CLARIFICATION OF A TWO-MAN CASE ONLY BY DNA MARKERS

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In a two-man case none of the men could be excluded from paternity in 36 blood group systems including HLA - A,B,DR. The men are not related.
On the base of mother-child combination the exclusion ratio of non-fathers came up to 99,96%. The calculation of the EM value for the accused man resulted lg Y/X + 10 = 6.3711 (W= 99,976%) PI= 4.255 and for the witness lg Y/X + 10 = 5.1862 (W= 99,998%) PI= 65.128 .
To resolve the matter we used the following combination of DNA probes:
1. EcoRI polymorphism of Hras onkogen (Wyman et al., 1980)
2. TaqI polymorphism of pAW101 (Capon et al., 1983)
3. Single-locus probes: MS1, MS31, MS43, g3, MS8
4. Two complementary multi-locus probes: 33.15 and 33.6 (Jeffreys et al 1988).
All nine DNA probes assued an exclusion of the accused man, but not of the witness.

MATERIALS AND METHODS

High molecular DNA was isolated from 5-10 ml EDTA blood by standard procedures. Individual DNA samples of 12 µg each were digested with 70 U of restriction endonuclease Hinf I (for 2 gels). Agarose gel electrophoresis was carried out in a 0,7% gel using a 134 mM Tris, 74,9 mM Boric acid, 2,55 mM Disodium ethylendiaminetatracetic acid pH 8,8 electrophoresis buffer. The gels (20x20 cm) were run at a constant voltage of 75 V for 24 hours. Treatment with 0,25 M HCl for 15 min., then with 1,5 M NaCl, 0,5 M NaOH for 30 min., and finally with 3,0 M NaCl, 0,5 M Tris ph 7,5 for 30 min. The gels were blotted overnight in 20xSSC auto nylon membranes (Amersham Hybond-N). Prehybridisation was carried out in 1 x Denhardt's solution for 30 min. at 62°C and in hybridisation solution for 60 min. at 62°C. Hybridisation was performed overnight at 62°C in the same buffer containing 20 mg 32P-labelled probe 33.15 and 33.6 or pooled inserts of MS1, MS31, g§ and MS43, MS8 (2 ng for each).

Hybridisation solution: 0,2 % Bovine serum albumin, 0,2 % Ficoll 400, 0,2 % Polyvinylpyralidone, 6,0 % Polyethylen glycol 6000, 50 µg/ml degraded Herring DNA 1 x SSC, Blots were washed 3 times in 1 x SSC, 0,1 % SDS at 62°C. Filters were exposed for 24-72 hours to X-ray film.

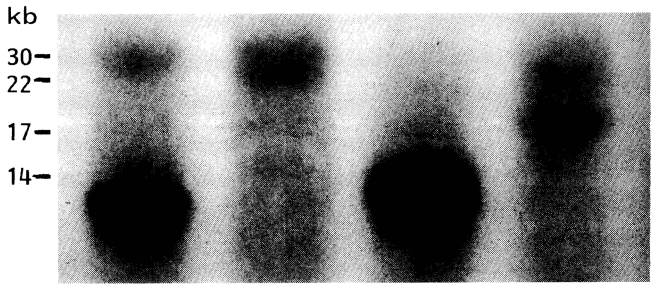

Fig. 1
EcoRI polymorphism of pAW 101 shows the exclusion from paternity of AF, but not of W.
(M=mother, C=child, AF= alleged father, W= witness)

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Edited by H. F. Polesky and W. R. Mayr

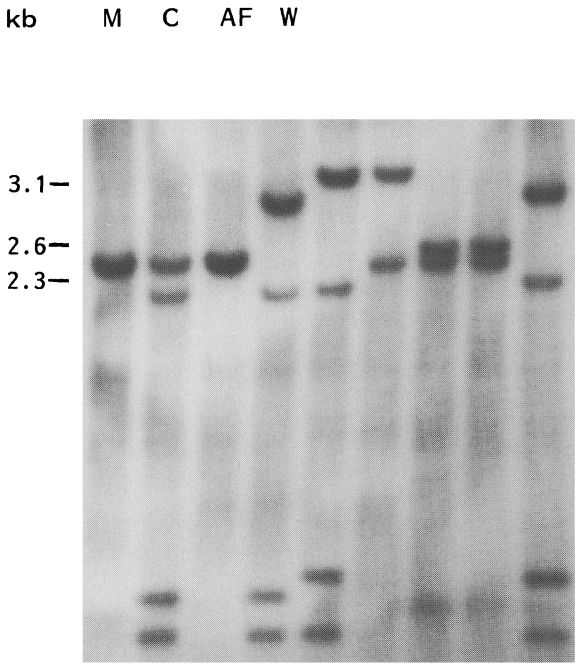


Fig. 2

TaqI polymorphism of Hras onkogen.

