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DNA POLYMORPHISMS - PRACTICAL USE

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INTRODUCTION

The Home Office Forensic Science laboratories in England and Wales group blood and blood-stains in the following systems: ABO, PGM, ACP, Hp and Gc. In combination these systems provide a mean discriminating power (DP) of 0.99. For semen stains the situation is much less satisfactory, only two systems are used routinely PGM and ABO. A third system, Lewis, is used to provide information to aid the interpretation of the results obtained by the ABO tests. Both ABO and PGM may give equivocal results particularly when a mixture of body fluids is present eg. semen and saliva, semen and vaginal secretion. Indeed in the examination of vaginal swabs taken after rape ABO testing will only reveal an antigen that is foreign to the victim in approximately 36% of cases (Werrett and Lang 1987). PGM tests on vaginal swabs are complicated by the stimulation of vaginal PGM production in the presence of semen, which tends to obscure the group of the seminal PGM (Garlo 1983). There is, therefore, a need for a sensitive highly discriminating test, particularly for the analysis of body fluids other than blood.

DNA ANALYSIS

Recently there have been several reports of the successful analysis of DNA extracted from both blood and semen stains (Gill et al 1985, 1987; Kanter et al 1985; Giusti et al 1985). In each case highly informative Restriction Fragment Length Polymorphisms (RFLP) have been examined which have originated from sequence re-arrangement rather than simple mutation of restriction sites. Probes which identify a particular type of sequence re-arrangement, variable numbers of tandem repeats, have been used either to identify multiple alleles at a single locus under hybridisation conditions of high stringency (Wong et al 1986; Nakamura et al 1987) or alternatively under low stringency conditions to reveal, simultaneously, alleles from many highly polymorphic loci (Jeffreys et al 1985a; Vassart et

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al 1987). The ability to discriminate between individuals is potentially much greater with the latter approach, Jeffreys et al calculated that the mean probability that all DNA fragments detected in one individual by a single probe, 33.15, would be detected in a second individual chosen at random is $3x10^{-11}$. The degree of individuality achieved by locus specific probes is many orders of magnitude less. Even so the combination of selected, highly informative, locus specific RFLPs has been successfully applied to the individual identification of cell lines and, in particular, for genotypic analysis following bone marrow transplantation when recipient and donor are often closely related (Knowlton et al 1986).

Traditionally for forensic scientists using conventional test systems: ABO, PGM etc the amount of variation revealed by a single analysis may be of crucial importance, since the available material is often limited. However, novel DNA techniques allow the multiple testing of one stain extract; DNA extracted from precious case material can be examined under low and high stringency conditions using a variety of probes sequentially. Thus probes that provide an efficient and informative analysis of the genomic DNA may be selected according to the requirements of the case.

DNA fingerprinting has become an established technique at the Central Research Establishment over the past two years, it has been used for a limited number of criminal cases. These include the investigation of two associated murders which has necessitated the screening of a large number of samples. For routine operational use the technique has been simplified. Recently a probe for the HVR 3' to the alpha-globin gene has been used to produce RNA transcripts that detect many loci simultaneously. Finally the use of locus specific probes, as a useful adjunct to DNA fingerprinting, is now under investigation.

DNA FINGERPRINTING

Samples for Analysis

Sufficient DNA for several analyses can be obtained from 500µl of EDTA treated blood by high speed centrifugation. The efficient extraction of DNA from untreated samples requires the physical disruption of clots. Post mortem blood samples often yield greatly reduced amounts of high molecular weight DNA, presumably native nucleases degrade the genomic DNA as membranes rupture. Indeed because of the potential degradation of DNA by native nucleases all blood samples should be stored frozen. This may require the donor to supply two samples, one for DNA analysis and one for conventional tests, if the samples are not to be brought to the laboratory promptly.

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The successful analysis of bloodstains requires approximately 50µl equivalent of whole blood compared with approximately 5-10µl of semen. Wide differences in success rate were found when vaginal swabs, taken at known times after intercourse, were examined from laboratory donors (Table 1).

