A Modified Multiplex PCR System for 13 RM Y-STRs with Separate Amplification of Two Different Repeat Motif Structures in DYF403S1a

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Introduction

Y chromosomal STRs are very useful markers in forensic science and human genetics. However, 17 Y-STRs which are commonly used in the forensic field have low haplotype diversity in some populations and fail to discriminate between relative males. To overcome these limitations, the 13 rapidly mutating (RM) Y-STRs, named due to their high mutation rate, are suggested as revolutionizing new tool that can yield high-resolution paternal lineage differentiation and discriminate between related males. In this study, we constructed two multiplex PCR sets to amplify the 13 RM Y-STRs and determined haplotypes distribution and discriminatory capacity in 400 individuals of Korean. In addition, we investigated sequence structure of DYF403S1 intensively and suggested another classification method according to their sequence structures.

Materials and Methods

DNA samples

Buccal swab samples were collected from 400 unrelated Korean males. Genomic DNA was extracted using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol.

Primer design for PCR amplification

We referred to the previous reports by Ballantyne et al. (Am J Hum Genet. 2010 and Forensic Sci Int Genet. 2012) to obtain the information about 13 RM Y-STRs. Primers for PCR amplification were designed using programs Primer 3 (http://frodo.wi.mit.edu/primer3). In particular, we designed allele-specific primers of DYF403S1 to separately amplify 3 different sequence structures and renamed subtypes into a, b1 and b2.

Multiplex PCR and genotyping

Two multiplex PCR sets were constructed to amplify 13 RM Y-STRs. Multiplex PCRs were performed in a final volume of 10 µl that contained 1 ng of DNA, 1.0 µl of 10× buffer (BioQuest, Seoul, Korea), 2.0 U of DNA Taq polymerase (BioQuest) and appropriate concentrations of primers. The reaction mixture was subjected to an initial denaturation at 95°C for 11 min, followed by 30 cycles of amplification consisting of denaturation at 94°C for 20 s, annealing at 59°C for 90 s, and extension at 72°C for 60 s and final extension at 60°C for 45 min. The PCR products were analyzed by capillary electrophoresis using ABI PRISM 310 Genetic Analyzer and 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA) and genotypes were determined using GeneMapper ID Software versions 3.2 (Applied Biosystems).

Results

Fig. 1. Modified classification of DYF403S1 subtypes by difference in their sequence structures

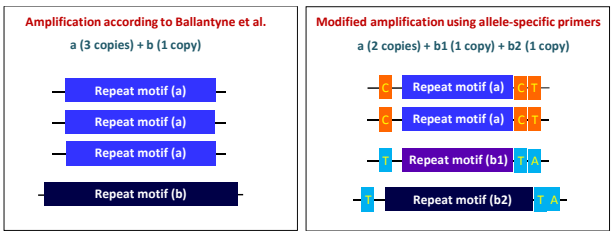
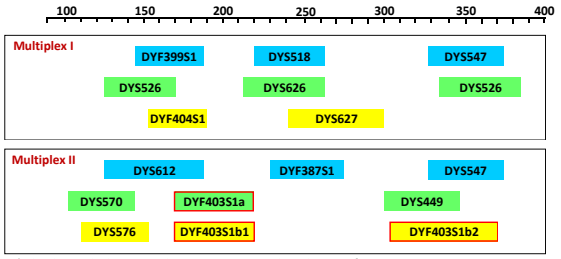


Fig. 2. Schematic of 2 multiplex PCRs for analysis of 13 RM Y-STRs



* The three which are in red squares represent the subtypes of DYF403S1.

Fig. 3. Electropherograms of two multiplex PCRs using 2800M standard DNA

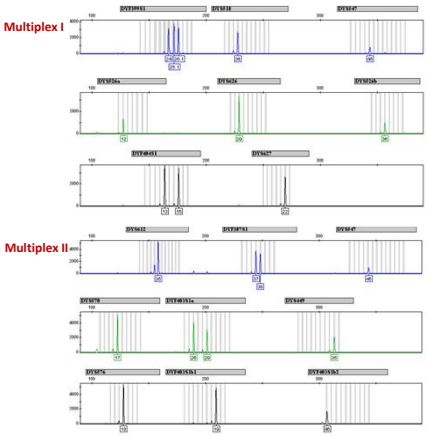
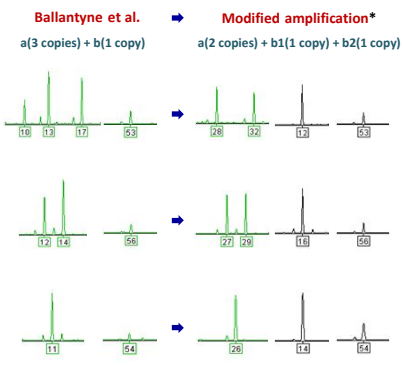


Fig. 4. Comparison of DYF403S1 PCR products by two different amplification methods



* In the modified multiplex PCR, allele nomenclature was revised according to the recommendations of the International Society of Forensic Genetics (ISFG) Commission.

Table 1. Haplotype diversity of each Y-STR multiplex system in 400 Korean males

	No. of haplotypes	No. of unique haplotypes	Haplotype diversity*
13 RM Y-STRs	400	400	1.0000
17 Yfiler Y-STRs	381	367	0.9996

* The following formula was used to calculate haplotype diversity: haplotype diversity = $[n/(n-1) \times (1-\sum p_i^2)]$, where p_i = allele frequency at the *i*th locus.

Table 2. Comparison of haplotype diversity at DYF403S1 in 400 Korean males

Subtypes of DYF403S1	No. of haplotypes	Haplotype diversity
Ballantyne et al.		
a	129	0.9866
b	14	0.9028
a+b	**284**	**0.9971**
Modified amplification		
a+b1	183	0.9923
b2	14	0.9028
a+b1+b2	**305**	**0.9978**

Conclusions

- Using the 13 RM Y-STRs, all of 400 Korean males showed different haplotypes each other, and this outstanding haplotype resolution was caused by high mutation rate of the 13 RM Y-STRs.
- In the modified multiplex PCR system, the alleles of DYF403S1 were separated into three distinct subtypes according to their sequence structures, thereby resulting in more polymorphic genotypes and increased haplotype diversity.
- The modified PCR system will not only facilitate the analysis of 13 RM Y-STRs using two multiplexes, but also increase the discrimination capacity even more by separate amplification of DYF403S1a, DYF403S1b1 and DYF403S1b2.

Acknowledgement

- We referenced the allelic ladder and nomenclature information for RM Y-STRs which were provided by Prof. Manfred Kayser (Eramus MC University Medical Centre Rotterdam, The Netherlands) and the International RM Y-STR Study Group as part of a multicenter study.
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