D1S80 AMP-FLP ATTRIBUTES IN TWO DIFFERENT ETHNIC GROUPS OF ARGENTINIAN POPULATIONS.

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INTRODUCTION

The highly polymorphic *locus* D1S80 is the most investigated and used AMP-FLP system in the field of the molecular identification. This is, due in part, to the moderate to high number of alleles (25), the frequency distribution of the alleles, and even amplification of alleles in most samples, inspite of the considerable fragment length differences that range from 401 to 801 bp. Optimization of amplification protocols and amplicon detection systems has yielded high reproducibility of results, fullfiling basic requirements for forensic case work. Since a large number of studies have been performed in distantly located populations around the world, a great deal of genetic information, concerning this *locus*, is available for comparison.

The present study focused on the comparison of D1S80 allelic distributions exhibited by two distinct populations from Argentina: 1- a caucasian group and 2- an autochthonous population of Mapuche indians. Moreover , this study enabled the generation of a local database to be employed in forensic casework and paternity testing.

MATERIALS AND METHODS

Populations:

1- Caucasoids: 191 unrelated donors inhabiting the metropolitan area of Buenos Aires city (Argentina).

2- Aborigines: 61 unrelated Mapuche indians inhabiting three geographically closed communities: Cerro Policía, Aguada Guzmán and Loncovaca Arriba, all located in Río Negro province (Argentina).

DNA isolation:

DNA was extracted from 5 mm^2 blood stains spotted on watmann 3MM paper (about 200 µl/sample). After hydration and removal of paper debris, DNA was purified by means of a standard organic extraction procedure. Briefly: digestion with proteinase K /

SDS in TEC (10mM, 10mM, 100mM), incubation at 56ºC overnight, phenol / chlorophorm / isoamyl alcohol (25:24:1) extraction, and washing and concentration with Microcon 100 (Amicon) concentrators.

DNA quantitation:

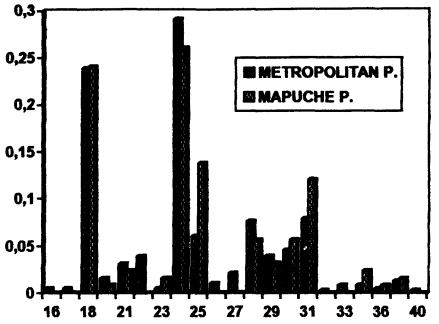
In order to obtain similar quantities of DNA samples to be amplified Slot-blot hybridization approach using the human probe D17Z1, was performed (ACES 2.0 Human DNA Quantitation System, GIBCO BRL).

PCR Amplification of *Locus* D1S80

Primer sequences: 1- 5' GAA ACT GGC CTC CAA ACA CTG CCC GCC G 3'
2- 5' GTC TTG TTG GAG ATG CAC GTG CCC CTT GC 3' .Reaction mix included:5 µl 10X PCR buffer (GeneAmp, Pekin Elmer Cetus), 4 ul 2.5 mM dNTP's, 1 µl 12.5 uM each primer, 2.5 units AmpliTaq DNA Polymerase (Perkin Elmer Cetus), 5 ng genomic DNA, and sterile water added to a final volume (50µl). Amplifications were carried out in a Perkin Elmer Cetus Thermal cycler GeneAmp PCR System 9600. Cycling was as follows: denaturation: 10 s, 95ºC; annealing 10 s, 67ºC; extension 30 s, 70ºC, for a total of 27 cycles. PCR products were separated by electrophoresis in a discontinuous buffer system using ultrathin (0.4 mm) polyacrylamide gels (7.5 % T, 2 % C) with piperazine diacrylamide as the crosslinker. Gels contained 60 mM tris-formate buffer, pH 9. Gels were cast onto a gel support medium (19.5 x 37 cm, Gelbond, FMC). The trailing ion was 0.28 M tris borate, pH 9.0. Electrophoresis settings were 1000 v (mA and W values setted at maximun). An SA32 electrophoretic apparatus (BRL Life technologies, USA) was used. The separated amplification products were visualized by silver staining the gel using the method of Budowle et al. (1991).

RESULTS

The D1S80 locus met Hardy-Weinberg expectations in both sample populations. The allele frequency distributions of the two populations generally were similar. The absence of some alleles and the higher frequencies for allelles 25 and 31 (figure 1) in the Mapuche population might be due to sample size. Nevertheless, genotype frequency distributions showed the presence of very unusual allele combinations, such as 21-34 and 31-37 which were carried by 4.8% of the Mapuche individuals analyzed. These two genotypes were not previously reported in caucasian populations, such as Dutch (Kloosterman et al, 1993), Finns (Sajantila et al, 1992), US Caucasians (Budowle et al, 1991), Spanish (Alonso et al, 1993) Portuguese (Pontes et al, 1994) and Argentineans, representing a total of 1010 urelated individuals screened. This observation should be investigated further in order to determine the possible causes of the presence of such combinations.



STATISTICAL PARAMETERS	Met.	Map.
Power of discrimination	95.70 %	93.90 %
Observed heterozygosity	82.72 %	87.06 %
Expected heterozygosity	84.22 %	83.97 %

Met.: Metropolitan population
Map.: Mapuche population

Figure 1

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