HAPLOTYPE FREQUENCIES OF TWO STRs OF THE CHROMOSOME 8q: D8S344 AND D8S323

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At the Region I of chromosome 8q, two synthenic tetranucleotide repeats, D8S323 (Lu 1993) and D8S344 (Ward 1993), are adscribed. In this work, a diplex amplification of both STRs is described, and the technical conditions in a population study are applied. On the basis of allele and haplotype frequencies, the utility of both systems in human genetic profiling is discused.

MATERIALS AND METHODS

DNA was extracted from 209 individuals from Galician population (NW Spain) by using the standard phenol-clhoroform (Maniatis 1982) or chelating resins (Singer-Sam 1989).

The sequences of the primers were as discused (Lu 1993) and (Ward 1993) for D8S344 and D8S323 respectively:

D8S344	1: 5' -CCA CCT TCC TGT CCA GTC GCA AG -3'
	2: 5' -AAA CAA AAA TAG CTG GGC ATG GTG A -3'
D8S323	1: 5' -CAC CAC TAC ACT CCA GCC TGT AA -3'
	2: 5' -ACT CTT ACA TTC CCA CAC CCC CAT A -3'

The PCR reactions parameters, in a final volume of 12.5 μL, consisted of: 20-50 ng of DNA, 200 μM dNTPs, 0.5 μM each primer, 2.5 mM $MgCl_2$, the reaction buffer (200 mM Tris-HCl pH 8.4, 500 mM KCl), 0.325 units of Taq DNA Polymerase (Gibco BRL) specifically bound to a monoclonal antibody anti Taq DNA polymerase. Initial denaturation was at 95°C for 5 min, followed by 30 cycles of 94°C (30 s), 59°C (30 s), 72°C (1 min).

Electrophoretic separation of amplify samples, was performed on polyacrylamide native gels (6% T, 5% C, 0.4 mm thickness and 19 cm long). 375 mM Tris-HCl pH 8.8 was used as gel buffer and 125 mM Tris-Glycine pH 8.8 for the bridge. Electrophoresis was accomplished in horizontal plates at constant V= 300 for 2 h at 18°C. Alleles were visualised using the silver staining method (Budowle 1991).

RESULTS AND DISCUSSION

Phenotype patterns diplex amplification of D8S323 and D8S344 are displayed in Fig. 1. Given that the alleles of both STRs differ between each other around 100 bp, a co-migration without overlapping is perfectly feasible. However, special attention has to be paid to the size of the pore, length of gel and temperature of electrophoretic running. With regard to PCR co-amplification, we have observed that the most critical parameter lies in the temperature of annealing; so, higher temperatures than 59°C lead us to non-specific amplification bands for D8S344, and lower temperatures than 57°C produce a decrease in the efficiency of amplification for D8S323 (Fig. 2). Even so, the use of monoclonal antibodies to Taq DNA polymerase (Kellogg 1994) is advisable in order to clarify the banding pattern.

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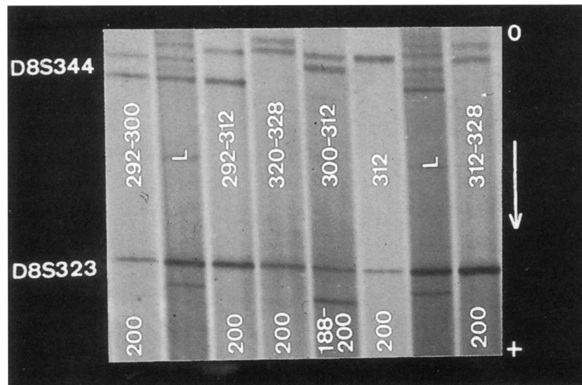

Figure 1. Electrophoretic patterns of the diplex amplification. Ladder consists of alleles of 192, 200, 292, 296, 300, 320, 328 bp.

An initial survey in the Galician population displays 8 alleles for D8S344 which configure values of H= 0.621 and PIC= 0.590. With regard to D8S323, up to 4 alleles were found and values of unbiased H= 0.449 and PIC= 0.352. Allele frequencies for both markers are shown in Table 1. No significant statistical departures from the Hardy-Weinberg proportions were observed either loci. Given that both loci are found in the same chromosome region (8q Region I), a genetic characterization in terms of haplotype frequencies would be feasible. Notwithstanding, no significant evidence of association of phenotypes for both STRs (p> 0.10), were observed (Table 2), which allows us to consider each system independently when carrrying out the statistical calculations.

Table 1. D8S344 and D8S323 allele frequencies in 209 unrealated Galicians.

D8S344		D8S323	
Size (bp)	Frequency ± s.e.	Size (bp)	Frequency ± s.e.
328 bp	0.0167 ± 0.0062		
324 bp	0.0694 ± 0.0124		
320 bp	0.0598 ± 0.0116	200 bp	0.6651 ± 0.0231
312 bp	0.5789 ± 0.0241	196 bp	0.0024 ± 0.0024
308 bp	0.0120 ± 0.0053	192 bp	0.3301 ± 0.0230
300 bp	0.1531 ± 0.0176	188 bp	0.0024 ± 0.0024
296 bp	0.0024 ± 0.0024		
292 bp	0.1077 ± 0.0152		
H= 0.621	PIC= 0.590	H= 0.449	PIC= 0.392

In conclusion, multiplexing of D8S344 and D8S323 allows for a clear diagnosis of the phenotype patterns of both STRs. The relatively good relation between the degree of polymorphism and the number of alleles displayed, expresses the interest of the use of these systems in genetic characterization studies.

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Table 2.Estimated haplotype frequencies according to the maximum-likelihood procedure outlined by Hill (1974). The most common allele of each marker was treated as one allele, and the remaining ones were combined (AC). D= linkage disequelibrium coefficient, D′= percentage of maximal disequilibrium, χ^2_G= the goodness-of-fit statistic.

A4-A1	A4-AC	AC-A1	AC-AC
0.4137	0.1652	0.2514	0.1697

D= 0.0287
D′= 14.8 %
χ^2_G= 3.17
p> 0.10

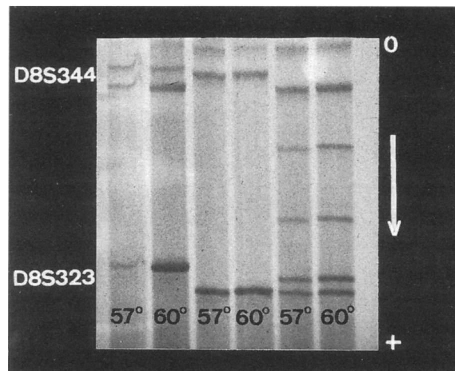

Figure 2. Effect of the annealing temperature (°C) in the efficiency of the amplification.

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