

## Results on Selected Trios Tested with Two Single Locus DNA Probes

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The purpose of this paper is to report on the results obtained from selected paternity cases in which two single locus DNA probes were used. Many of the trios in this study were referred from other laboratories because of a previously low likelihood of paternity or single exclusion using conventional methods.

### MATERIALS AND METHODS

DNA was extracted for all samples using a non organic solvent procedure (Miller 1988). Samples were quantitated and adjusted to a concentration of 0.35ug/ul with Tris-EDTA buffer. Each sample was tested with pS194 (locus D7S104) after restriction with Pst I and pL336 (locus D1S47) after restriction with Rsa I. The probes were obtained from Collaborative Research, Inc. (Bedford, MA) and biotin labeled with a nick translation kit obtained from ONCOR, Inc. (Gaithersburg, MD). All gels contained a visible lambda Hind III marker, a human control, and size markers in every ninth lane. The trios were run on the same gel and included a sample mix of the alleged father and child. The gels were run approximately 46 hours and Southern blotted as described by Dykes (1988). The nylon membranes were hybridized and stained using a kit from ONCOR. A high resolution video camera was used to obtain a computer image of each membrane. Analysis of this image to determine band position and kilobase size was done by a program developed in house.

### RESULTS

Table one lists the results of 158 cases. Exclusions were found in 23 cases. In 85 cases, where only the probes were used, the PI ranged from 12.3 to 16,667 ( $<50 = 16$ ,  $50 - 100 = 18$ ,  $>100 = 51$ ).

In 73 cases data from other systems were available. Table two shows results in six of these cases in which an apparent indirect exclusion was observed in another system. Based on the high residual index, we concluded that a null allele was responsible for the apparent exclusion (Polesky 1988). In two of the 73 cases where the probes failed to provide evidence of exclusion, direct exclusions were observed in other systems (BF & F13B; HLA).

Table three shows the results in three additional cases. In each of these there appears to be a mutation detected by pL336.

In three cases of the 158, the initial results using pL336 and Rsa I showed a single band of different sizes for the alleged father and child. The use of another restriction enzyme (Pst I) showed an additional band shared by both the alleged father and child.

### SUMMARY

Both of the probes described have been helpful in establishing an exclusion for some additional men, but alone will not exclude all non-fathers. The use of these probes has resolved cases with low paternity indexes and helped establish paternity in cases with null alleles. Based on our observations, pL336 many have an mutation rate in excess of 0.1.

Table One

## RESULTS OBSERVED WITH TWO SINGLE LOCUS PROBES

	Number	%
Excluded by DNA	23	14.6
<i>Two probes</i>	<i>15</i>	<i>65</i>
<i>pL336 (Rsa I) only</i>	<i>7</i>	<i>30</i>
<i>pS194 (Pst I) only</i>	<i>1</i>	<i>5</i>
No exclusion by DNA	135	85.4
Total	158	

Table Two

## CASES WITH PROBABLE SILENT ALLELES

<i>System</i>	<i>Child</i>	<i>Mother</i>	<i>Alleged Father</i>	<i>Residual PI All systems</i>	<i>Residual PI 2 Probes</i>	
Case 1	Rh	R2	R1R2	R1	859	145.0
Case 2	PGM1	2+	2+	1+	1,635	7.6
Case 3	PLG	2	12	1	289	52.0
Case 4*	FY	a	a	b	265,417	8325.0
Case 5*	FY	a	a	b	2,454	71.0
Case 6	GLO	1	12	2	34,724	51.0

\*Hispanic, 2 children, same alleged father

Table Three

**CASES WITH POSSIBLE  
MUTATION IN pL336 (Rsa I)**

	<i>Child</i>	<i>Mother</i>	<i>Tested Man</i>
<i>Case 1</i>	7.41	7.85	13.0 *
	4.12	4.84	4.12
<i>Case 2</i>	6.24	6.03	4.96
	4.12	4.27	4.12
<i>Case 3</i>	6.53	5.07	6.31 +
	4.17	4.17	4.17

\*Direct exclusion of paternity in ACP

+Residual index using 10 systems is 2,247,726

#### REFERENCES

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