomposed body remains, it has been difficult to obtain reliable DNA typing results because of the drastically reduced yield of PCR products larger than 350 bp. The multiplex PCR system consisting of these five STRs that we reported in our previous paper was sufficiently applicable to fresh DNA samples, but insufficient for typing from blood and semen stains stored for several years or longer at room temperature. Here we describe an improvement of our multiplex PCR method that is practically applicable to DNA typing from aged samples.

2. Materials and methods

2.1. Samples

Blood and semen samples were collected from healthy unrelated Japanese individuals, after obtaining their informed consent. Blood and semen stains were prepared on clean cotton sheets, air-dried for one day, and stored at room temperature until required for the typing. The amount of blood or semen spotted on the sheets was 16 or

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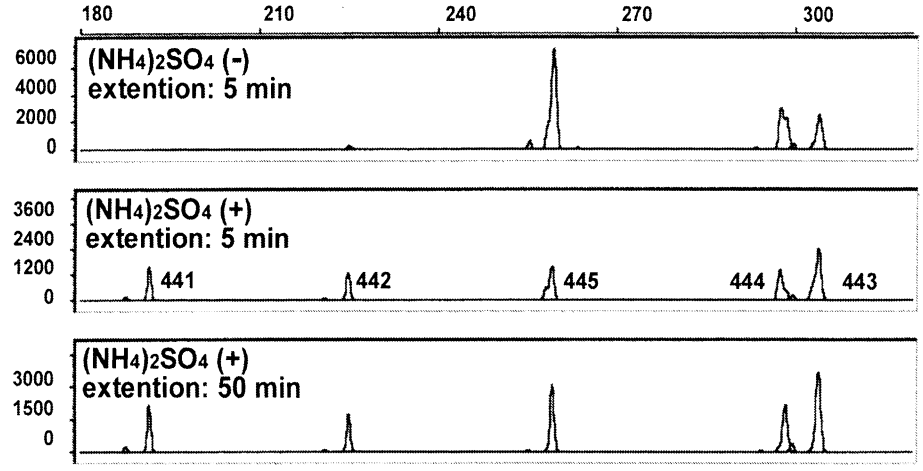

Fig. 1. Fluorescent DNA typing analysis of blood samples (DYS441/DYS442/DYS443/DYS444/DYS445: 15/11/15/12/12) under different PCR conditions.

9 μl/cm^2, respectively. DNA was extracted using a QIAamp DNA Mini Kit (Qiagen, Chatsworth, CA) from 200 μl of whole blood or 2 cm^2 of each stain according to the manufacturer's instructions for whole blood or dried blood spots, except that in the case of extraction from semen stains proteinase K treatment was performed in the presence of 50 mM dithiothreitol. The amount of DNA extracted from each stain was semi-quantitated by ethidium bromide fluorescent quantitation (saran wrap method) [6]. The use of these samples was approved by the Human Ethics Committee of University of Fukui.

2.2. PCR primers

The sequences of the PCR primers for DYS443, DYS444 and DYS445 (DYS443, YRE43S and YRE43A; DYS444, YRE44S and YRE44A; DYS445, YRE45S and YRE45A) were those described in our previous paper [4]. The sequences of the new primers for DYS441 were: 441-S2, 5′-GGCATT GCAGTATTTATTTCCTT-3′ (nt 20120-20142 of GenBank AC004474); 441-A2 5′-GCCTGGGTGACAGAGCAAG-3′ (nt 20304-20286 of GenBank AC004474). Those for DYS442 were: 442-S2, 5′-GCCTCCAATCTCATCCAAGC-3′ (nt 48756–48775 of GenBank AC004810); 442-A2, 5′-GGCATGTTTATAGCAGCACAACT-3′ (nt 48984–48962 of GenBank AC004810). Forward primers for DYS441, 444 and 445 were labeled with 6-FAM, DYS442 and DYS443 with HEX, respectively.

2.3. PCR conditions and analysis of PCR products

From the results of optimization of the PCR conditions, multiplex-PCR for the five Y-STRs was finally performed in a volume of 10 μl in 67 mM Tris–HCl (pH 8.3), 3 mM $MgCl_2$, 0.25 mM dNTPs, 0.16 mg/ml bovine serum albumin, 0.16% (w/v) $(NH_4)_2SO_4$, 0.5 U of AmpliTaq Gold (Applied Biosystems, Foster City, CA), 200 nM 441-S2 and 441-A2, 40 nM 442-S2 and 442-A2, 80 nM YRE43S and YRE43A, 250 nM YRE44S and YRE44A, 190 nM YRE45S and YRE45A, and 0.05–2 ng of genomic DNA, using a GeneAmp PCR System 9700 (Applied Biosystems). Cycling conditions were: 95 °C for 10 min, 32 cycles at 94 °C for 1 min, 64 °C for 1 min, 72 °C for 1 min, and then 60 °C for 50 min. Aliquots of 1.5 μl of the PCR products mixed with 24 μl of deionized formamide and 0.5 μl of GeneScan size standard (GENESCAN-350 ROX) were analyzed with a capillary electrophoresis apparatus (ABI PRISM 310 genetic analyzer, Applied Biosystems) with a 470×0.05 mm capillary and performance optimized polymer 4 (POP-4). Analysis of the data was performed with

Table 1
Typing from blood and semen stains obtained from different male volunteers and stored for various periods at room temperature

Period, no. of samples	Blood			Semen		
	2 years, 3	7 years, 5	12 years, 3	2 weeks, 6	7 years, 6	11 years, 2
DYS441	3[a] (3)[b]	5 (3)	3 (0)	6 (6)	6 (5)	2 (2)
DYS442	3 (3)	5 (3)	3 (0)	6 (6)	6 (4)	2 (2)
DYS443	3 (3)	5 (3)	1 (0)	6 (6)	6 (5)	2 (2)
DYS444	3 (3)	5 (3)	0 (0)	6 (6)	6 (5)	2 (2)
DYS445	3 (3)	5 (3)	3 (0)	6 (6)	6 (6)	2 (2)

[a] The values indicate the number of correct results obtained in this study by our improved method.
[b] The values in parentheses are those obtained by our previous method [5].