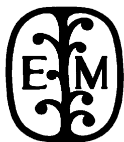

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Optimisation of Y-STR multiplexing combining established and newly described loci

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Abstract

In order to extend Y databases for forensic and anthropological applications, some recently described markers were selected to be co-amplified with established ones in a multiplex reaction. The markers chosen were DYS391/434/437/439, with non-overlapping size ranges for the alleles of each marker, allowing its use in both monochromatic and polychromatic platforms. For the design of this tetraplex, new primers were used and several technical conditions were assayed. This newly constructed tetraplex has proved to be very useful in population genetic studies and forensic casework.
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Keywords: Y chromosome; STRs; PCR multiplex

1. Introduction

Much attention is being paid to the determination of human Y-STR polymorphisms for anthropological studies, as well as for paternity testing and forensic casework. Until now, only a few Y-STR loci have been studied and only seven of them fulfil the criteria necessary for application in both forensic and population genetics [1]. The validation of more Y-STRs would increase the discriminating power of the Y-haplotypes. Recently, primers for a series of Y-specific STRs have been developed and tested [2,3]. In this paper, we describe the

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optimisation of a Y chromosome STR tetraplex which includes some of these novel markers (DYS434/437/439) and an established one (DYS391), which can be useful for extending Y databases with a fast and low-cost method.

2. Material and methods

PCR was performed using the primers described in the literature [3,4], in a 25-μl reaction volume containing 5–50 ng of genomic DNA, 1.5 mM $MgCl_2$, 200 μM of dNTPs, 1 × Gold buffer (Applied Biosystems, AB) and 0.5 U of *Taq* Gold Polymerase (AB). Concentrations were 0.08 μM for DYS391 and 434, 0.06 μM for DYS437 and 0.3 μM for DYS439. PCR cycling conditions consisted in a pre-incubation for 11 min at 95 °C, followed by 30 cycles of 30 s at 94 °C, 30 s at 60 °C, 45 s at 70 °C, and then a final incubation step of 60 °C for 45 min. Detection of the amplified products was carried out using an ABI Prism 310 (AB) and fragment sizes was determined automatically using the GeneScan Analysis 3.1 Software (AB).

For each system, a ladder was constructed with previously sequenced alleles. For DYS391, DYS434 and DYS437 the ladder was reamplified using 2 μl of a 1:10.000 dilution of the original ladder; for DYS439 the ladder was reamplified using 2 μl of a 1:2000 dilution of the original ladder. For DYS391, DYS434, DYS437 and DYS439, PCR amplification was performed in a 50-μl reaction volume containing 1.5 mM $MgCl_2$, 200 μM of dNTPs, buffer, 2 U *Taq* DNA polymerase (in the case of DYS391 Taq Gold (AB) was used). Primer concentrations were 0.3 μM for DYS434, DYS437 and DYS439 and 0.1 μM for DYS391. Cycling conditions: pre-incubation for 5 min at 95 °C, followed by 30–32 cycles of 30 s at 95 °C, 30 s at 60 °C, 1:30 min at 72 °C, and then a final incubation step of 72 °C for 10 min. For DYS391, the final incubation step was at 72 °C for 30 min.

3. Results and discussion

The combination of DYS391/434/437/439 and the use of the reverse primer for DYS391 designed by Gusmão et al. [4], which amplifies a smaller and male-specific PCR product, allow its use in both monochromatic and polychromatic platforms. In the case of DYS391 a double peak was observed, probably due to a tendency for non-template adenine nucleotide addition. The problem was easily solved by changing the dye label to the reverse primer. The DYS391 ladder had to be reamplified with Taq Gold. An additional peak was also observed in the range of 360–370 bp (Fig. 1), which can be explained by the presence of a sequence complementary to the reverse primer of DYS437 located between DYS434 and DYS437 stretches, giving rise to an amplification product with the forward primer of DYS434 and the reverse primer of DYS437. However, this unspecific product does not lead to typing errors.

The best strategy in constructing the tetraplex ladder consisted in the reamplification and mixture of each locus specific ladder.

The tetraplex described here proved to be a useful tool for the analysis of a large number of samples in population studies and for forensic casework, since there was no amplification in females and we were able to genotype the male portion in mixed stains. Moreover, this

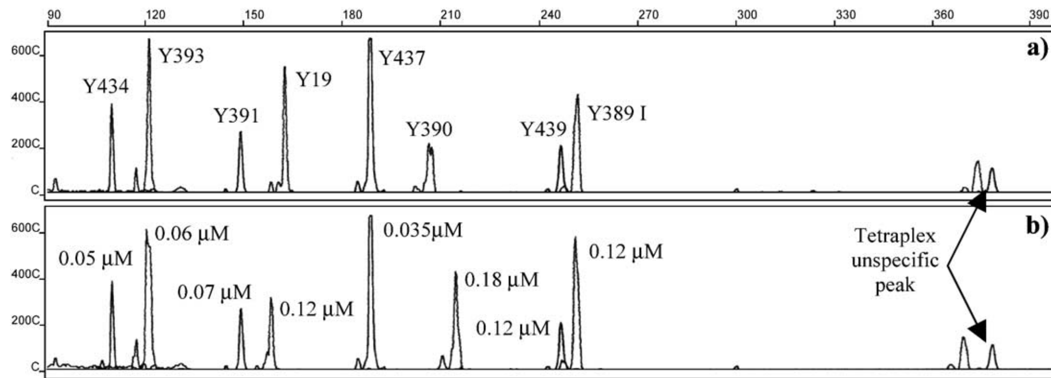

Fig. 1. Tetraplex + pentaplex co-amplification in two male individuals. (a) Y-STR markers and (b) primer concentrations. DYS19 reverse primer is modified from Szibor et al. (2000), having the following sequence: GGG TTA AGG AGA GTG TCA CTA. Annealing temperature was 58 °C.

tetraplex can be co-amplified with a multiplex composed by the markers DYS19/389I and II/390/393, modified from a previous one developed by Gusmão et al. [5] in order to avoid overlap between the alleles of all markers. For that reason a new reverse primer for DYS19 (modified from Szibor et al. [6]) was used (Fig. 1).

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