POLYMORPHISM OF THE D1S80 LOCUS IN BASQUE COUNTRY POPULATION AND ITS FORENSIC APPLICATIONS

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INTRODUCTION

The D1S80 locus is a VNTR region, whose moderate repetition (units of 16 bp repeated in tandem 15-40 times) allows its analysis after amplification by the polymerase chain reaction (Kasai et al. 1990) and its genotyping after fractionation of the resulting fragments in high resolution PAGE followed by silver staining (Budowle et al. 1991). The possibility of analyzing this locus by PCR makes it a useful tool for the analysis of forensic samples which provide degraded or very low quantities of DNA. Moreover, the D1S80 locus displays a high number of different alleles in all the populations studied, and due to its high power of discrimination, this locus is becoming a widely used system in genetic identification testing. So, in order to develope a simple system of amplification of the D1S80 locus, we have developed a new protocol using a micro thermal cycler, its advantages consisting of a reduction in time and costs. On the other hand, a study of the allele distribution of the D1S80 locus in a sample of 150 individuals residing in the Basque Country has been carried out using our procedure. We can also see that this technique is applicable to DNA samples obtained by different extraction procedures (phenol-chloroform, Chelex resin, rapid digestion) as well as from different sources (blood, blood or semen stains, hair roots).

METHODOLOGY

Extraction of DNA from different samples

Whole blood samples were obtained by venipuncture from 150 healthy and unrelated individuals resident in the Basque Country. DNA was extracted using the phenol-chloroform method. The DNA from blood or semen stains was extracted with Chelex resin (Jung et al. 1991). With the aim of increasing the concentration of the DNA extracted, the following modifications were made: a 30% stock solution of Chelex resin was prepared and 30 µl of this were added to the sample. The DNA from hair roots was extracted by rapid digestion.

Amplification

4 µl of the DNA (100ng) extracted using the phenol-chloroform method or 4 µl of each sample extracted by the above mentioned methods were amplified with 6µl of the reaction mix containing 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 200 µM of each dNTP, 1µM of each primer (Budowle et al. 1991) and 0.2 U of Taq I polymerase (Ampli Taq ™ Perkin-Elmer Cetus) without adding mineral oil.

Alternatively, 6 µl of D1S80 PCR reaction mix from Cetus Corp. were used adding 0.1 U of Taq polymerase. Amplification was carried out using a micro thermal cycler (Linus Microcycler) with the following cycles: one cycle of 94°C 2 min - 65°C 5 s - 72°C 35 s, five cycles of 94°C 30 s - 65°C 5 s - 72°C 35 s , thirty cycles of 94°C 5 s - 65°C 5 s - 72°C 35 s -30 cycles and one final cycle of 72°C 1min.

PAGE and silver staining

PAGE was performed using 0.4 mm thick, 12 cm long polyacrilamide-piperazine gels (4% T, 3% C), 0.375 M Tris-HCl pH 8.8 buffer, with 3% agarose electrodes in 0.25 M Tris-glicine buffer pH 8.8. The gels were run at 15 mA, keeping the current constant. Detection of the genotypes was perfomed by silver staining (Budowle et al. 1991).

Statistical calculations

Hardy-Weinberg equilibrium was tested using the three-allele model of Skowash et al. (1992). The expected number of alleles was estimated as by Chakraborty et al. (1991) and the expected heterozygosity as by Nei (1978).

RESULTS and DISCUSSION

Up to 22 different alleles were found in the Basque sample, ranging in size from 17 to 40 repetitions. The alleles of 34 and 39 repetitions were not observed, while the alleles of 35 and 38 repetitions, not previously reported in the studied European populations, have been detected. The gene frequency distribution of the sample here studied and those from the European populations of Galicia and Portugal (Lareau et al, in press), Germany (Skowash et al., 1992), Finland (Sajantila et al., 1992) and that from the U.S. Caucasian population (Budowle et al. 1991) are shown in Fig.1. All the populations display similar distributions of their D1S80 gene frequencies.

The Hardy-Weinberg equilibrium test was performed by binning alleles together (Brenner and Morris, 1990) and using a three-allele model (17-23, 24 and 25-40; $X^2_{5d.f.}$= 1.584).

The observed Heterozygosity in the Basque population sample for the D1S80 locus was 0.74, the expected Heterozygosity being 0.794±0.016 (Nei, 1978). The Index of discrimination, calculated as by Wong et al. (1987) in the Basque population was 0.076. This Index is slightly higher than in other populations, and therefore, this system is equally useful for forensic purposes in our population. The Chance of Exclusion, calculated as by Smouse and Chakraborty (1986) is 0.621, a figure a little higher than that observed in other populations.

Genotyping of the amplified 5 ng samples of DNA both from homozygous and heterozygous individuals was possible using the procedure here described (Fig. 2), while the resolution was not enough in the case of the 2.5 ng samples. These results show that the lowest limit of DNA that can be amplified using this technique is 5 ng.

The application of the micro thermal cycler technique to criminal cases requires the modification of the DNA extraction protocol for stains with Chelex resine. The concentration of DNA extracted by Chelex resin was increased using a 30% resin stock solution, and adjusting the final volume of extraction to 30 µl. The same modification can be applied to semen stains, which enables stronger signals to be obtained after silver staining. Figure 3 shows the results obtained when forensic samples are amplified using this new procedure.

