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Analysis of 12 X-chromosomal short tandem repeats in the North-West Italian population by means of two multiplex PCRs

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Abstract. Twelve X-chromosomal short tandem repeat (STR) markers were typed by means of two multiplex PCR systems. Multiplex I included DXS6789, HumARA, GATA172D05, DXS101, DXS8378, and DXS8377; multiplex II comprised DXS7132, DXS6800, DXS6803, DXS7424, HPRTB, and DXS10011. Allelic frequencies for these loci were determined in a North-West Italian population sample ($n = 140$; 70 females and 70 males). © 2005 Published by Elsevier B.V.

Keywords: X chromosome; STR; Multiplex PCR; Italian

1. Introduction

The analysis of short tandem repeats (STRs) located on the X chromosome can effectively complement autosomal STR data in paternity testing of female children (deficiency cases and cases involving close blood relatives as alternative alleged fathers) and maternity testing of male children. The use of X-STRs in forensic practice requires a precise knowledge of their population genetics properties. At present, X-STR population data available in the literature are still limited, and only a few multiplex PCR systems for rapid and efficient typing of these loci have been developed. In this study, 12 STRs were analysed in an Italian population sample by means of two multiplex PCRs.

2. Materials and methods

Buccal swabs were collected from 140 unrelated Italians (70 females and 70 males) residing in Piedmont (North-West Italy). Genomic DNA was isolated by means of spin

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Table 1
Characteristics of multiplex PCR systems I and II

	STR	Primer sequences		μM	Label	bp
Multiplex I	DXS6789	5′ gttggtacttaataaaccctcttt	5′ aagaagttatttgatgtcctattgt	0.5	FAM	154–198
	HumARA	5′ tccagaatctgttccagagcgtgc	5′ gctgtgaaggttgctgttcctcat	0.5	FAM	231–307
	GATA172D05	5′ tagtggtgatggttgcacag	5′ ataattgaaagcccggattc	0.5	JOE	108–136
	DXS101	5′ actctaaatcagtccaaatatct	5′ aaatcactccatggcacatgtat	1	JOE	179–233
	DXS8378	5′ cacaggaggttttgacctgtt	5′ acaagaacgaaactccaactc	0.5	TAMRA	163–187
	DXS8377	5′ cacttcatggcttaccacag	5′ gacctttggaaagctagtgt	0.5	TAMRA	204–261
Multiplex II	DXS7132	5′ gagcccattttaataaatcc	5′ gccaaactctattagtcaac	0.5	FAM	131–155
	DXS6800	5′ gtgggaccttgtgattgtgt	5′ ctggctgacacttagggaaa	0.25	FAM	194–218
	DXS6803	5′ gaaatgtgctttgacaggaa	5′ caaaaagggacatatgctactt	0.1	JOE	105–128
	DXS7424	5′ ctgcttgagtccaggaattcaa	5′ gaacacgcacatttgagaacata	2	JOE	147–180
	HPRTB	5′ atgccacagataatacacatcccc	5′ ctctccagaatagttagatgtagg	0.25	JOE	259–303
	DXS10011	5′ ggagtgaactctgaaaaaaaa	5′ tgaaatcatctatctttctttc	1	TAMRA	137–257

columns (Macherey–Nagel). X-STRs included in the multiplex PCR assays, primer sequences, concentrations and dye-labelling are listed in Table 1. PCRs were performed in a 25 μl volume containing 1 ng of template DNA, 10 mM Tris–HCl, 50 mM KCl, 1.5 or 2 mM $MgCl_2$ (in multiplex I and II, respectively), 100 μM each dNTP, and 2.5 U of AmpliTaq Gold polymerase (Applied Biosystems). PCR protocols consisted of a 10 min pre-PCR heat step at 95 °C, followed by 32 cycles of denaturation at 95 °C for 1 min, annealing at 57 °C (multiplex I) or 53 °C (multiplex II) for 45 s and extension at 72 °C for 45 s, with a final 60 min extension step at 72 °C. Typing was done on ABI PRISM 310

Table 2
Combined allelic frequencies of X-STR loci in the NW Italian population and *P*-values (*P*) for Hardy–Weinberg equilibrium (standard error ± 0.001)

Allele	DXS6789	HumARA	GATA 172D05	DXS101	DXS8378	DXS7132	DXS6800	DXS6803	DXS7424	HPRTB	Allele	DXS8377	Allele	DXS10011
6			0.210								41	0.005	26	0.005
8		0.005	0.200							0.005	42	0.033	27	0.005
9			0.048		0.010			0.033	0.005	0.014	43	0.043	28	0.010
10			0.319		0.314			0.267	0.010	0.014	44	0.071	28.2	0.005
1.3								0.019			45	0.100	29	0.010
11			0.133		0.400	0.005		0.267		0.162	46	0.095	30	0.014
11.3								0.143			47	0.124	3.2	0.014
12		0.010	0.090		0.262	0.119		0.114	0.029	0.314	48	0.090	31	0.019
12.3								0.143			49	0.110	31.2	0.057
13			0.087		0.014	0.276			0.048	0.262	50	0.100	32	0.014
13.3								0.014			51	0.100	32.2	0.086
14						0.371			0.167	0.181	52	0.038	33	0.024
15	0.028			0.029		0.176	0.005		0.310	0.033	53	0.048	33.2	0.024
16	0.014	0.005		0.010		0.038	0.357		0.357	0.014	54	0.019	34	0.086
17		0.010		0.005		0.014	0.043		0.067		55	0.019	34.2	0.024
18		0.048		0.095			0.105				56	0.005	35	0.100
19	0.009	0.100		0.033			0.305		0.010				35.2	0.005
20	0.412	0.119		0.043			0.033						36	0.071
21	0.289	0.167		0.057			0.114						36.2	0.005
22	0.161	0.071		0.024			0.038						37	0.062
23	0.062	0.152		0.048									38	0.086
24	0.024	0.095		0.171									39	0.095
25		0.086		0.167									40	0.043
26		0.071		0.152									41	0.033
27		0.024		0.076									42	0.029
28		0.014		0.067									43	0.019
29		0.010		0.024									44	0.029
30		0.005											45	0.014
31		0.005											46	0.005
32		0.005											47	0.010
P	0.632	0.852	0.464	0.130	0.502	0.186	0.156	0.534	0.363	0.675		0.229		0.150

Genetic Analyzer in comparison to sequenced allelic ladders and control DNA from K562 and NA9947A cell lines (Promega) [1]. Allelic designation was according to ISFH recommendations [2]. Arlequin Software version 2.000 was used to perform: exact test for Hardy–Weinberg equilibrium in the female subsample; exact test of population differentiation between male and female allele frequencies; exact test for linkage disequilibrium between all pair of markers in the male subsample; exact test of population differentiation between the North-West Italian sample and other Italian population data from the literature.

3. Results and discussion

No significant differences in the allelic frequencies of female and male samples were found by exact test. Therefore, combined frequencies are given in Table 2. All loci were found to be in Hardy–Weinberg equilibrium in the female subsample. Inter-marker linkage disequilibrium analysis in the male subsample showed significant linkage disequilibrium ($P = 0.0002 \pm 0.00004$) between HumARA and DXS7132, the two markers with the shortest pairwise genetic distance (4.5 cM) among the 12 analysed by multiplex PCRs I and II [3].

No statistically significant differences were observed, when comparing by exact test of population differentiation North-West Italians and other Italian population samples previously described in the literature [4–6].

At locus DXS6789, a male individual displayed a biallelic genotype (22,23), suggesting the occurrence of a segment duplication in Xq21 followed by a mutational change in the number of repeats within the STR locus.

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