AF excluded
W included

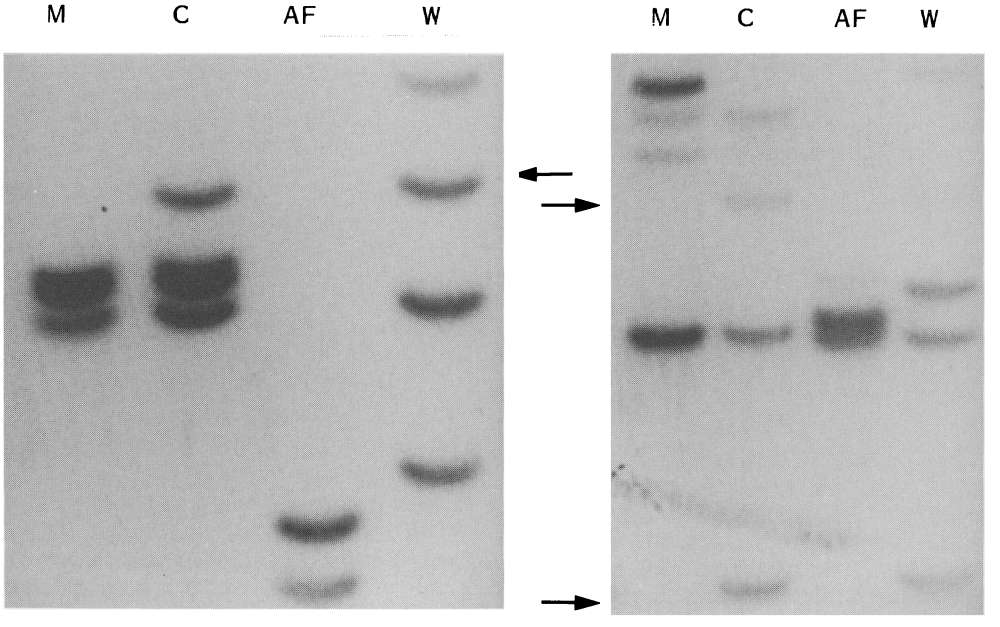
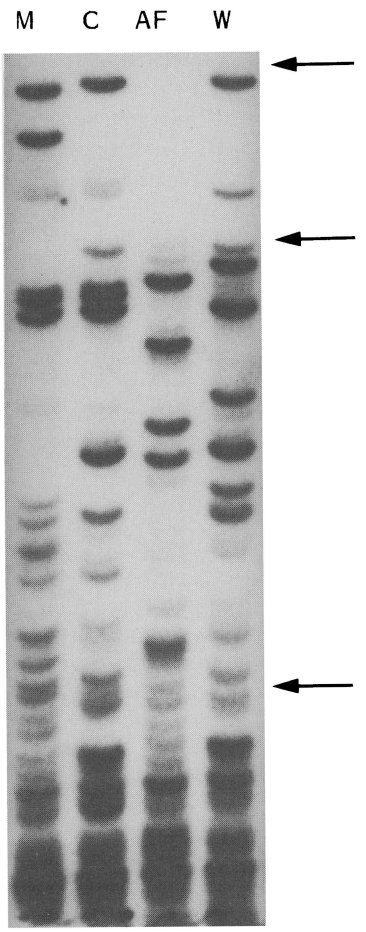


Fig.3: Mixture of single-locus probes MS31 and MS1

Fig.4: Mixture of single-locus probes MS8, MS43, g3



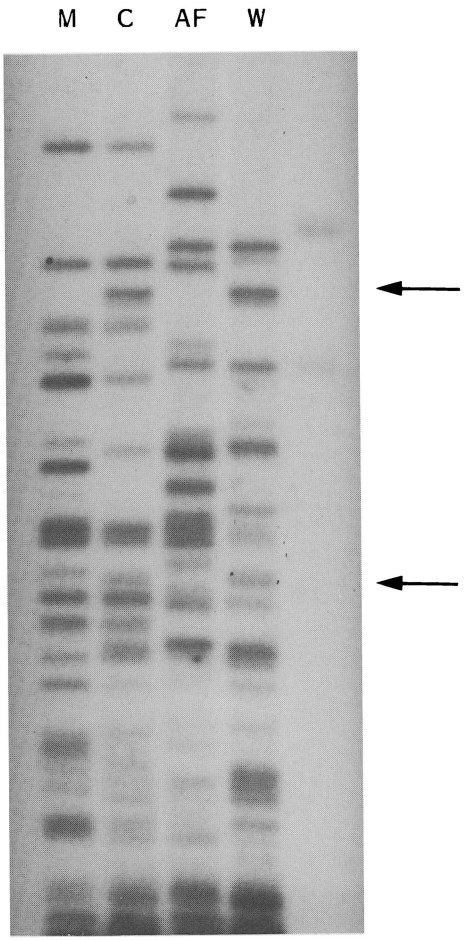


Fig. 5: DNA 'fingerprint' with minisatellite probe 33.15. Exclusions of the AF are indicated by arrows.

Fig. 6: DNA 'fingerprint' with minisatellite probe 33.6

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