To summarize, based on a micro thermal cycler apparatus, we have developed a new protocol for amplification of the D1S80 locus.The advantages of this system consist of a reduction in time and costs of the amplification process, which greatly facilitates the analysis of the D1S80 locus at the population level as well as in paternity testing and forensic identification.

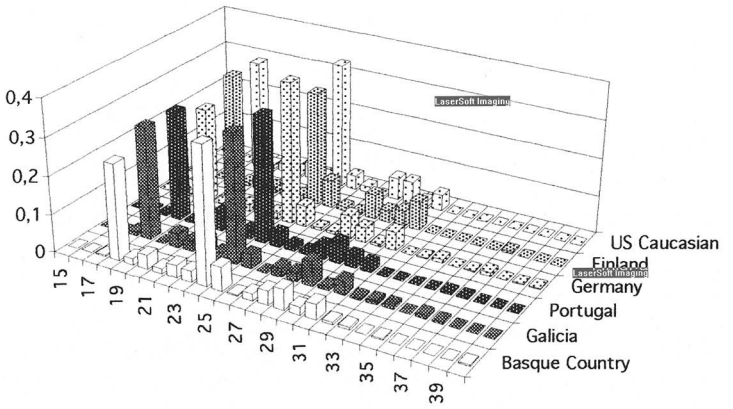


Figure 1.-Gene frequency distributions of several populations

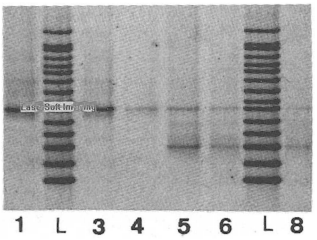
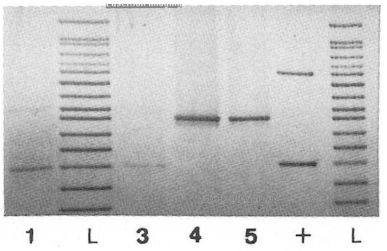


Figure 2.-D1S80 typing of a serial dilution of DNA. Allelic ladder (L). DNA from individual 1(T24,T24): lane 1 (50 ng), lane 3 (25 ng), lane 4 (5 ng). DNA from individual 2 (T18,T24) : lane 5 (50 ng), lane 6 (25 ng), lane 8 (5 ng).



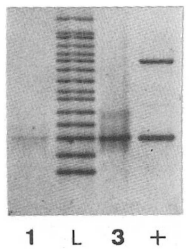


Figure 3.-D1S80 typing of different forensic samples. Allelic ladder (L). Positive control (+). (A) Case 1(T18,T25): lane 1 (DNA from whole blood extracted by the phenol-chloroform method), lane 3 (DNA from a hair root extracted by rapid digestion). Case 2 (T24,T24): lane 5 (DNA from whole blood extracted by the phenol-chloroform method), lane 4 (DNA from a semen stain extracted with 30 µl of 30% Chelex resin). (B) Case 3 (T18,T18): lane 1 (DNA from a blood stain extracted with 30 µl of 30% Chelex resin), lane 3 (DNA from whole blood extracted by the phenol-chloroform method).

REFERENCES

Brenner C, Morris JW (1990) Paternity index calculations in single locus hypervariable DNA probes: validation and other studies. Proceedings for the International Symposium on Human Identification, Promega corporation, Madison, U.S.A.

Budowle B, Chakraborty R, Giusti A M, Eisenberg J, Allen R C (1991) Analysis of the VNTR locus D1S80 by the PCR followed by high-resolution PAGE. Am J Hum Genet 48: 137-144

Chakraborty R, Daiger S (1991) Polymorphisms at VNTR loci suggest homogeneity of the white population of Utah. Hum Biol 63:571-587

Jung JM, Comey CT, Baer DB, Budowle B (1991) Extraction strategy for obtaining DNA from bloodstains for PCR amplification and typing of the HLA-DQa gene. Int J Leg Med 104: 145-148

Kasai K, Nakamura Y, White R (1990) Amplification of a variable number of tandem repeats (VNTR) locus (pMCT118) by the polimerase chain reaction (PCR) and its application to forensic science. JFSCA 35:1196-1200

Lareu M.V, Muñoz I, Pestoni C, Rodriguez M S, Vide C, Carracedo A (1993) The distribution of HLA DQA1 and D1S80 (pMCT118) alleles and genotypes in the populations of Galicia and Central Portugal. Int J of Legal Medicine (in press)

Nei M (1978) Estimation of average Heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-590

Sajantila A, Budowle B, Strom M, Johsson V, Lukka M, Peltonen L, Ehnholm C (1992) PCR amplification of alleles at the D1S80 locus: comparison of a finnish and a north american caucasian population sample, and forensic casework evaluation. Am J Hum Genet 50:816-825

Skowasch K, Wiegand P, Brickmann B (1992) pMCT 118 (D1S80): a new allelic ladder and an improvided electrophoretic separation lead to the demonstration of 28 alleles. Int J Leg Med 105:165-168

Smouse PE, Chakraborty R (1986) The use of restriction fragment length polymorphisms in paternity analysis. Am. J. Hum. Genet. 38: 918-939

Wong Z, Wilson V, Patel I, Povey S, Jeffreys A J (1987) Characterization of a panel of highly variable minisatellites cloned from human DNA. Ann Hum Genet 51:269-288