

**ALLELE FREQUENCY DISTRIBUTION OF FIVE VNTR LOCI AND
PATERNITY TESTING IN NORTH-EAST OF SPAIN**

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System and locus: MS1 / Hinf I (D1S7)
MS31 / HinfI (D7S21)
MS43a /Hinf I (D12S11)
MS8 / Hinf I (D5S43)
YNH24 / Hinf I (D2S44)

Population and sample size: Catalonia (N.E. of Spain). N=208

Methods:

Extraction method: proteinase K/SDS, phenol/chloroform [1]

Restriction enzyme: Hinf I (Promega)

Electrophoresis and blotting: The gels (0.7% agarose in TBE buffer) were electrophoresed and Southern blotted in a nylon membrane according to the EDNAP group protocol and recommendations [2]. NICE DNA analysis ladder (BRL) was used as size marker and K562 DNA as genomic control.

Hybridization: The membranes were sequentially hybridised with the YNH24 (Promega), MS8, MS43a, MS31 and MS1 (Cellmark) alkaline phosphatase labelled probes, following Cellmark protocol.

Size measurement and data analysis: DNA band size was measured following Elder & Southern method [3] with a computerised analytical imaging system (BioImage). Frequency distribution was analysed using sliding window approach [4] with 5 base pairs (bp) intervals and a 5.6% window. Within and between sample comparisons have been made (intergel comparison of the genomic control (K562 DNA) bands, intergel comparison of duplicated sample analysis and inter/intragel comparison between mother/child shared bands).

Results:

Table 1. Data from repeated sizing of the genomic control.

	MS1		MS31		MS43a		MS8		YNH24	
	HMW	LMW	HMW	LMW	HMW	LMW	HMW	LMW	HMW	LMW
Number of bands	78	78	85	85	74	74	64	64	70	70
Mean	4814	4465	7814	6957	13448	5259	5532	4770	4008	2898
Maximum	4885	4525	7934	7056	13779	5327	5583	4823	4052	2942
Minimum	4766	4421	7728	6897	13169	5203	5446	4728	3967	2868
SD	26	21	46	31	133	25	25	21	17	14
Maximum difference	2.46%	2.33%	2.65%	2.28%	4.54%	2.35%	2.47%	1.99%	2.13%	2.55%

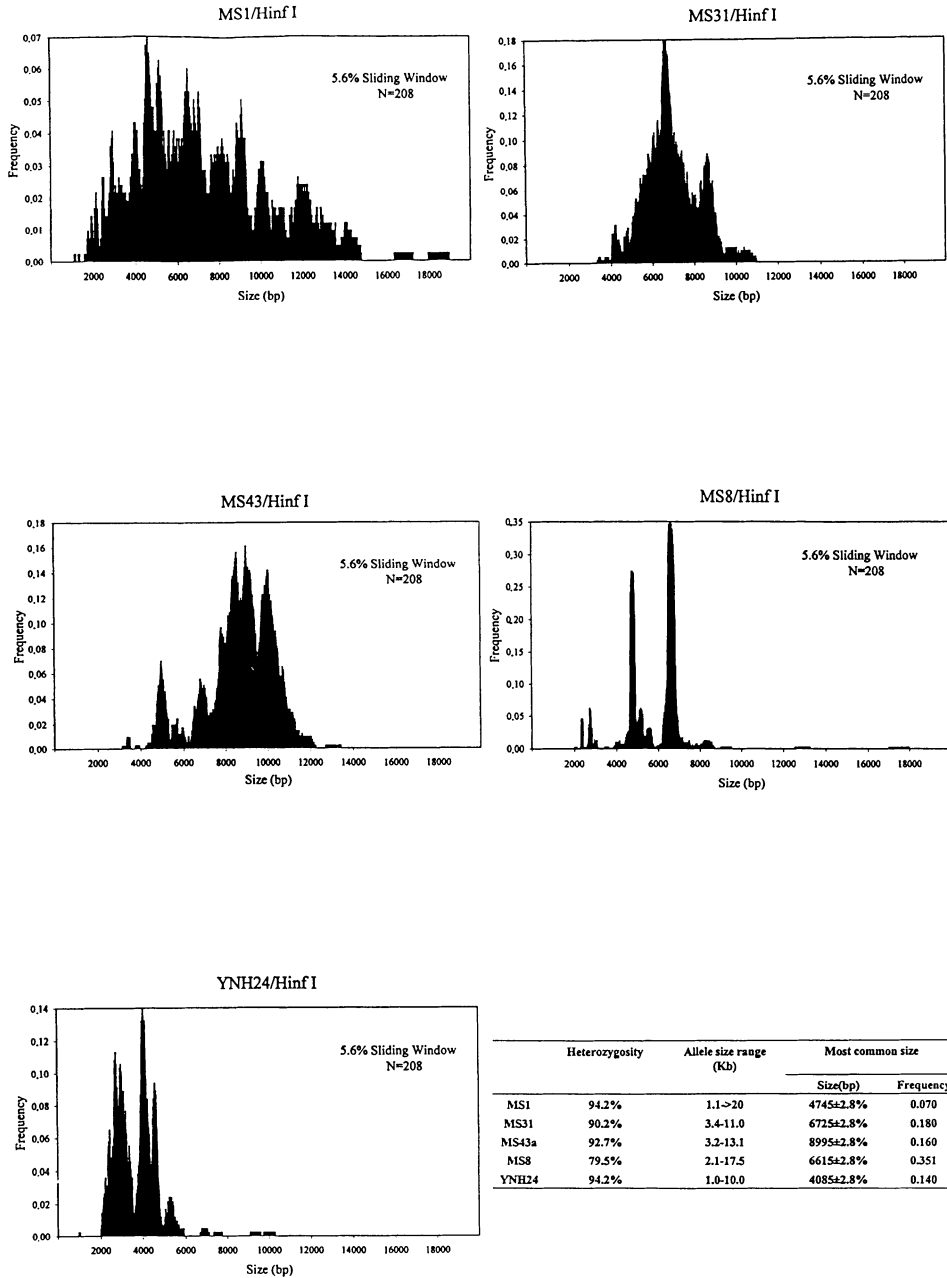


Figure 1. Allele frequency distribution for the five VNTR loci (D1S7, D7S21, D12S11, D5S43 and D2S44) using sliding window method and data summary.

Table 2. Intra and intergel comparisons between the mother/child shared bands from paternity testing and between bands of the same individual analysed in different gels.

	Mother/child shared band		Duplicated sample
	Intragel	Intergel	Intergel
Number of band comparisons	531	536	1593
Mean of deviation	0.38%	0.87%	0.82%
Maximum deviation	1.96%	4.17%	5.08%
Percentage of match in $\pm 2.8\%$ guideline	100%	98.5%	98.6%

Comments:

The allele frequency distribution of the five VNTR loci in north-east of Spain is similar to others Caucasian populations [5, 6, elsewhere].

In the intra and intergel comparisons, it seems that there is not correlation between deviation and band size. However, over 12000 bp the deviation is higher. The deviation intergel is similar in mother/child and within person comparisons, and lower in the mother/child intragel comparisons. In this one, 100% of comparisons fall into the 2.8% match criterion (max. 1.96%), thus, mother-child-alleged father samples in paternity testing must be coelectrophoresed if it is possible. In the intergel comparisons 98.5% and 98.6% of samples match within 2.8% range. Reviewing the data, most of the samples over 2.8% range are maternal mutation (mainly with MS1 probe), molecular weight over 12000 bp or distorted bands.

References:

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