International Congress Series 1288 (2006) 292–294

www.ics-elsevier.com

Allele distribution of 6 X-chromosome STR loci in an Italian population sample

A. Coletti *, L. Lottanti, M. Lancia, G. Margiotta, E. Carnevali, M. Bacci

Section of Legal Medicine, University of Perugia, Italy

Abstract. ChrX markers represent an efficient supplementation of autosomal and Y-chromosomal STR analysis and ChrX haplotyping can elucidate complicated kinship situations. This is the reason why it needs to increase the population data for ChrX STR allelic frequencies and to create national or local databases. An esaplex PCR was developed to amplify DXS6789, HumARA, DXS7423, DXS6807, DXS101 and DXS8377 in some Italian samples from Perugia and Terni. This system represents a protocol for the ChrX analysis with a shorter procedure. The DNA was extracted from 100 blood samples using the QIAmp DNA Minikit produced by Qiagen. The samples were detected on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems), using the same dye labels, run conditions, standard (GeneScan 500 Liz) and matrix file of AmpFlSTRIdentifiler. We performed statistical analysis for all the loci. © 2005 Published by Elsevier B.V.

Keywords: X-chromosome; STR; Population data

1. Introduction

X-chromosome (ChrX) STR loci have been proven to be useful tool for paternity testing, especially in some cases as specific paternity deficiency and complex kinship. The X-STRs were recently recognized as important tools in forensic application, particularly in complex cases of kinship testing.

The aim of this study was to increase the population data for ChrX STRs allele frequencies using a new esaplex in a population sample living in Umbria a region in the middle of Italy.

2. Materials and methods

The analysis was carried out using a protocol for the ChrX with a short procedure. DNA was collected from 100 Umbrian unrelated native individuals: 50 from Perugia and

* Corresponding author. Tel.: +39 744 205055; fax: +39 744 220236.
E-mail address: alessiocoletti@libero.it (A. Coletti).

0531-5131/
doi:10.1016/j.ics.2005.10.025

Table 1
STR loci with chromosomal locations, fragment size, fluorescent dye labels and primer sequencies

System	Primer sequence	Location	Fragment size (bp)	Dye
DXS6789	GTTGGTACTTAATAAACCCTCTT AAGAAGTTATTTGATGTCCTATTGT	Xq22.3	154–198	VIC
HumARA	TCCAGAATCTGTTCCAGAGCGTGC GCTGTGAAGGTTGCTGTTCCTCAT	Xcen-q13	258–312	VIC
DXS101	ACTCTAAATCAGTCCAAATATCT AAATCACTCCATGGCACATGTAT	Xq21.33–Xq22.3	200–227	NED
DXS7423	TAGCTTAGCGCCTGGCACATA GTCTTCCTGTCATCTCCCAAC	Xq27–28	175–199	FAM
DXS6807	GAGCAATGATCTCATTTGCA AAGTAACATGTATAGGAAAAAGCT	Xpter–Xp22.2	248–273	FAM
DXS8377	CACTTCATGGCTTACCACAG GACCTTTGGAAAGCTAGTGT	Xq28	216–270	PET

50 from Terni, extraction was carried out by QIAmpDNAMiniKits (Qiagen) according to the manufacturer's protocols.

PCR amplifications were performed using QIAGEN multiplex PCR Kit in 12,5 μl containing 3 ng of genomic DNA. Primers sequences, dye-labeling are listed in Table 1.

Resulting PCR products were resolved and detected by capillary electrophoresis in the ABI PRISM 310 Genetic Analyzer (Applied Biosystems).

After several pilot experiments to optimise the amplification of all markers in the same reaction tube, was prepared a reaction mix containing appropriate amounts of esch primer (DXS6789, HumARA, DXS7423 and DXS6807 0,16 μM; DXS101 0,26 μM; DXS8377 0,21 μM).

PCR protocol, consisting of initial denaturation step at 95° for 15 min, followed by 33 cycles with denaturation at 94° for 30 sec, annealing at 58,5° for 90 sec and extension at 72° for 1 min; followed by a final extension at 72° for 60 min. We analyzed genotype profiles on DNAs from two established cell lines 9947A (Applied Biosystems, USA) and K562 (Promega, USA) as recommendation of Szibor et al. [1].

Alleles were assigned according to the recommendations of the International Society of Forensic Genetics (ISFG) Commission [2].

