Simultaneous DNA Probing of Paternity Cases Using Non-isotopic Methods

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INTRODUCTION

The use of DNA restriction fragment length polymorphisms (RFLP's) in the field of parentage testing has recently become a reality. Commercial availability of probes and the introduction of non-isotopic methods of detection (Dykes et al. 1986, 1988) has gradually made phenotyping of RFLP's more practical for the normal laboratory setting. The common complaint is that non-isotopic methods do not permit probe stripping and rehybridization. Historically the real problem was due to the fact that most polymorphic loci had broad overlapping ranges of band sizes. However, with careful selection of DNA probes it has become possible to identify highly polymorphic loci with non-overlapping band sizes which permit simultaneous probing.

This study was performed to determine the number of exclusions and the level of inclusion estimates when testing single versus multiple hybridizations. Multiple hybridizations were considered to be two to three probes applied to the same membrane. The results confirm the expectation that multiple probing is an advantage over rehybridization.

METHODS

Blood samples were supplied to Analytical Genetic Testing Center for complete parentage testing using our routine testing protocol of 2-4 DNA probes, serum protein and red cell enzyme polymorphisms, exclusion probability >99%, or as referrals from other laboratories. Referral cases involved non-excluded fathers with PI<100 which required further testing. DNA was extacted using the non-toxic extraction method of (Miller et al. 1988). Probes were selected from Collaborative Research and Epitope and labeled with biotin using the ONCOR labeling kit. DNA was restricted with Pst I and electrophoresed 36 hours at 1.2 V/cm in 0.8 gm% agarose. Each sample was run on two separate gels to permit hybridization with different probes. DNA was blotted to Sureblot and detected using the non-isotopic alkaline phosphatase-streptavidin detection system of ONCOR. All paternity cases were run using the mother, child, alleged father and a child/alleged father mix.

Size markers were a mixture of lambda restricted with Hind III, Bst EII and Sph I. Population data provided by Collaborative Research, Epitope and our laboratory and generally ranged from 500-1200 individuals. PI values were calculated using a binning system where the frequency of the obligatory gene of the random

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man was based upon a summation of all band frequencies +/- 1% from obligatory band shared by the child and alleged father.

RESULTS

Table 1 shows the probes used to detect the various polymorphic loci detected in this study. Although pL336 was always run alone on a single gel because of it's size range, various combinations of multiple hybridizations were used for the second gel, for example pS194 and pL427-4 or pL355-8, pL159-1 and pL427-4 (data not included). 85 referral cases and 29 private cases were reviewed which demonstrated an exclusion rate of 27-28%, Table 2.

In Table 3 are the 31 observed exclusions seen in the 114 cases tested. In all but one case pL336 was probed on one gel whereas the second gel contained either a second probe or a combination of two non-overlapping loci which could be detected with simultaneous probing. The heterozygosity of pL427-4 was expected to demonstrate more exclusions than observed but we suspect that sample size affected the lower exclusion rate. Table 4 breaks down the results of the probe used in table 3 into two and three probe combinations. pL336 probed on one gel and either pS194, pL427-4 or p144-D6 on the other gel all showed similiar rates of

TABLE 1. DNA LOCI, PROBES AND SIZE RANGES

LOCUS	PROBE	SIZE RANGE	SOURCE
D1S47	pL336	2-21	Collaborative Res.
D7S107	pS194	5.2-12.4	" "
D18S17	pL159-1	5-7	" "
D21S112	pL427-4	1.4-3.4	" "
D11S129	pR365-1	1.5-3	" "
D20S15	pL355-8	6-8	" "
D17S34	p144-D6	3.4-7.9	Epitope

TABLE 2. TABULATED RESULTS FROM PRIVATE AND REFERRAL CASES

	REFERRALS	PRIVATES
EXCLUDED	23	8
NON-EXCLUDED	62	21
TOTAL	85	29
% EXCLUDED	27	28

TABLE 3. OBSERVED EXCLUSIONS USING CRI AND EPITOPE PROBES

336	194	427-4	144-D6	159-1	365
yes	yes				
yes			yes	no	
yes	no	yes			
yes	yes				
yes			yes		
yes	yes				
yes		no	no		
yes		no	no		
no		yes	yes		
yes	yes				
yes		yes			
yes	no	yes			
no	no		yes		
yes	yes				
no	yes				
yes		no	no		
yes		yes	yes		
yes		yes	no		
yes	yes				
yes	yes				
yes			yes		
					yes
yes	yes		yes		
yes					
yes	yes				
yes	yes				
no		yes	yes		
yes	yes	yes			
yes	yes	yes			
yes	yes				
	yes		yes		yes
(a) 25/29	14/18	9/13	8/12	0/1	2/2
(b) 86.2%	77.8%	69.2%	66.7%		100%

(a) = exclusion count, (b) = % total exclusion

single and double exclusions. Simultaneous probing of a single membrane also proved effective, for example pS194 and pL427-4. Using three probes clearly demonstrated the best combination due to multiple exclusions.

Table 5 lists the observed PI values in the non-excluded cases. Using simultaneous probing on the same gel was as effective as a single probe on two separate membranes. Using a simultaneous

probing in conjunction with single probing demonstrated a five fold increase in PI values.

Considering the cost of isotopic techniques and the time required to rehybridize membranes (days), the use of simultaneous probing will maximize results at a considerable savings. Careful selection of non-overlapping loci will enable the testing laboratory to provide clients the best results at a reasonable price.

TABLE 4. EXCLUSION RESULTS FOR PROBE COMBINATIONS *

PROBE	NO. EXCLUSIONS			
COMBINATIONS	0	1	2	3
336 + 194	1/18	5/18	12/18	
336 + 427-4		6/13	7/13	
336 + 144-D6		4/12	8/12	
336 + 159-1		1/1	0/1	
194 + 427-4		0/3	3/3	
194 + 144-D6		1/2	1/2	
194 + 365-1		0/1	1/1	
427-4 + 144-D6	4/7		3/7	
THREE PROBES		5/15	5/15	5/15

* Results taken from Table 3 using both two and three probe combinations. 336 + 194 and 427-4 + 144-D6 include "0" exclusions with these combinations but alleged father was excluded by a third probe.

TABLE 5. PI VALUES FOR NON-EXCLUDED CASES

	2 PROBES 2 MEMBRANES		2 PROBES SAME MEMBRANE		3 PROBES	
PI	FREQ	AVERAGE	FREQ	AVERAGE	FREQ	AVERAGE
<100	0.38	49	0.42	28	0.18	76
100-1000	0.50	415	0.48	278	0.44	342
>1000	0.12	1955	0.10	2432	0.38	10,372

* pL336 on one membrane and two probes on second membrane.

REFERENCES

Dykes D, Fondell J, Watkins P, Polesky H (1986) The use of biotinylated DNA probes for detecting single copy human restriction fragment length polymorphisms separated by electrophoresis. Electrophoresis 7:278-282.

Dykes D (1988) The use of biotinylated DNA probes in parentage testing: Non-isotopic labeling and non-toxic extraction. Electrophoresis 8:359-368.

Miller SA, Dykes DD, Polesky HF (1988) A salting out procedure for extracting DNA from human nucleated cells. Nucleic Acid Res 16:1215.