

ALLELE FREQUENCY DISTRIBUTIONS OF FIVE VNTR LOCI (D1S7, D7S21, D12S11, D5S43 AND D2S44) IN SPAIN.

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INTRODUCTION

Since the discovery of a hypervariable locus [1] numerous such loci have been found [2-4]. Of particular value and interest are the RFLP-analysis of hypervariable VNTR regions with the single locus probe hybridization technique. These markers have great potential for individual identification in forensic analysis and paternity testing [5-7]. However, before they can be adopted for routine use in these applications, the allele frequency distributions must be determined [8].

Here, we report the allele frequency distributions for the loci D1S7, D7S21, D12S11, D5S43 and D2S44 in the spanish population as determined by using oligonucleotide conjugated with alkaline phosphatase single locus probes (MS1, MS31, MS43a, MS8 and YNH24).

MATERIALS AND METHODS

Samples

The following population samples were analyzed:

- a) EDTA blood samples from about 200 unrelated individuals living in Madrid (Central Spain).
- b) EDTA blood samples from about 200 unrelated individuals spanish involved in criminal cases from different areas of Spain.

DNA profiling procedure

Genomic DNA was extracted by the standard phenol/chloroform protocol [7]. DNA was restricted with Hinf I. Separation of the DNA fragments was performed by agarose gel electrophoresis in TBE buffer according to the EDNAP group protocol. Gels contained a control DNA size marker (Hind III/lambda), three DNA control ladders (BRL) and two genomic controls (K562). Gels were depurinated, denatured and Southern blotted onto nylon membranes. Membranes were hybridised with the probes MS1, MS31, MS43a, MS8 (Cellmark Diagnostic) and YNH24 (Promega Corporation) according to the Non-Isotopic Chemiluminiscent Enhancement protocol (Cellmark Diagnostic). Autoradiography was carried out for 16h-3days.

Image analysis

All autoradiographs were analysed by means of the Bioimage equipment (Millipore) using the method Elder and Southern [9] for band size calculation with reference to the control DNA ladder at three differing positions on the autoradiographs.

