J Forensic Sci, September 2003, Vol. 48, No. 5
Paper ID JFS2002379_485
Available online at: www.astm.org

TECHNICAL NOTE

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A Novel Multiplex PCR System Consisting of Y-STRs DYS441, DYS442, DYS443, DYS444, and DYS445*

ABSTRACT: We have developed a new sensitive multiplex PCR system consisting of five male-specific and polymorphic tetranucleotide STRs—DYS441 (GDB: 10013873), DYS442 (GDB: 10030304), DYS443 (GDB: 10807127), DYS444 (GDB: 10807128), and DYS445 (GDB: 10807129) on the Y chromosome. Fifty pg DNA per 10 μL reaction volume was required for the correct typing of five STRs. Using this system, the five Y-STRs were correctly typed from blood and semen stains that had been stored for several years at room temperature.

KEYWORDS: forensic science, DNA typing, DYS441, DYS442, DYS443, DYS444, DYS445, multiplex polymerase chain reaction, short tandem repeat, semen stain

Y-chromosomal STRs are a powerful tool in forensic examination, paternity testing, and evolutionary studies (1,2). Recently, we identified five novel Y-STRs—DYS441, DYS442, DYS443, DYS444, and DYS445 through a search of sequence database information (3,4). The cumulative haplotype diversity of these five Y-STRs was calculated to be 0.95 (4), and therefore it was shown that application of these STRs would provide much additional information for forensic and anthropological analyses. In order to utilize these STRs more availably in real forensic casework such as seminal individualization in cases of sex crime, a sensitive and simultaneous typing method using a single PCR and electrophoretic run has been anticipated. Here, we describe the development of a new reliable multiplex PCR system consisting of these five Y-STRs and the results of typing from blood and semen stains stored for various periods at room temperature.

Materials and Methods

Samples

Blood and semen samples were collected from healthy unrelated Japanese individuals, after obtaining 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 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.

PCR primers

The sequences of the PCR primers for the five STRs (DYS441, YRE41S and YRE41A; DYS442, YRE42S and YRE42A; DYS443, YRE43S and YRE43A; DYS444, YRE44S and YRE44A; DYS445, YRE45S and YRE45A) were those described in our previous papers (3,4). Forward primers for DYS441, 444, and 445 were labeled with 6-FAM, DYS442 with TET and DYS443 with HEX, respectively.

Optimization of PCR conditions and analysis of PCR products

PCR conditions for correct and simultaneous typing of the five STRs were examined. Annealing temperature was varied from 61 to 68°C at intervals of 1°C. Primer concentrations for each of the five STR loci were altered between 0.005 and 0.25 (0.005, 0.025, 0.05, 0.063, 0.083, 0.125, and 0.25) μM. From the results of optimization of PCR conditions, PCR was performed finally in a volume of 10 μL in 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2 mM $MgCl_2$, 800 μM dNTPs, 0.75 U of AmpliTaq Gold (Perkin Elmer-Applied Biosystems, Foster City, CA), 0.25 μM YRE41S, YRE41A, YRE44S, YRE44A, YRE45S and YRE45A, 0.025 μM

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* This work was supported in part by Grants-in-Aid from Japan Society for the Promotion of Science (12670390 to R. I., 11470119 to T. M., 12307011, 14657111 to K. K. and 12770216 to T. Y.).
Received 19 Oct. 2002 and in revised form 22 Dec. 2002, and 29 Mar. 2003, accepted 29 Mar. 2003; published 4 Aug. 2003.

TABLE 1—*Typing using blood stains obtained from different male volunteers and stored for various periods at room temperature.*

Period (No. of Samples)	1 Day (7)	20 Days (7)	2 Years (3)	7 Years (5)	12 Years (3)
DYS441	7*	7	3	3	0
DYS442	7	7	3	3	0
DYS443	7	7	3	3	0
DYS444	7	7	3	3	0
DYS445	7	7	3	3	0

* The values indicate the number of correct results.

TABLE 2—*Typing using semen stains obtained from different male volunteers and stored for various periods at room temperature.*

Period (No. of Samples)	1 Day (6)	2 Weeks (6)	7 Years (6)	11 Years (2)
DYS441	6*	6	5	2
DYS442	6	6	4	2
DYS443	6	6	5	2
DYS444	6	6	5	2
DYS445	6	6	6	2

* The values indicate the number of correct results.

