SOME CRITICAL COMMENTS AND EXPERIMENTAL CALCULATIONS USED AS VALIDATION STANDARDS IN THE IMPLEMENTATION OF RFLP ANALYSIS.

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INTRODUCTION

Analysis of restriction fragment length polymorphisms (RFLP's) has become one of the most powerful methods in forensic DNA analysis. In the last few years we have implemented in routine case work in our laboratory the use of 5 single locus probes. The forensic use of this technique is regarded as valid and reliable (NRC, 1992; Lander and Budowle, 1994). However, a critical interpretation of test results should be carried out according to the potency of the technique (i.e. reproducibility or sizing error and power of resolution) and the distribution of the allele of a locus in a given population.

The aim of this study was to assess the validity of RFLP analysis and to comment some interpretation difficulties in parentage testing. Moreover we present a conflicting paternity case with band sizing similarities between non-related mother and alleged father, in which matching decisions conducted either to father or to mother exclusion.

MATERIALS AND METHODS

DNA was extracted from blood samples according to the method of Gill et al (1987) using phenol/chloroform and was restricted with the enzyme Hinf I. Size separation of restricted DNA and K562 / Hinf I digest genomic control DNA was achieved by electrophoresis in a 0.7% agarose gel running in TBE buffer for 18 hours (Gill et al, 1992). Alkaline phosphatase conjugated single locus probes MS31, MS43a, G3, MS8 and MS43a from Cellmark Diagnostics and YNH24 from Promega Corporation were used sequentially. All autoradiographs were analysed using a computerized digital image analysis system (Bioimage, Millipore Corp.) which utilised the Elder and Southern method (1987) for band size calculation with reference to molecular weight markers (BRL/NICE).

The sizing error or measurement of reproducibility (sigma) of the electrophoretic system was estimated by determining the size variation of the genomic control (K562 / Hinf I digest) run on every gel. The ability of the system to resolve close bands (defined as delta, power of resolution) was estimated through a critical analysis of samples in which closely spaced bands occur in the same lane (heterozygous individuals or DNA mixtures from alleged father and child).

Percent homozygosity and heterozygosity for each VNTR-locus was calculated from the study of DNA from blood samples of 110 unrelated individuals of Southern Spain. The average power of exclusion ($A=h^2(1-hH^2)$) and the typical paternity index ($TPI=1/2H$) were estimated according to Brenner and Morris (1990).

RESULTS AND DISCUSSION

The sizing error (sigma) obtained for the system ranged from 0.54 to 2.7 % depending on the VNTR-locus and the size of the K562 / Hinf I digest fragment (Table 1). In this sense, sigma was higher for the low molecular weight fragment for all the loci studied, except for the D12S11. The power of resolution (delta) ranged from 2.6 to 3.6 %.

Table 1: Measurement reproducibility of the electrophoretic system calculated as allele sizing error (sigma) determining the size variation of the two fragments (high and low molecular weight fragments) of the genomic control (K562 / Hinf I digest) run on every gel (n=25). The power of resolution (delta) was calculated from closely spaced bands of the same lane (n > 10).

VNTR locus	D2S44		D12S11		D7S21		D7S22		D5S43	
DNA-probe	YNH24		MS43a		MS31		G3		MS8	
Fragment	HMW F	LMW F	HMWF	LMW F	HMW F	LMW F	HMW F	LMW F	HMW F	LM WF
Mean (bp)	4011	2909	13599	5253	7812	6963	7059	1964	5538	4774
SD (bp)	21.8	55.5	183.2	29.2	58.3	54.0	51.5	53.2	35.7	33.1
Sigma (%)	0.54	1.91	1.35	0.55	0.75	0.78	0.73	2.71	0.64	0.69
Maximum	4051	3079	13950	5309	7905	7079	7163	2027	5613	4836
Minimum	3986	2858	13270	5191	7694	6844	6963	1799	5481	4716
Resolution power (δ)	2.6		3.6		2.8		2.8		2.9	

Table 2: Percent homozygosity and heterozygosity for each VNTR-locus, average power of exclusion and typical paternity indexes were calculated for the andalusian population (S Spain), (N=110).

Genetic locus	D2S44	D12S11	D7S21	D7S22	D5S43
DNA-probe	YNH24	MS43a	MS31	G3	MS8
Heterozygosity (%)	95.75	95.10	95.05	83.33	85.06
Homozygosity (%)	4.25	4.90	4.95	16.67	14.94
Average power of exclusion (%)	88	86	86	61	76
Typical paternity index	0.118	0.102	0.101	0.030	0.033

The heterozigosity of the andalusian population was higher than 95 % for the D2S44, D12S11 and D7S21 loci, with an average power of exclusion higher than 86 % (Table 2). The tipical paternity index was particularly high for the D2S44 locus, and very low for the D7S22 and D5S43 loci. The average power of exclusion was very high for the

D2S44, D12S11 and D7S21 loci. This data confirms the validity of RFLP analysis, but also shows that some locus are more useful than others.

The results of a paternity case are presented in Table 3. Clear father exclusion was produced for the D12S11 locus. D7S22 locus showed also a clear exclusion of the father, however a possible mother exclusion was also present. The run of a mixture of the alleged father and the child DNA was very useful to detect exclusions when alleles from the mother and the father have similar molecular weights, as occurred with D7S21 and D2S44. In these case the mixture of child and mother DNA should be also included.

Table 3: RFLP analysis of a paternity case showing the allele size (bp) of the child, the mother, the alleged father and the mixture of the child and the father DNA. The conclusion of the study are expressed as excluded (E) or non-excluded (NE).

VNTR locus	D2S44		D12S11		D7S21		D7S22		D5S43	
DNA-probe	YNH24		MS43a		MS31		G3		MS8	
Fragment	HMW F	LMW F	HMWF	LMW F	HMW F	LMW F	HMW F	LMW F	HMW F	LM WF
Child	4059	2636	7895	5150	7882	5379	3216	3216	6349	6257
Mother	2652	2652	11919	7957	6364	5414	6674	6674	6257	4776
Putative father	4321	2684	9522	4867	7774	5685	6212	5790	6303	4688
Mixture (child + p. father)	4351	2701	9739	5182	8032	5722	6257	3216	6395	4688
	4089	2668	7957	4897	7882	5396	5825		6303	
Conclusion	F or M E		FE		FE		F or M E		F NE	

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