Table 1. Semen contaminated vaginal swabs from laboratory donors

Donor	Time *post coitus*	Number of bands	Random Assoc.
1	1	10	$2.9x10^{-7}$
	3	10	$2.9x10^{-7}$
	7	none	
	8	9	$1.8x10^{-8}$
	8	none	
	13	3	$1.4x10^{-2}$
	13	none	
	18	10	$2.9x10^{-7}$
	21	none	
	21	10	$2.9x10^{-7}$
	22	10	$1.7x10^{-7}$
	24	10	$2.9x10^{-7}$
	24	10	$4.2x10^{-6}$
	25	9	$1.6x10^{6}$
	27	none	
	27	none	
	36	none	
	36	none	
	48	10	$2.9x10^{-7}$
2	2	8	$3.3x10^{-6}$
	7	8	$3.3x10^{-6}$
	11	none	
	19	8	$3.3x10^{-6}$
	22.5	4	$4.6x10^{-3}$
	32.5	4	$4.6x10^{-3}$
	42.5	4	$4.6x10^{-3}$

Chance association has been calculated for each molecular weight range: 4-6kb, 6-10kb and 10-20kb and the product of these quoted. Only the bands which could not be attributed to the female are recorded.

DNA of seminal origin was recovered on some swabs up to 48 hr after intercourse, however swabs from the same donor gave negative results just 7 hr after intercourse. The casework analyses (Table 2), which includes semen stains on textile material as well as vaginal swabs, has mirrored the wide difference in success rate achieved with the laboratory donors.

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Table 2. Examination of semen stained case material/swabs

Case Number	Stain Substrate	Sperm Density	Result
1	Knickers	4+H	none
	Knickers	3+H	none
2	Knickers	4+H	$5.2x10^{-4}$
3	Mac st1	4+H	$6.4x10^{-7}$
	Mac st2	4+H	$6.4x10^{-7}$
4	N.gown	2+H	$1x10^{-2}$
5	Sheet	2+H	none
	Anal swab	3+H	$5.2x10^{-4}$
6	Vag.fluid	3+H	none
7	V.S.	3+H	none
	Blouse	3+H	$9.7x10^{-4}$
8	Skirt	4+H	$5.8x10^{-8}$
	V.S.outer	2+H	$1.2x10^{-6}$
	V.S.inner	2+H	$5.8x10^{-8}$
	Pubic hair	2+H	$5.8x10^{-8}$
9	Petticoat	4+H	$2x10^{-7}$
10	Dress st1	4+H	none
	Dress st2	1+H	none
	Dress st3	4+H	$5.4x10^{-6}$
	Petticoat	2+H	none
11	Skirt	2+H	$3.8x10^{-6}$
12	Knickers	3+H	none
13	Knickers	3+H	$5.4x10^{-6}$
	V.S.lower) V.S.upper)	1+H	none
14	N.gown	2+H	none
	V.S.upper	2+H	none
	Sheet	3+H	none
	Sheet	4+H	none
15	Slip	2+H	none
16	V.S.lower	3+H	none
	V.S.mid	2+H	none
	V.S.upper	3+H	$1.1x10^{-3}$
	Knickers st1	3+H	$6x10^{-2}$
	Knickers st2	3+H	$6x10^{-2}$
	Shoe	3+H	$2.1x10^{-5}$
	Anorak	4+H	none
17	Dress st1	2+H	$8x10^{-5}$
	Dress st2	3+H	none
18	Vulval Swab	3+H	none
	V.S.outer	3+H	$5x10^{-8}$
	V.S.inner	3+H	$5x10^{-8}$
	Shirt	2+H	$5x10^{-8}$
19	Duvet cover	3+H	$2x10^{-5}$
20	Knickers	4+H	none

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The overall success rate for seminal DNA detected for all substrates was 51%. Success rate for stains was 53% (17/32) and for swabs (V.S.) 46% (6/13). The strength of the stain was estimated on a scale 0,1+H, 2+H, 3+H, 4+H. H is shorthand notation for sperm head. Chance association has been calculated using either 0.22 as the probability for band sharing (Gill et al 1987) or 0.26 depending on the hybridisation protocol (see below).

The poor correlation between assessment of sperm content, as scored on a scale 0 to 4+H, may be caused by a number of factors including storage conditions. Vaginal swabs, if not kept frozen, will create high humidity conditions within the plastic storage sleeve. DNA in bloodstains kept at high relative humidity at room temperature degrades rapidly (Gill et al 1987).

Modifications to the Method

The processing of large numbers of samples requires that the method is as simple and robust as possible. The technique has, therefore, been modified to facilitate its operational use.

