International Congress Series 1288 (2006) 313–315

www.ics-elsevier.com

A multiplex PCR design for simultaneous genotyping of X chromosome short tandem repeat markers

I. Gomes [a,b,*], A. Carracedo [b], A. Amorim [a,c], L. Gusmão [a]

[a] *IPATIMUP, Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Portugal*
[b] *Unidad de Genética Forense, Instituto de Medicina Legal, Universidad de Santiago de Compostela, Spain*
[c] *Faculdade de Ciências da Universidade do Porto, Portugal*

Abstract. A tetraplex system for the X chromosome genetic markers DXS7423, DXS101, DXS8377 and HPRTB (human phosphoribosyl transferase) was optimized in a single PCR reaction. These short tandem repeat (STR) markers were typed for 65 individuals (29 female and 36 male samples) from a Galician population sample (Northern Spain). Allele frequencies were estimated for all loci. Optimization of STR multiplexes is a practical and simple method to obtain large amounts of information important in forensic and population genetic studies, therefore, in this context, they should be the tool of approach for X-STR studies.

Keywords: X chromosome; Short tandem repeat; DXS7423; DXS101; DXS8377; HPRTB

1. Introduction

The human X chromosome (ChrX) has so far played a minor role in forensic and in population studies, but the application of ChrX markers in these fields is now accomplishing detailed attention and new doors are opening in population and forensic research [1]. Analysis of ChrX short tandem repeat markers (STRs) can successfully embrace the answer that unravels the challenge presented in particular cases of kinship analysis, when the offspring is female [2]. One of the implications of population studies on ChrX markers is to evaluate informative power of loci, useful for forensic practice and also to address questions regarding our genetic history. The distinctive properties of inheritance

* Corresponding author. IPATIMUP, Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Portugal. Tel.: +351 22 5570700; fax: +351 22 5570799.
E-mail address: igomes@ipatimup.pt (I. Gomes).

0531-5131/
doi:10.1016/j.ics.2005.09.124

of the X chromosome are responsible for its importance in population genetic studies. Only one copy in males, allows effortless haplotype reconstruction and on the other hand, the presence of recombination in females, divides genetic information in each generation, so different regions have different histories [3].

2. Material and methods

A total of 65 individuals (29 female and 36 male samples) from a Galician population sample were typed for the genetic markers DXS7423, DXS101, DXS8377 and HPRTB (human phosphoribosyl transferase). A tetraplex system was optimized in a single PCR reaction using fluorochrome-labelled primers. Primer sequences were selected according to published studies: DXS7423 and DXS8377 [4], DXS101 and HPRTB [5]. The final reaction mix contained 0.2 μM of DXS101, DXS8377 and HPRTB primers and 0.12 μM of DXS7423 primers, 1.5 mM $MgCl_2$, 200 μM dNTPs and 1 U Taq Polymerase (AmpliTaq gold, Applied Biosystems; Foster City, California) in a final volume of 25 μl. Thermocycling conditions used in this work were the following: initial denaturation at 95 °C for 11 min; 10 cycles: 94 °C for 30 s, 60 °C for 30 s, 72 °C for 45 s; 20 cycles: 90 °C for 30 s, 58 °C for 30 s, 72 °C for 45 s and final extension at 60 °C for 60 min. For capillary electrophoresis system, ABI 310 (AB Applied Biosystems) was used and fragment sizes were analyzed with software Genescan 2.1 Analysis. Allele assignment was performed according to control DNA samples 9947A (female) and 9948 (male) from Promega (Madison, WI) [6]. Allelic frequencies and standard errors were calculated using software ARLEQUIN ver 2.000 [7].

3. Results and discussion

Optimization of the tetraplex PCR reaction of DXS7423, DXS101, DXS8377 and HPRTB genetic markers was effectively achieved using conditions previously described. Consistent results of allele assignment were observed in the DNA control samples and nomenclature of allele designation was based on previous studies [4–6]. Locus DXS7423 revealed 5 alleles with size ranging 175–191 bps followed by 8 alleles observed for the HPRTB locus, with fragment lengths 267–295 bps (Table 1). For STR DXS101 fragment sizes ranged from 187 to 223 bps, revealing 12 alleles and for DXS8377, 17 alleles were found, corresponding to sizes 211–262 bps (Table 2). Allele frequencies were estimated for all loci. Following analysis of individual frequency results, male and female

Table 1
DXS7423 and HPRTB loci, respective allele frequencies and standard errors (S.E.)

