Forensic Science International: Genetics 26 (2017) 85–90

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Short communication

A multiplex PCR system for 13 RM Y-STRs with separate amplification of two different repeat motif structures in DYF403S1a

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ARTICLE INFO

Article history:
Received 15 April 2016
Received in revised form 28 September 2016
Accepted 26 October 2016
Available online 27 October 2016

Keywords:
Rapidly mutating Y-STR
DYF403S1
Haplotype
Discriminatory power
Koreans

ABSTRACT

In forensic science and human genetics, Y-chromosomal short tandem repeats (Y-STRs) have been used as very useful markers. Recently, more Y-STR markers have been analyzed to enhance the resolution power in haplotype analysis, and 13 rapidly mutating (RM) Y-STRs have been suggested as revolutionary tools that can widen Y-chromosomal application from paternal lineage differentiation to male individualization. We have constructed two multiplex PCR sets for the amplification of 13 RM Y-STRs, which yield small-sized amplicons (<400 bp) and a more balanced PCR efficiency with minimum PCR cycling. In particular, with the developed multiplex PCR system, we could separate three copies of DYF403S1a into two copies of DYF403S1a and one of DYF403S1b1. This is because DYF403S1b1 possesses distinguishable sequences from DYF403S1a at both the front and rear flanking regions of the repeat motif; therefore, the locus could be separately amplified using sequence-specific primers. In addition, the other copy, defined as DYF403S1b by Ballantyne et al., was renamed DYF403S1b2 because of its similar flanking region sequence to DYF403S1b1. By redefining DYF403S1 with the developed multiplex system, all genotypes of four copies could be successfully typed and more diverse haplotypes were obtained. We analyzed haplotype distributions in 705 Korean males based on four different Y-STR subsets: Yfiler, PowerPlex Y23, Yfiler Plus, and RM Y-STRs. All haplotypes obtained from RM Y-STRs were the most diverse and showed strong discriminatory power in Korean population.

1. Introduction

Y-chromosomal short tandem repeats (Y-STRs) are useful markers in forensic genetics, genealogical, and evolutionary study [1–3]. Such Y-STRs are suitable for identifying male DNA profiles from admixed stain samples obtained from crime scenes, and are especially useful for cases of sexual assault. However, the Y-STRs generally used in forensic sciences show low haplotype diversity in some populations and cannot distinguish between closely or distantly related males [4]. A recently identified set of 13 rapidly mutating (RM) Y-STRs (DYF387S1, DYF399S1, DYF403S1, DYF404S1, DYS449, DYS518, DYS526, DYS547, DYS570, DYS576, DYS612, DYS626, and DYS627) provides a substantially higher discrimination between haplotypes than other commercially available Y-STR sets and enables differentiation between closely and distantly related males [5,6]. Actually, a worldwide population study have reported that 742 of 2528 male pairs (29.0%) related by one to 20 generations could be differentiated by RM Y-STR set [6]. Another report on Italian father-son pairs was in line with the previous study [6] in that 85 of 422 pairs (20.1%) could be discriminated by RM Y-STRs set [7]. In a study with Korean father-son pairs, three RM Y-STRs, DYS449, DYS576, and DYS627 showed far higher mutation rates (above 1%) than other commonly used Y-STRs [8]. Recently, an improved empirical evidence was provided in support of differentiating closely related men, most notably beyond father-sons; 705 of 1568 pairs (45.0%) related by 1–6 meioses were differentiated by at least one of RM Y-STRs in a study with 572 Pakistani males [9].

A very large-scale international study into RM Y-STR was performed by worldwide collaboration among 52 laboratories and confirmed the differentiating potential for the RM Y-STR set in 14,644 related and unrelated males from 111 worldwide populations; however, some groups have concerns regarding the quality control of DYF403S1 with its typing using multiplex PCR designed by Ballantyne et al. [6]. Among the 13 RM Y-STRs,

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http://dx.doi.org/10.1016/j.fsigen.2016.10.019
1872-4973/

DYF403S1 has the most complex sequence structure and comprises four copies. Three of these four copies belong to subtype a, and their amplification in the multiplex system resulted in an overlap in fragment size on capillary electrophoresis [6]. The presence of partial (0.1, 0.2, and 0.3) alleles was a challenge for interpretation, even when formed using a single source; however, no other multiplex, including those that have been recently reported, could adequately address this issue [7,9,10].

In this study, we constructed two multiplex PCR sets to amplify 13 RM Y-STRs and investigated the DYF403S1 sequence structure. We confirmed the genotypes for these markers with 705 samples from Korean males. Further, based on the observed sequence structure, we suggested revised allele nomenclature for DYF403S1. Finally, we determined the haplotype distributions and discriminatory capacities for four Y-STR panels (13 RM Y-STR, Yfiler Plus loci, PPY23 loci, and Yfiler loci) in a Korean population.

