

Development of a heptaplex PCR system to analyse X-chromosome STR loci from five Italian population samples. A collaborative study

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Abstract. A heptaplex PCR has been developed to amplify DXS6789, HUMARA, DXS10011, DXS7423, HPRTB, DXS6807 and DXS101 on Italian samples from Bologna, Modena, Padova, Ancona and Pisa. Statistical analyses were performed for all the loci. © 2003 Elsevier B.V. All rights reserved.

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1. Introduction

The aim of this study was to increase the population data for ChrX STRs allele frequencies using a new heptaplex PCR including DXS6789, HUMARA, DXS10011, DXS7423, HPRTB, DXS6807 and DXS101 chosen because of their informativeness and their localization along the ChrX.

2. Materials and methods

DNA was extracted from 556 Italian samples: 101 unrelated native individuals from Bologna, 121 from Modena, 100 from Padova, 74 from Ancona and 160 from Pisa by QIAampDNABlood MiniKits (Qiagen, Germany). PCR was performed in 12.5 µl:10 ng

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Table 1

STR loci with chromosomal locations, fragment sizes, primer concentrations, fluorescent dye labels, and references used in this study

System	Location	Fragment sizes (bp)	Primer concentration (μM)	Dye	Reference
DXS6789	Xq22.3	154–198	0.2	5-FAM	[5]
HUMARA	Xcen-q13	204–273	0.2	5-FAM	[6]
DXS10011	Xq27-28	137–257	1.0	JOE	[7]
HPRTB	Xq26	147–179	0.08	NED	[8]
DXS7423	Xq27-28	185–197	0.16	NED	[9]
DXS6807	Xpter-Xp22.2	251–275	0.24	NED	[10]
DXS101	Xq21.33-Xq22.3	179–233	0.4	ROX	[11]

DNA, $1 \times$ PCR buffer with 1.5 mM MgCl_2 , 150 μM each dNTP, 1 U AmpliTaq DNA Polymerase (Applied Biosystems, USA). Primer sequence references, concentrations and dye-labeling are listed in Table 1. The temperature profile was 1 min at 94 °C, 1 min at 58 °C and 1 min at 72 °C for 33 cycles and a final extension of 10 min at 72 °C. The samples were detected by using an ABI Prism 310 Genetic Analyzer (Applied Biosystems). Polymorphism information content (PIC) [1], heterozygosity (Het) [2], power of discrimination (PD) in males and females and power of exclusion (PE) [3] were performed; Hardy–Weinberg equilibrium (HWE) was tested following the likelihood ratio test criterion [4].

3. Results

The seven ChrX STRs were conveniently amplified under the above reported conditions (Fig. 1) except for DXS10011 in the Pisa sample. No differences in the allelic distribution patterns for male and female individuals were found. Observed genotype frequencies in females conformed to Hardy–Weinberg expectations, except for DXS7423 ($p = 0.02$). The statistical parameters PIC, Het, PD^{F} , PD^{M} and PE for each locus are listed in Table 2.

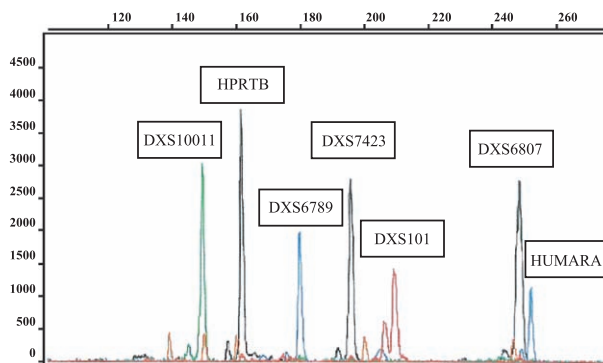


Fig. 1. Electropherogram of K562 heptaplex PCR products.

Table 2

Forensic efficiency of seven ChrX markers in a sample of 268 females and 288 males

	DXS6789	HUMARA	DXS10011 ^a	HPRTB	DXS7423	DXS6807	DXS101
PIC	0.69	0.88	0.93	0.73	0.64	0.59	0.87
PD ^F	0.891	0.979	0.992	0.91	0.852	0.818	0.973
PD ^M	0.744	0.883	0.944	0.787	0.701	0.566	0.885
Het	0.729	0.892	0.937	0.768	0.695	0.645	0.877
PE	0.57	0.782	0.892	0.625	0.501	0.373	0.788
P	0.056	0.999	0.998	0.783	0.02 ^a	0.838	0.64

^a Sample size: 188 females and 208 males.

4. Discussion

Nowadays intense efforts of research are addressed to ChrX to increase the number of markers and to quantify linkage disequilibrium between them. Our heptaplex PCR represents a convenient procedure to type more ChrX STRs in a single reaction. The DXS10011 proved difficult to amplify and needed more primer concentration and DNA template for typeable results. In addition, the HUMARA locus amplification was more difficult than others and frequently showed stutter bands of $n - 1$ repeat units high up to 20% the true band. In this study, a new allele for DXS101 (allele 14) in Modena sample and a new inter-allele (allele 19.2) for DXS10011 in the samples of Bologna and Ancona were found. The statistical analysis showed similar allele frequencies in males and females, Het ranging from 0.6451 for DXS6807 to 0.9370 for DXS10011. As expected DXS10011, HUMARA and DXS101 showed a similar very high PIC. Linkage disequilibrium studies are now in progress for a powerful ChrX haplotyping.

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