# MOLECULAR PHENOTYPING OF TWO TRINUCLEOTIDE REPEATS (XT00444 AND D5S373). EXPERIMENTAL CONDITIONS.

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In this work the genetic analysis of two trinucleotide repeats XT00444 (Yandava et al., 1994) and D5S373 (Dixon and Dixon, 1993) located in the chromosomes 13 and 5q32 respectively, is carried out. The main objectives consist not only in analysing their degree of variability in this initial approach, but mainly to study their molecular behaviour with a view towards developing robust phenotyping conditions for routine analyses.

## MATERIAL AND METHODS

DNA samples were extracted from whole blood of 200 autochtonous Galician individuals (NW Spain) by standar chelating resins method (Singer-Sam et al., 1989) or phenol/chloroform (Maniatis et al., 1982).

The primer sequences for the D5S373 were as described by Dixon and Dixon (1993):
5' GGT AAC AAG AGA GAA ACT CC 3'
5' CAA TTT CTT AGT GCA CAC ATC 3'.
The temperature cycling conditions were 92°C/30 sec, 60°C/30 sec, 72°C/30 sec for 35 cycles and an additional extension step at 72°C for 10 min in the Linus Dualcicler. The DNA was amplified in a final volume of 12.5 $\mu$l containing 1$\mu$M of each primer, 200 $\mu$M dNTPs, 0.5 units of Taq DNA Polymerase (Boehringer Mannhein), 1.5 mM Mg $Cl_2$.

The XT00444 amplification was carried out according to the following primer sequences (Yandava et al., 1994):
5' GAA TAA AGT GCC CAG CTT GT 3'
5' GTT GTC CTT AAA GCC CCG T 3'.
The temperature profile consisted of an initial denaturation at 94°C for 6 min, followed by 35 cycles of 94°C/15 sec, 62°C/23sec, 72°C/30sec and an final extension step at 72°C for 5 min, in the Gene Amp PCR 2400 System (Perkin Elmer). The amplification mixture consisted of 20-50 ng of DNA, 0.4 $\mu$M of both primers, 200 $\mu$M dNTPs, 0.75 units of Taq DNA Polymerase (Boehringer Mannhein), 3 mM Mg $Cl_2$ in a total volume of 12.5 $\mu$l.

PCR amplified products were separated by semy-dry discontinuous polyacrylamide gel electrophoresis, in horizontal plates with 400 $\mu$m of thickness. The gel composition was 9% T, 4% C for XT00444 and 10% T, 5% C for D5S373 using piperazine diacrylamide as a crosslinker, Glycerol (7.1% v/v). A 0.375 M Tris-HCl pH 8.8 buffer was used for the gel and a 0.125 M Tris-Glycine pH 8.8 buffer for the bridge. Electrophoresis was carried out at constant 12.5 V/cm for 2 hours, and the bands were visualised by a specific DNA Silver Staining (Budowle et al., 1991).

## RESULTS AND DISCUSSION

A non-specific constant band in the reading zone (Fig.1) of D5S373 was observed according to the conditions reported elsewhere (Dixon and Dixon, 1993). In our experience, the concentration primers and temperature of annealing are the critical factors. Thus, increasing the temperature up to 60°C leads to a progressive decrease of the non-specific bands which gives a remarkable improvement for the phenotype diagnosis (Fig. 1a). With regard to XT00444 a poor signal of amplification was obtained, despite diverse PCR parameters (reagents, cycling conditions) being assayed.

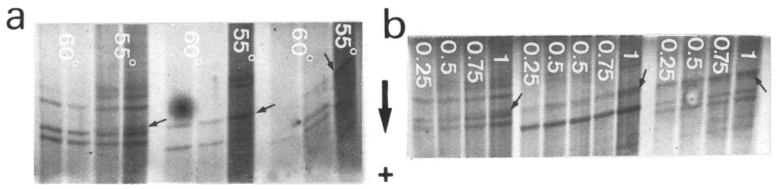


Figure. 1. Influence on the amplification of the D5S373 of the annealing temperature (a) and of the primer concentration ($\mu$M)(b). Arrows indicate the constant bands referred in the text.

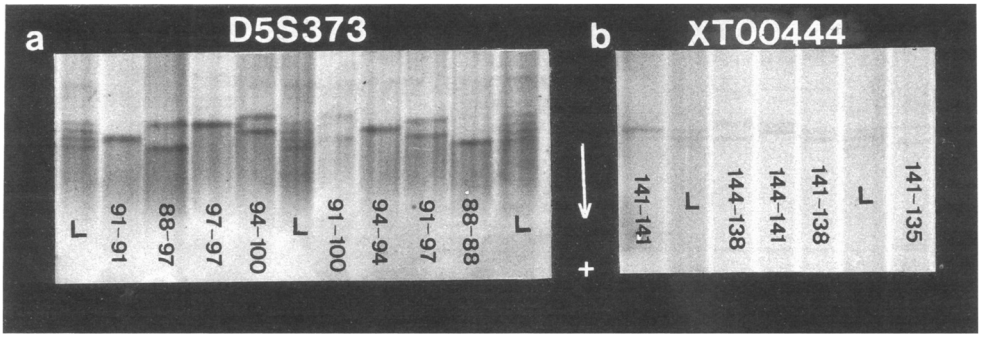


Figure. 2. Phenotype patterns after polyacrylamide gel electrophoresis : a) D5S373 and b) XT00444.

Polyacrylamide supports are revealed as a suitable modality for molecular separation of the alleles of both systems (Fig 2a/2b), regardless of the efficiency of the amplification as mentioned above.

The allele frequencies distributions in 200 individuals of the population of Galicia are summarised in Table I. No deviations from the Hardy-Weinberg proportions, were registered ($\chi^2$= 31.541, $0.6<p<0.7$ for XT00444 and $\chi^2$=4.035, $0.9<p<0.95$ for D5S373). Finally, the weak bands frequently observed after PCR amplification of XT00444, leads us to not recommend this marker for routine analysis. Conversely, the robustness of D5S373 phenotyping and its degree of polymorphism deserves its potential inclusion as a suitable marker for these type of studies.

Table 1.- Allele frequencies distribution of the XT00444 and the D5S373.

| XT0044 | |
|---|---|
| Alleles (bp) | Frequencies ± s.e. |
| 147bp | 0.0100 ± 0.005 |
| 144bp | 0.2125 ± 0.020 |
| 141bp | 0.2275 ± 0.021 |
| 138bp | 0.4225 ± 0.025 |
| 135bp | 0.0950 ± 0.015 |
| 132bp | 0.0050 ± 0.004 |
| 129bp | 0.0025 ± 0.002 |
| 126bp | 0.0050 ± 0.004 |
| 123bp | 0.0200 ± 0.007 |
| PIC = 0.672; H = 0.715 | |

| D5S373 | |
|---|---|
| Alleles(bp) | Frequencies ± s.e. |
| 100bp | 0.0125 ± 0.006 |
| 97bp | 0.1800 ± 0.019 |
| 94bp | 0.2825 ± 0.023 |
| 91bp | 0.3700 ± 0.024 |
| 88bp | 0.1550 ± 0.018 |
| PIC = 0.678; H = 0.727 | |

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