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SHORT COMMUNICATION

M. Gené · E. Piqué · N. Borrego · A. Carracedo
E. Huguet · P. Moreno

Catalonian population study of the tetranucleotide repeat loci D3S1358, D8S1179, D18S51 and D19S253

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Abstract Allele frequencies for four short tandem repeat loci were determined in a population sample from Catalonia (NE Spain). After denaturing PAGE electrophoresis, 8 alleles were identified for D3S1358 (n = 201), 10 alleles for D8S1179 (n = 198), 13 alleles for D18S51 (n = 197) and 11 alleles for D19S253 (n = 201). No deviation from Hardy-Weinberg equilibrium was found. Complete and relative uniformity in Caucasoid populations has been observed for D18S51 and D8S1179 respectively. Pronounced differences were found between different ethnic groups for both systems. Catalonia and Portugal do not differ for D3S1358 locus. Multiplex PCR amplifications of three loci (D3S1358, D18S51 and D19S253) without overlapping fragment size ranges could be interesting for monochrome automated laser fluorescence devices.

Key words D3S1358 · D8S1179 · D18S51 · D19S253 · Triplex PCR amplification · Forensic genetics

Introduction

A multiplex STR system with three loci (D3S1358, D18S51 and D19S253) has been developed, which combined with the D8S1179 singleplex reaction can be performed and studied in a single electrophoretic lane using a monochrome automated laser fluorescent sequencer (ALF Pharmacia) due to absence of overlapping size ranges. A population sample from people living in Catalonia (N.E. Spain) has been analyzed with this method.

M. Gené (✉) · E. Piqué · N. Borrego · E. Huguet
Forensic Genetics Laboratory. Department of Legal Medicine, Faculty of Medicine, University of Barcelona, C. Casanova 143, E-08036 Barcelona, Spain
e-mail: mgene@medicina.ub.es.
Fax +34 (3) 403 5260

A.Carracedo
Institute of Legal Medicine, Faculty of Medicine, E-15705 Santiago de Compostela, Galicia, Spain

P. Moreno
Department of Animal Biology, Section of Anthropology, Faculty of Biology, University of Barcelona, Diagonal 645, E-08028 Barcelona, Spain

Material and methods

DNA was extracted from blood samples from healthy unrelated individuals living in Catalonia using the phenol-chloroform-isoamyl alcohol method [1]. Triplex amplification of D3S1358, D18S51 and D19S253 was achieved using primers (Table 1) described by Li et al. [2], Straub et al. [3] and Weber et al. [4] respectively. Hot start PCR reactions were carried out in a 20 µl volume containing 5 ng template DNA, 200 µM each dNTP and 8 pmol (D18S51 and D19S253) or 15 pmol (D3S1358) of each primer, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.3 mM $MgCl_2$ and 0.8 units of AmpliTaq Gold polymerase. Temperature conditions were: after 11 min at 95 °C (hot start and denaturing step), samples were processed through 28 cycles consisting of 1 min at 94 °C, 1 min at 59 °C and 1 min at 72 °C. The last elongation step was 30 min at 60 °C.

Singleplex amplification of D8S1179 was achieved using primers (Table 1) (CHLC, accession nr. 374). The PCR reaction was carried out in a 20 µl volume containing 5 ng template DNA, 200 µM each dNTP, 12 pmols of each primer, 50 mM KCl, 10 mM Tris (pH 9), 1.3 mM $MgCl_2$, 0.1% Triton X-100 and 0.5 units of Taq DNA polymerase. Temperature conditions were: after 1 min 30 s at 95 °C denaturing step, samples were processed through 32 cycles consisting of 1 min at 95 °C, 1 min at 59 °C and 1 min 20 s at 72 °C. The last elongation step was extended to 6 min 20 s at 72 °C.

All reactions together with negative and positive control samples were performed in MJ200 (triplex reactions) and Perkin Elmer PE1 (singleplex reactions) thermocyclers.

Table 1 Primer sequences

Locus	
D3S1358	5′-F-ACTGCAGTCCAATCTGGGT-3′
	5′-ATGAAATCAACAGAGGTCTG-3′
D8S1179	5′-F-TTTTTGTATTTCATGTGTACATTCG-3′
	5′-CGTAGCTATAATTAGTTCATTTTC-3′
D18S51	5′-F-CAAACCCGACTACCAGCAAC-3′
	5′-GAGCCATGTTCATGCCACTG-3′
D19S253	5′-F-ATAGACAGACAGACGGACTG-3′
	5′-GGGAGTGGAGATTACCCCT-3′

Table 2 Allele frequency distribution for D3S1358, D8S1179, D18S51 and D19S253 in a Catalonian population and statistical parameters for the four STR systems