DXS7423		HPRTB	
Allele	Frequency ± S.E.	Allele	Frequency ± S.E.
13	0.085 ± 0.027	9	0.011 ± 0.011
14	0.223 ± 0.044	10	0.011 ± 0.010
15	0.489 ± 0.051	11	0.149 ± 0.037
16	0.191 ± 0.041	12	0.340 ± 0.049
17	0.011 ± 0.011	13	0.245 ± 0.046
		14	0.149 ± 0.036
		15	0.074 ± 0.027
		16	0.021 ± 0.015

Table 2
DXS8377 and DXS101 loci, respective allele frequencies and standard errors (S.E.)

DXS8377		DXS101	
Allele	Frequency ± S.E.	Allele	Frequency ± S.E.
40	0.011 ± 0.011	17	0.011 ± 0.012
41	0.021 ± 0.015	18	0.064 ± 0.025
42	0.043 ± 0.021	19	0.032 ± 0.019
43	0.053 ± 0.023	21	0.032 ± 0.017
44	0.064 ± 0.025	22	0.032 ± 0.018
46	0.096 ± 0.031	23	0.096 ± 0.029
47	0.074 ± 0.027	24	0.223 ± 0.043
48	0.096 ± 0.030	25	0.170 ± 0.039
49	0.096 ± 0.030	26	0.149 ± 0.035
50	0.096 ± 0.030	27	0.117 ± 0.031
51	0.085 ± 0.028	28	0.053 ± 0.023
52	0.064 ± 0.025	29	0.021 ± 0.015
53	0.096 ± 0.031		
54	0.032 ± 0.019		
55	0.021 ± 0.015		
56	0.021 ± 0.016		
57	0.032 ± 0.018		

frequencies were combined into the same group, since no significant differences were observed (Tables 1 and 2). Our data were compared to those obtained in German population studies [5,8] and as expected, revealed similar results.

The simultaneous typing of ChrX markers for population and forensic studies is a practical and simple method to obtain large amounts of information as proven in many other studies using autosomal and Y-chromosomal markers [9]. With the recent interest demonstrated by the scientific community in ChrX STRs (particularly in forensic and population studies) it is noteworthy that PCR multiplexes should continue to be optimized and considered for large amount of microsattelite genotyping.

References

[1] Szibor, R. Hering, S. Edelmann, J. A new Web site compiling forensic chromosome X research is now online. Int. J. Leg. Med. (in press).
[2] R. Szibor, et al., Chromosome X haplotyping in deficiency paternity testing principles and case report, Int. Congr. Ser. 1239 (2003) 815–820.
[3] S.F. Schaffner, The X chromosome in population genetics, Nat. Rev., Genet. 5 (2004) 43–51.
[4] J. Edelmann, et al., Sequence variation and allele nomenclature for the X-linked STRs DXS9895, DXS8378, DXS7132, DXS6800, DXS7133, GATA172D05, DXS7423 and DXS8377, Forensic Sci. Int. 129 (2002) 99–103.
[5] D. Athanasiadou, et al., Development of a quadruplex PCR system for the genetic analysis of X-chromosomal STR loci, Int. Congr. Ser. 1239 (2003) 311–314.
[6] R. Szibor, et al., Cell line DNA typing in forensic genetics: the necessity of reliable standards, Forensic Sci. Int. 138 (2003) 37–43.
[7] S. Schneider, D. Roessli, L. Excoffier, Arlequin ver. 2.000: a software for population genetics data analysis, Genetics and Biometry Laboratory, University of Geneve, Switzerland, 2000.
[8] U. Edelmann, 16 X-chromosome STR loci frequency data from a German population, Forensic Sci. Int. 124 (2001) 215–218.
[9] L. Gusmão, C. Alves, Y chromosome STR typing, Methods Mol. Biol., vol. 297, Humana Press, 2004, pp. 67–82.