2. Materials and methods

2.1. DNA samples

The study was approved by the institutional review board of Severance Hospital, Yonsei University, Seoul, Korea. We analyzed 705 DNA samples of unrelated Korean males. These samples had been completely typed for 17 Yfiler loci included in the AmpFlSTR® Yfiler® PCR Amplification Kit (Applied Biosystems, Foster City, CA,

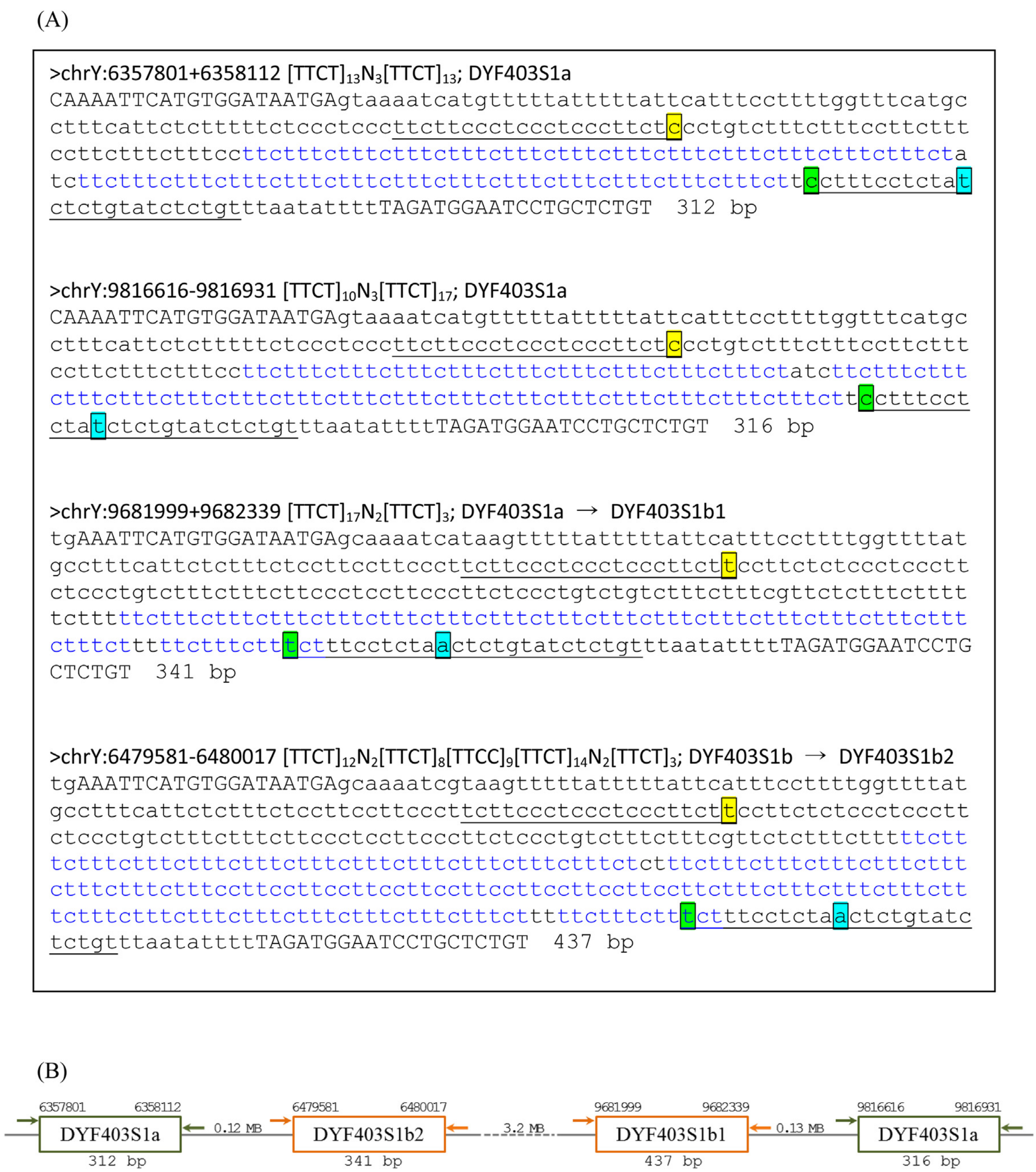

Fig. 1. Different repeat motif structures of DYF403S1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
(A) All sequences were obtained from UCSC *in silico* PCR program based on Human GRCh38/hg38. Two fragments of 341 bp and 437 bp were renamed as DYF403S1b1 and DYF403S1b2, respectively. The capitals indicate primer sites reported in an earlier study [6], and repeat motifs are marked in blue letters. The newly designed primer sites are underlined and the sequence differences between multiple copies are marked in colored boxes.
(B) Relative location and primer binding position of each copy of the DYF403S1 marker on the Y chromosome