Allele	D3S1358 n = 201	D8S1179 n = 198	D18S51 n = 197	D19S253 n = 201
3				0.00249
5				0.26866
7				0.03234
8		0.01515		
9		0.02020		0.00995
10		0.06565	0.01015	
11		0.07070	0.01776	0.03483
12	0.00249	0.11868	0.15482	
13	0.00249	0.29545	0.11421	0.10448
14	0.09204	0.21464	0.15736	
15	0.26368	0.17424	0.17258	0.29104
16	0.25373	0.02020	0.13959	
17	0.17164	0.00505	0.10659	0.19652
18	0.18905		0.06345	0.00249
19	0.02488		0.03807	0.05224
20			0.01776	
21			0.00507	0.00498
22			0.0025	
HWE: Exact-Test	P = 0.2773	P = 0.2443	P = 0.1441	P = 0.2032

System	h	PD	CE
D3S1358	0.7918	0.9245	0.5879
D8S1179	0.8118	0.9401	0.6336
D18S51	0.8713	0.9697	0.7395
D19S253	0.7885	0.9245	0.5907

h: Heterozygosity value
PD: power of discrimination
CE: chance of exclusion

Separation of the PCR products was carried out on 6% (w/v acrylamide/bisacrylamide) polyacrylamide denaturing high-performance DNA sequencing gels (Ready Mix Gel ALF grade, Pharmacia). All PCR products were typed twice. The electrophoresis was carried out on the Automated Laser Fluorescent (ALF) DNA sequencer (Pharmacia) at 1450 V, 38 mA and 50 °C with laser power at 3 mW for 220 min.

Amplified DNA was mixed with internal fluorescently labeled size standards (100 and 250 bp, Pharmacia) and external lane ladders (50–500 bp, Pharmacia) were also used for adjustment. Sequenced allelic ladders were used for each system as recommended by the DNA Commission of the International Society for Forensic Haemogenetics [5, 6].

Possible divergence from Hardy-Weinberg equilibrium (HWE) was determined by calculating the exact test proposed by Guo and Thompson [7]. From a forensic point of view, the power of discrimination (PD) [8], heterozygosity value (h) [9] and the "a priori" chance exclusion value (CE) [10] were calculated. The Catalonian data were compared with Spanish and other populations using a R × C contingency table χ^2-test for homogeneity [11].

Results and discussion

Allele frequencies for the four STR systems obtained in the Catalonian population sample are shown in Table 2.

For D3S1358 a total of 22 different genotypes and 8 alleles were observed in 201 individuals. For D8S1179 there were 32 genotypes and 10 alleles observed in 198 individuals. A total of 47 genotypes from 13 alleles were observed for D18S51 locus in 197 individuals and 28 genotypes and 11 alleles were observed in 201 individuals typed at the D19S253 locus. The distribution of the genotypes at all four loci were in Hardy-Weinberg equilibrium.

Polymorphisms obtained in Catalonia were compared with other populations. For D3S1358 no differences were observed between Catalonia and Portugal [12], the only comparative population found for the D3S1358 system (χ^2 = 5.354, df = 5, P = 0.3742).

Data obtained in Catalonia for D8S1179 and D18S51 were compared with French Caucasians [13] and three British ethnic groups (British Caucasian, Afro-Caribbeans and Asians) [14]. From comparisons (Tables 3 and 4) it can be deduced that the Caucasian population is more uniform for the D18S51 system than D8S1179. From the rest of comparisons it can be deduced that for the two loci there is heterogeneity among the different ethnic groups. No comparative samples have been found for D19S253 system.

Table 3 Comparison of allele frequencies for different populations for the D8S1179 system

	Catalonia	French population	British Caucasians	Afro Caribbeans
French population [13]	P = 0.0073			
British Caucasians [14]	P = 9.949E-04*	P = 0.6529		
Afro-Caribbeans [14]	P = 3.641E-04*	P = 4.394E-07*	P = 4.486E-07*	
Asians [14]	P = 8.331E-08*	P = 8.062E-07*	P = 4.486E-07*	P = 4.670E-10*

* Significative differences

Table 4 Comparison of allele frequencies for different populations for the D18S51 system

	Catalonia	French population	British Caucasians	Afro Caribbeans
French population [13]	P = 0.6311			
British Caucasians [14]	P = 08894	P = 0.9193		
Afro-Caribbeans [14]	P = 3.885E-09*	P = 3.661E-07*	P = 1.450E-12*	
Asians [14]	P = 3.781E-04*	P = 3.571E-06*	P = 1.344E-07*	P = 4.486E-07*

* Significative differences

From a forensic point of view, theoretical values were calculated from gene frequencies obtained in our population (Table 2). The observed heterozygosity and the power of discrimination reveal that the four combined systems have a high forensic efficiency.

From a technical point of view triplex PCR amplification of the three loci is easy to perform and gives a good amplification signal with small amounts of DNA template, so we consider that it could be used in a monochrome automated laser fluorescence sequencer.

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