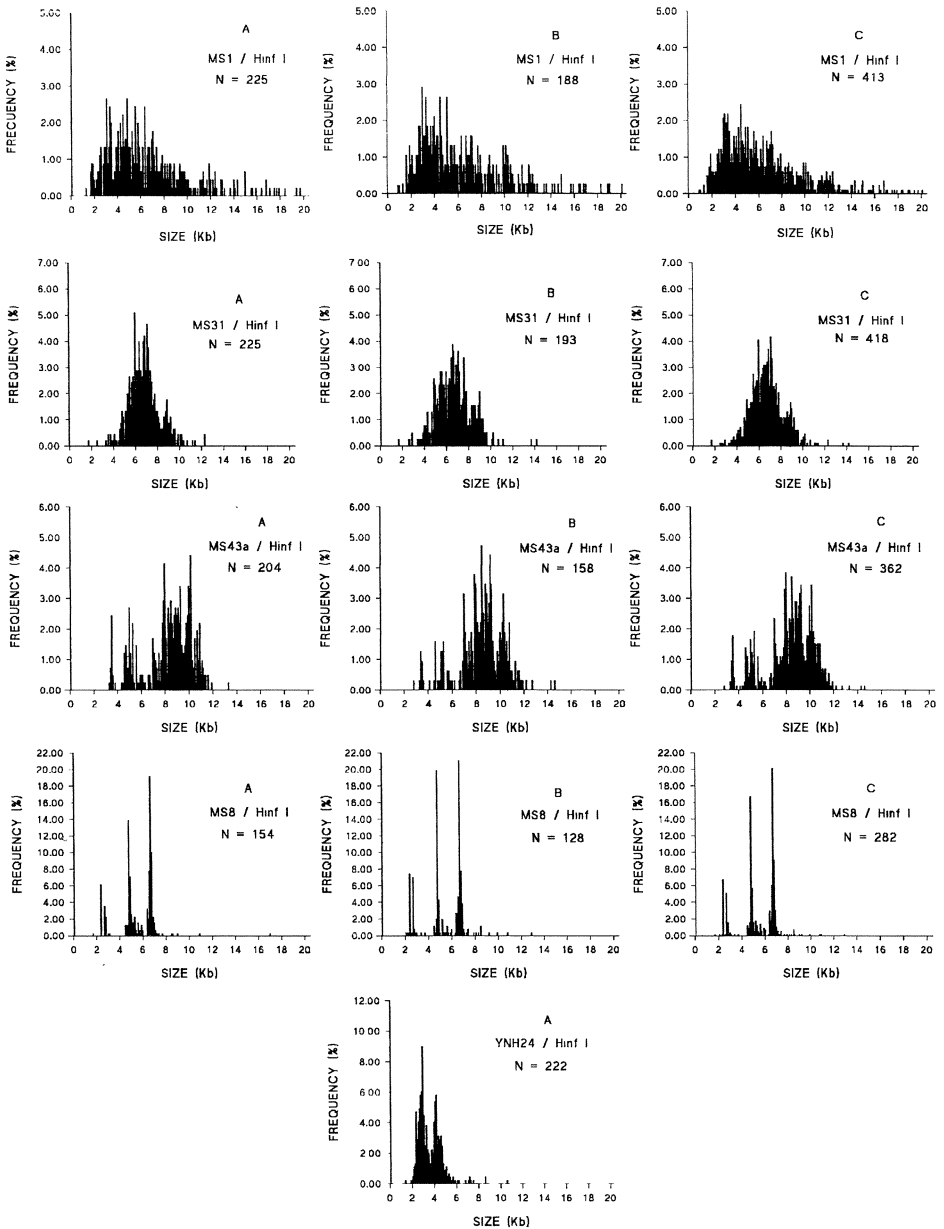


Fig 1. DISTRIBUTION OF MS1, MS31, MS43a, MS8 AND YNH24 ALLELES.

A. Unrelated individuals resident in Madrid B. Unrelated spanish involved in criminal cases C. Cobined data (A + B)

RESULTS AND DISCUSSION

The allele sizes and the allele frequency distributions were determined for 5 VNTR loci: D1S7, D7S21, D12S11, D5S43 and D2S44.

The resolution limit and measurement reproducibility ranges of our electrophoretic system was estimated by determining the size variation of the genomic control (K562) run in duplicated on every gel (Table 1).

	MS1		MS31		MS43A		MS8		YNH24	
	HMWF	LMWF	HMWF	LMWF	HMWF	LMWF	HMWF	LMWF	HMWF	LMWF
N	106	106	99	102	120	73	73	75	37	36
MEAN	4835	4482	7858	7003	13587	5290	5550	4789	4028	2906
MAXIMUM	4912	4527	7997	7095	13930	5367	5634	4845	4070	2929
MINIMUM	4759	4432	7694	6852	13323	5239	5465	4716	3978	2867
SD	29	23	60	46	120	27	33	27	21	15
MVI	2.19%	1.10%	3.82%	3.48%	4.47%	2.42%	3.05%	2.70%	2.27%	2.12%

Table 1. Resolution limit and measurement reproducibility. HMWF=High Molecular Weight Fragment; LMWF=Low Molecular Weight Fragment; N= number of genomic controls examined; MVI=Maximal Variation Inter gel.

The allele frequency distributions of five VNTR loci in a population sample of individuals living in Madrid and individuals from different areas of Spain are shown in Fig 1A and 1B respectively. As can be seen the allele frequency profiles are very similar in both populations, and no differences were observed when the data of these populations were combined (Fig. 1C).

The observed heterozygosity, range of band size and the most common fragment size range of the five loci analyzed are shown in the following table :

	MS1	MS31	MS43a	MS8	YNH24
Heterozygosity (%)	94.6	92.6	90	77.5	91
Allele size range (Kb)	0.9 - >20	1.7 - 14.2	2.8 - 14.6	1.7 - 17	1.4 - 10.6
Common size range (Kb) - [Frequency]	4.5 - 4.6 [2.4 %]	7.1 - 7.2 [4.2 %]	8 - 8.1 [3.8 %]	6.7 - 6.8 [20.2 %]	3 - 3.1 [8.9 %]

On the other hand, the allele frequency distributions determined in Spain are in agreement with other data previously reported from Caucasian populations, where loci and restriction enzyme are in common [10-14].

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