Table 2
Allele frequency distribution in female, male and total

DXS6789				DXS7423				DXS6807			
Allele	F %	M %	T %	Allele	F %	M %	T %	Allele	F %	M %	T %
15	6.3	5.4	5.92	13	5.2	3.6	4.61	11	61.5	44.6	55.26
16	2.1	0.0	1.32	14	33.3	26.8	30.92	12	3.1	3.6	3.29
17	0.0	0.0	0.0	15	30.2	48.2	36.84	13	0.0	1.8	0.66
18	0.0	0.0	0.0	16	27.1	17.9	23.68	14	16.7	35.7	23.68
19	5.2	3.6	4.61	17	4.2	3.6	3.95	15	16.7	10.7	14.47
20	34.4	32.1	33.55					16	1.0	3.6	1.97
21	25.0	35.7	28.95					17	1.0	0.0	0.66
22	18.8	7.1	14.47								
23	6.3	12.5	8.55								
24	2.1	3.6	2.63								

Table 3
Allele frequency distribution in female, male and total

DXS101				HumARA				DXS8377			
Allele	F %	M %	T %	Allele	F %	M %	T %	Allele	F %	M %	T %
15	4.2	7.1	5.26	10	0.0	1.8	0.66	40	1.0	0.0	0.66
16	0.0	0.0	0.0	18	3.1	5.4	3.95	41	3.1	0.0	1.97
17	3.1	0.0	1.97	19	7.3	5.4	6.58	42	0.0	5.4	1.97
18	20.8	12.5	17.8	20	12.5	7.1	10.5	43	7.3	1.8	5.26
19	5.2	3.6	4.6	21	36.5	17.9	29.6	44	8.3	8.9	8.55
20	5.2	0.0	3.3	22	8.3	12.5	9.9	45	4.2	3.6	3.95
21	2.1	3.6	2.6	23	11.5	16.1	13.16	46	4.2	14.3	7.89
22	1.0	7.1	3.3	24	6.3	12.5	8.55	47	10.4	10.7	10.53
23	5.2	3.6	4.61	25	9.4	10.7	9.87	48	14.6	7.1	11.84
24	15.6	21.4	17.76	26	2.1	7.1	3.95	49	9.4	16.1	11.84
25	14.6	10.7	13.16	27	3.1	0.0	1.97	50	11.5	7.1	9.87
26	6.3	14.3	9.2	28	0.0	1.8	0.66	51	11.5	7.1	9.87
27	10.4	8.9	9.87	29	0.0	0.0	0.0	52	3.1	7.1	4.61
28	3.1	3.6	3.29	30	0.0	0.0	0.0	53	6.3	3.6	5.26
29	2.1	3.6	2.63	31	0.0	1.8	0.66	54	2.1	0.0	1.32
30	1.0	0.0	0.66					55	1.0	5.4	2.63
								56	2.1	1.8	1.97

Table 4
Statistical parameters for the 6 STR loci in female population

	DXS6789	DXS7423	DXS6807	DXS101	HumARA	DXS8377
Het %	76.8	72.0	47.9	86.5	80.5	74.7
Ho %	68.6	79.2	52.7	87.5	60.4	77.1
PIC	0.74	0.67	0.52	0.87	0.79	0.90
PD	0.905	0.839	0.759	0.96	0.918	0.970
PE	0.409	0.584	0.206	0.745	0.296	0.546

3. Results and discussion

For each locus was calculated allele frequency and was evaluated Hardy–Weinberg equilibrium[3], we did not find significant deviations from Hardy–Weinberg equilibrium for each individual marker ($p > 0{,}05$).

Tables 2 and 3 show the allelic classes for every STR and his respective frequencies in male and female population. The allelic range changes among the 5 alleles that presents DXS7423 and the 17 alleles detected in case of DXS8377.

Frequencies were similar to those reported in other Italian populations, however, overall larger population studies are needed to confirm this issue.

Table 4 shows some statistical values calculated using Software PowerStats v 2.1 [4]: Expected Heterozygosity (Het), Observed Heterozygosity (Ho), Polymorphic Information Content (PIC), Power of Discrimination (PD) and Power of Exclusion (PE).

References

[1] R. Szibor, et al., Cell line DNA typing in forensic genetics—the necessity of reliable standards, Forensic Sci. Int. 138 (2003) 37–43.
[2] W. Bar, et al., DNA recommendations. Further report of the DNA Commission of the ISFH regarding the use of short tandem repeat system. International Society for Forensic Haemogenetics, Int. J. Leg. Med. 110 175–176.
[3] S.W. Guo, et al., Performing the exact test of Hardy–Weinberg proportions for multiple alleles, Biometrics 48 (1992) 361–372.
[4] PowerStats v.1.2.