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A pentaplex PCR assay for the genetic analysis of ChrX short tandem repeat (STR) loci

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Abstract. The paper describes the development of a multiplex PCR assay suitable for the simultaneous amplification of 5 X-chromosomal STR loci. © 2003 Published by Elsevier B.V.

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1. Introduction

So far, X-chromosomal short tandem repeat markers (ChrX-STRs) have played a minor role in forensic genetics. However, under certain circumstances, Chr X typing can complement the analysis of autosomal and Chr Y markers, especially in complicated kinship cases. Thus, this paper aims at introducing the development of a rapid pentaplex PCR assay suitable for fluorescent detection.

2. Methods

Recently, Athanasiadou et al. [1] have described a quadruplex PCR assay, which encompasses X-chromosomal loci STRX1, DXS101, HPRTB, and DXS8377. The recruitment of locus DXS6789 [2] to that multiplex required several modifications, which are outlined below.

2.1. PCR conditions

PCR is performed in a 10-μl volume containing 2 ng target DNA, 2 pmol of primers for loci DXS6789, STRX-1, and HPRTB, 1.7 pmol of DXS8377 primer, 3 pmol of DXS101 primer, 1 μl 10× Accuprime™ Buffer II (Invitrogen, Carlsbad, CA, U.S.A.), 0.25 μl Accuprime™ Taq DNA Polymerase, and 1 μMol dNTPs. Primer sequences and dye-labellings are compiled in Table 1.

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Table 1
Primer sequences of investigated ChrX STR systems

System	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)
STRX-1	6-FAM-GTTTCCTCCTgCAAAATACAgC	TCCAgCACCCAAggAAgTC
DXS6789	6-FAM-TgTCCTATTgTATTAgTCAgggATC	ATgTAAgTTggTACTTAATAAACCCTC
DXS101	NED-ACTCTAAATCAgTCCAAATATCTCCCTT	CAAATCACTCCATggCACATgTAT
HPRTB	NED-ATgCCACAgATAATACACATCCCC	CTCTCCAgAATAGTTAGATgTAgg
DXS8377	VIC-ACCACTTCATggCTTACCACAg	TATggACCTTTggAAAgCTAg

2.2. Touchdown PCR

Intitial denaturation at 95 °C for 8 min, followed by six cycles for 1 min at 94 °C, 61–58.5 °C for 1 min, respectively, lowering the annealing temperature by 0.5 °C per cycle, followed by 24 cycles at 94, 58, and 72 °C, respectively, for 1 min each, an extension at 72 °C for 1 h and a final extension at 60 °C for 14 h. This last step was undertaken to solve the ±A (adenine) problem (Malik and Bern, personal communication.). Phenotyped DNA samples kindly provided by P. M. Schneider (Mainz) and R. Szibor (Magdeburg) enabled us to construct the respective allelic ladders.

3. Results

The performance of our pentaplex is illustrated by the respective allelic ladders, which are depicted in Fig. 1. The allele designations are based on proposals from previous publications [3–5].

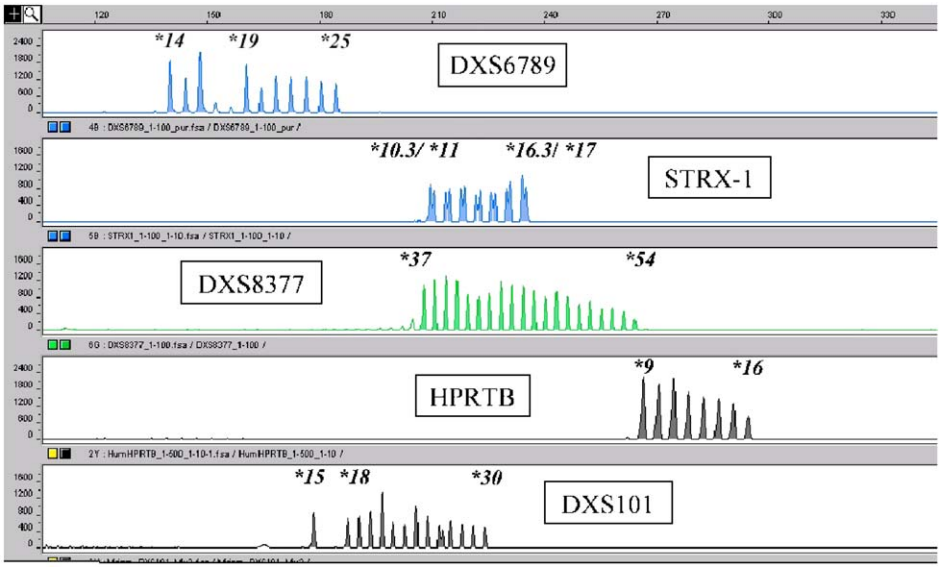

Fig. 1. Allelic ladders of ChrX pentaplex assay.

4. Discussion

After numerous efforts, we were able to adjust the experimental conditions to forensic demands and the above-described pentaplex assay is delivering reproducible typing results. Reviewing the arguments regarding the meaningful application of X-chromosomal STRs to forensic genetics [6], it can be emphasized that their employment is almost exclusively sensible in deficient paternity cases.

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