

Investigation of variation in fragment size determinations found when using single locus DNA probes

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When using single locus probes the assignment of a fragment's size is relatively straight forward, but it is essential to have knowledge of the variability that can occur in that assessment before the results can be fully interpreted. When 'matching' consideration must be taken of whether the samples were run on the same gel, on different gels within the same laboratory, or on different gels in different laboratories.

We have made assessments of variability of results by comparing bands from mother-child pairs, run in adjacent tracks of the same gel, and by repeated testing of the same DNA sample on different gels within the same laboratory. Also we have used a series of blood samples to prepare DNA autoradiographs in two separate laboratories using four probes. We have used both a ruler and image analysis methods to size the fragments on both sets of autoradiographs.

#### METHODS

3µg samples of AluI-digested DNA from whole blood samples were electrophoresed, blotted and hybridised by each laboratory's standard methods. The probes used were 3' $\alpha$ -HVR, MR24/1, Muc-7 (Amersham Int), TBQ7 and YNH24 (Promega Corp). Fragment sizes were estimated, either using a ruler or by image analysis (Biotrac: Foster and Freeman), using the local form of the reciprocal relationship  $c = (m - m_0)(L - L_0)$  as described by Elder and Southern (1987). The DNA size markers used were a 14-rung <sup>35</sup>S-labelled ladder (Amersham Int).

#### RESULTS

A series of 394 pairwise comparisons of fragment sizes of corresponding bands in mother-child pairs were made. The differences in the measured fragment sizes of these pairs are expressed as percentages of the child's fragment size (Fig. 1). Based on these results we consider that a named man is a possible father if he has a fragment within +/-2.5% of the paternal fragment in the child. This 2.5% corresponds to a confidence interval of 99.999%.

Table 1. Cases not resolved by conventional testing alone

CONVENTIONAL TESTING		DNA TESTING		
Possible exclusion evidence		Result		
	PI (Conventional)	No. probes excluding	PI (DNA)	
No exclusions - 2 other children in family excluded	15	Excluded	3/4	
No exclusions - incomplete test	2	Excluded	2/5	
Probable Rh exclusion	3	Excluded	3/4	
Fy 2 <sup>o</sup> order exclusion	10	Excluded	3/4	
EAP 2 <sup>o</sup> order exclusion	2	Excluded	5/5	
PLG 2 <sup>o</sup> order exclusion	24	Excluded	3/3	
Gc 2 <sup>o</sup> order exclusion	10	Excluded	4/4	
HLA only exclusion - B and C loci	11	Excluded	5/5	
Glo 1 <sup>o</sup> order exclusion	3	Excluded	3/4	
ESD 1 <sup>o</sup> order exclusion	350	Excluded	3/4	
Fy 2 <sup>o</sup> order exclusion	110	Not excluded	0/4	100
PLG 2 <sup>o</sup> order exclusion	36	Not excluded	0/4	500

The cases were not randomly selected. All cases are initially investigated using the conventional systems. DNA testing is performed only when these fail to produce sufficient evidence to resolve the case, or when specifically requested. The cases chosen for DNA testing are therefore biased towards those with low PI values, or those with only one system showing an exclusion of paternity after investigation of conventional polymorphisms.

Figure 1. illustrates that, using DNA testing alone (and with a wide +/-5% window to interrogate the database), paternity indices of greater than 100 were achieved in virtually all cases, provided at least four of the probes selected for this study were used. The combined use of these DNA probes and conventional polymorphisms gives consistently high PIs and corresponding RCPs: all cases (often selected for their low PI values) where no exclusion was found, gave an RCP of >99.9% when at least four probes had been successfully applied.

An estimation of the power of exclusion has been made for each probe by constructing false trios whereby mother-child pairs are compared with an unrelated man's sample on the same gel using the +/-2.5% band match criteria. Results from 335 false families (see Table 2) show a combined exclusion rate of 99.99989% (which increases to 99.999997% when combined with the expected exclusion rate for conventional tests). 63 men (18.8%) were not excluded by one out of the range of probes used, but only 3 men (0.89%) had not been excluded when at least two probes were used. Use of further probes excluded all false fathers.

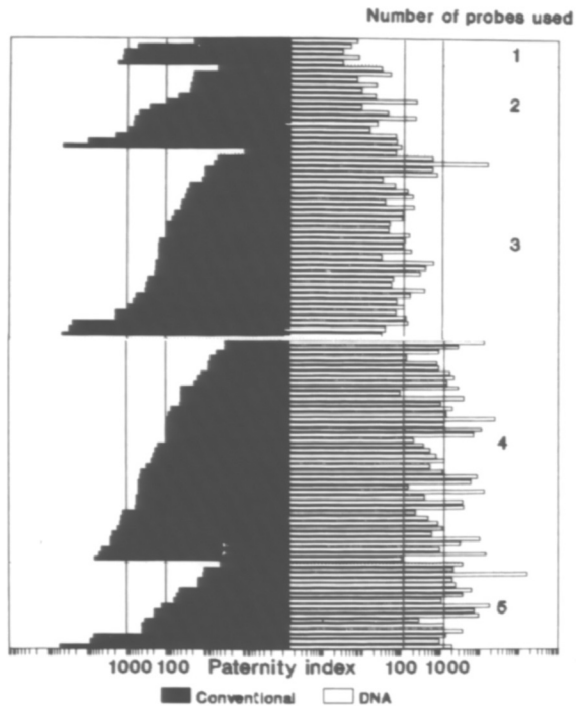


Fig. 1. Comparison of paternity indices provided by conventional testing and up to five single locus probes (n=114)

Table 2. Exclusion rates for DNA probes

	Muc 7	3' $\alpha$ -HVR	MR24	YNH24	TBQ7
False trios created	269	83	275	257	138
Exclusion rate	88.5%	85.8%	97.1%	94.9%	95.6%