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A new duplex PCR system for YCAII and DXYS156Y microsatellites analysis

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Abstract

The Y-chromosome polymorphisms represent an important tool in forensic genetics for different purposes: paternity testing, special problems of medical genetics, such as incest causing autosomal recessive diseases, analysis of mixed stains and other biological samples in criminal events. The number of Y-chromosome polymorphisms is huge, but only a well-defined number of them are used in official protocols of the international forensic genetics community. Recently, we presented a definitive haplotype of Y-STR in 107 unrelated individuals living in a Tuscany population (Central Italy) [Forensic Sci. Int. 120 (2001) 210]. To study this "minimal haplotype" we used the markers DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS385I/II [Forensic Sci. Int. 114 (2000) 31]. Subsequently, we introduced in our laboratory the use of an automated infrared DNA sequencer (LI-COR 4200). We applied this technology to study the hypervariable locus YCAII [Hum. Mol. Genet. 3 (1994) 115] for the "extended haplotype" and the polymorphism DXYS156 [Int. J. Legal Med. 110 (1997) 125], suggested by Kayser et al. [Hum. Biol. 70 (1998) 979]. We set up a new duplex PCR system for the simultaneous analysis of these loci with the automatic technology. This system was employed to obtain frequency data in the Tuscany population and to solve paternity dispute in which STR mutations were detected. A preliminary sensitivity study was also performed to evaluate the efficiency of this new duplex for stains analysis.

Keywords: Y-STR polymorphism; YCAII; DXYS156Y

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1. Introduction

The aim of this study is to present a new duplex amplification system for the pentanucleotide STR DXYS156 [5] and dinucleotideYCA-II marker [3]. The locus DXYS156 consists of two homologous microsatellite loci, mapping to the long arm of the X chromosome (DXYS156X) and to the short arm of the Y chromosome (DXYS156Y). The STR YCAII is a highly informative two-locus system. We used an infrared monolaser Automated DNA Sequencer (LICOR-4200) to detect the alleles. The forward primers were labelled with a new infrared molecule (IRDye™ 800, LICOR). Since this automatic detection system detects only one fluorocrome, its necessary that the PCR's fragments do not overlap in size.

A new primer pair for DXYS156 was then selected (GenBank AF257078), which efficiently amplifies this locus with the dinucleotideYCA-II marker [1]. The annealing temperature and PCR conditions were similar for the two STRs.

2. Materials and methods

2.1. DNA extraction

DNA samples were obtained from unrelated healthy males, referred to our Centre for genetic consultation. DNA was extracted by using Puregene® system (Gentra). For the sensitivity study, we used diluted samples of K562 DNA control.

2.2. Primer selection

DXYS156 original sequence was obtained from GenBank (Accession X71600). The new primer pair (GenBank AF257078) was the following:

Forward primer: 5′ ATATACTCAAATCAGTAGCA 3′
Reverse primer: 5′ TTTATAATTTCCTTGTAGTG 3′

This primer pair was selected considering the same annealing temperature for YCAII marker. The size of the DXYS156 amplicon (203–218 bp) did not overlap with YCAII amplified fragments (147–165 bp). The amplification products were detected by using a LI-COR Model 4200 monolaser automated DNA sequencer (LI-COR, Lincoln, NE) [6].

Because the automated DNA Sequencer uses high sensitivity infrared fluorescence technology, as previously mentioned, the forward primer of each primer set was labeled with an infrared molecule IRDye™ 800 supplied by MWG Biotech, Germany.

2.3. Fragment amplification of the STRs

PCR amplification was performed in a total volume of 12.5 μl: reaction buffer (75 mM of Tris–HCl pH 9.0 at 25 °C, 20 mM $(NH_4)_2SO_4$, 0.01%Tween), 1.5 mM $MgCl_2$, 200 mM of dNTPs, 0.2 μM of DXYS156F/R and 0.8 μM of YCAIIF/R. The amount of DNA

was 1–3 ng for each reaction and 0.25 U of Red Hot Thermostable Polymerase (Advanced Biotechnologies) was added to each tube with the hot-start method.

The amplifications condition were: 95 °C for 2 min for the initial denaturation, then 94 °C for 1 min, 45 °C for 1 min, 72 °C for 1 min, for 30 cycles, with a final extension at 72 °C for 10 min. At the end of the amplification reaction, 6 μl of stop solution (95% formamide, 10 mM EDTA, 0.1% blu bromofenol) was added to each sample. PCR fragments were separated in a 6%, 33-cm-long denaturing polyacrylamide gel, using the LI-COR® automated DNA Sequencer, 1.5 μl of each denatured sample was loaded with a Hamilton syringe by using a 48-sharkstooth comb. The gel was run at constant power of 31.5 W, with a constant temperature of 50 ± 1 °C. The image file was completed 2–3 h after sample loading.

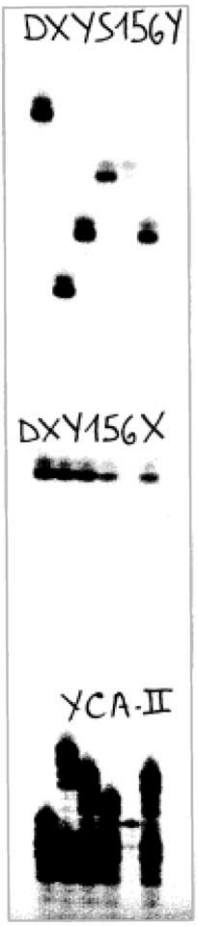

Fig. 1. Autoradiogram-like representation of the results obtained using the new duplex system for DXYS156 and YCAII.

3. Results

Fig. 1 shows an example of the results obtained using the new duplex system for DXYS156 and YCAII, detected with the LI-COR® Sequencer. The result of electrophoresis appears in a familiar autoradiogram-like image that is automatically registered as a TIF image in the computer. The size of the alleles was established by comparison with molecular ladder using "Gene ImageIR 4.00" software, which permits each Y-system to be analyzed separately.

DXYS156 primers amplify also a shorter polymorphic fragment derived from the X-chromosome (DXYS156X).

A sensitivity study with decreasingly diluted DNA samples confirmed the ability of the system to amplify as little as 10 pg of undegraded DNA. This data suggests the possibility of forensic applications of this new duplex Y-STR amplification system [2,4]. Moreover, this duplex system can be used also in manual electrophoretic apparatus, since the PCR-fragments do not overlap in size.

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