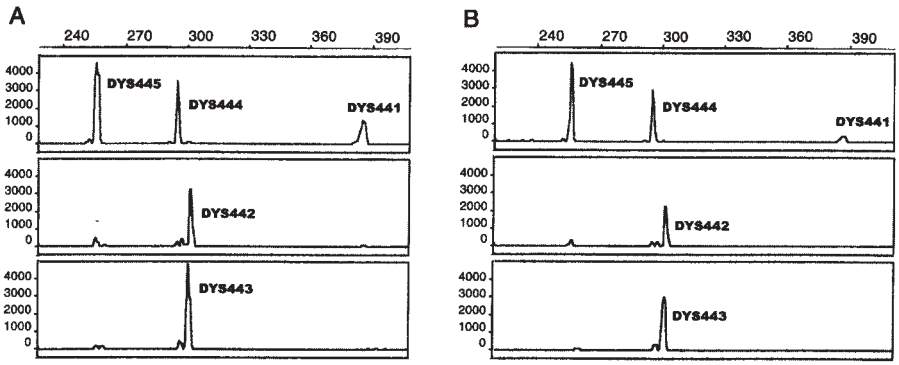

FIG. 1—*Fluorescent DNA typing analysis of semen stain samples (DYS441/DYS442/DYS443/DYS444/DYS445: 14/11/14/11/11) stored for two weeks (A) and seven years (B) at room temperature. Forward primers for DYS441, 444 and 445 were labeled with 6-FAM (top column), DYS442 with TET (middle column) and DYS443 with HEX (bottom column), respectively. The PCR products were run on an ABI PRISM 310 Genetic Analyzer. The results were analyzed using GeneScan analysis software.*

YRE42S and YRE42A, 0.083 μM YRE43S and YRE43A, and 2 μL of genomic DNA (0.05–50 ng), using a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA), and cycling conditions were: 94°C for 15 min, 35 cycles at 94°C for 30 s, 63°C for 30 s, 72°C for 20 s, and then 72°C for 5 min. Capillary electrophoresis using an ABI PRISM 310 genetic analyzer (Applied Biosystems) was carried out as described previously (3) except that GENESCAN-500 TAMRA (Applied Biosystems) was used as a size standard.

Results and Discussion

First, we examined the optimum PCR conditions including annealing temperature and primer concentrations for correct typing of the five STRs. The optimum annealing temperature was determined to be 63°C, because several non-specific peaks appeared at a temperature lower than 62°C and the amplification efficiency of DYS444 and DYS445 decreased markedly at a temperature higher than 64°C. When all five STRs were amplified using the same primer concentrations (0.25 μM), the fluorescent signal intensities of DYS442 and DYS443 were sufficiently higher than those of the rest. Therefore, in order to keep the same levels of intensity, the optimum concentrations of the primers were determined to be 0.025 μM for DYS442, 0.083 μM for DYS443, and 0.25 μM for DYS441, DYS444, and DYS445, respectively. Under the same conditions as those determined above, no signal was detected in any of the DNA samples (10–50 ng) from five different females. When much higher amounts of female DNA (100–250 ng) were used, several non-specific signals were detected, but these did not interfere with the correct typing of male samples because they lay outside the range within which the specific signals appeared. Only 50 pg male DNA per 10 μL reaction volume was required for the correct typing of five STRs, suggesting that this multiplex PCR system is practical and useful for real forensic casework.

We then investigated the possibility of typing from blood or semen stains obtained from different males and stored for an extended period at room temperature. All blood stains stored for two years were typed correctly, but two samples out of five stored for seven years and all samples out of three stored for 13 years were difficult to type because the fluorescent signals were weak or undetectable in all five STRs (Table 1). The amount of each blood stain used for typing was estimated to be a 13–20-mm^2 spot on a cotton sheet (equivalent to 2–3 μL of blood). In the case of semen stains, all stains stored for two weeks were typed correctly, but typing of DYS441, DYS443, and DYS444 was difficult in one sample out of six stored for seven years, and typing of DYS442 was difficult in two samples (Table 2, Fig. 1). The amount of each semen stain used for typing was estimated to be a 3–5-mm^2 spot on a cotton sheet (equivalent to 0.3–0.45 μL of semen). In forensic examination, semen stains are encountered not only in the form of uncontaminated semen but also as mixtures with vaginal secretion. Therefore, this new multiplex PCR system consisting of male-specific STRs is considerably advantageous for seminal individualization in cases of sex crime.

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