Preferential Extraction of Seminal DNA from Mixtures: The preferential extraction of seminal DNA is possible because sperm heads are impervious to extraction in SDS/proteinase K/extraction buffer (0.01M Tris-HCl, 0.01M EDTA, 0.1M NaCl (pH8.0)). Female cells can, therefore, be preferentially lysed by treatment in this extraction mixture and the sperm recovered by centrifugation. However experience has shown that three washes in the extraction mixture are required, without dithiothreitol (DTT), to reliably remove the female material. The addition of DTT to the mixture causes effective lysis of the sperm head.

Dialysis and Restriction: The partial restriction of the extracted DNA, which is clearly seen by the presence of a band at the molecular exclusion point of the gel, has been eliminated by the introduction of a dialysis procedure. After the extraction and purification of the DNA (Gill et al 1985), it was reconstituted in 20µl of sterilised, distilled water and dialysed against 0.01M Tris-HCl, 0.01M NaCl pH7.6 for 2h by placing it on a floating 0.05 m Millipore filter in a 100% humidity chamber. After dialysis the DNA was recovered with a micropipette and the filter carefully washed to recover any DNA on the surface of the membrane. Restriction was carried out overnight at 37°C in approximately a 10 fold excess of *Hinf1*/core buffer made 4mM with spermidene trihydrochloride. The introduction of the dialysis procedure before restriction eliminates the partial digestion of the extracted DNA.

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Electrophoresis and Blotting - Nylon Membranes: Nylon membranes offer the advantages of much greater resilience and, more importantly, after autoradiography they can be stripped of radioactivity and re-probed. Electrophoresis and Southern blotting was carried out as previously described (Gill et al 1987). Transfer of the DNA to either nitrocellulose (Schleicher and Schull, BA85) or nylon membranes (Amersham Hybond) gave equivalent results. The effect of ultra-violet irradiation on this type of nylon membrane was found to be minimal.

Probe Preparation and Hybridisation: A synthetic 400 base pair (bp) probe Ig5ls comprising 25 tandem repeats of the core sequence (AGAGGTGGGCAGGTGG) of 33.15 was used (the latter is a 592 bp probe with unique sequence DNA flanking the 29 repeats of the core sequence (Jeffreys et al 1985b)), in addition to 33.15, to develop robust, simplified protocols for hybridisation and probe preparation. Probes of high specific activity were prepared by either primer extension or random hexanucleotide priming (Amersham Multiprime Kit). Purified inserts of 33.15 and Ig5ls were used for random priming at a concentration of 0.5ng probe/ml. Primer extension probes were purified by digestion of the phage to liberate the labelled insert followed by electrophoresis in a 1.6% low melting point agarose gel. The insert was then excised from the gel after autoradiography to confirm its position. Unincorporated free radionucleotides were removed from randomly primed probes by spun columns (Maniatis et al 1982), Sephadex G-50, followed by precipitation of the DNA with 0.25 volumes of 6M ammonium acetate and 2.5 volumes of ethanol.

All hybridisation procedures were carried out at 61°C and were either as described by Jeffreys (1985a) or Reed and Mann (1985). The latter method is less complicated; dried milk is substituted for Denhardt's solution and carrier DNA. Furthermore the Reed and Mann method uses the same pre-hybridisation and hybridisation solutions 1.5xSSPE (1.5xSSPE is 0.27M NaCl, 15mM sodium phosphate (pH7.7), 1.5mM EDTA). Post-hybridisation the filters were washed in 3xSSC (1xSSC is 0.15M NaCl, 0.015M Sodium citrate), 0.1%SDS, until the unbound radioactivity was removed, followed by several washes in 1xSSC, 0.1% SDS.

Autoradiography and Stripping of Filters: The washed filters were covered in Saran Wrap and autoradiographed for 1-7 days with Amersham MP film and Dupont lightning plus intensifying screens at -70°C. After auto-radiography filters were routinely stripped of probe by repeated washing in 0.4M sodium hydroxide at 61°C (Gill 1987).

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Interpretation and Comparison of 'Fingerprints'

The removal of carrier DNA from the hybridisation solution increases the number of bands observed per individual, between 4-20kb, from a mean of 11 to 11.6 (Figure 1). The increase in band number effects the individual specificity of the fingerprints (Table 3). The chance of coincidental band sharing was re-calculated using the profiles of 112 individuals with reference to the control sample as previously described by Gill et al (1987). The increased probability calculated, P=0.26 compared with P=0.22, reflects the increase in bands per individual. However the overall probability of chance association is of the same order of magnitude.

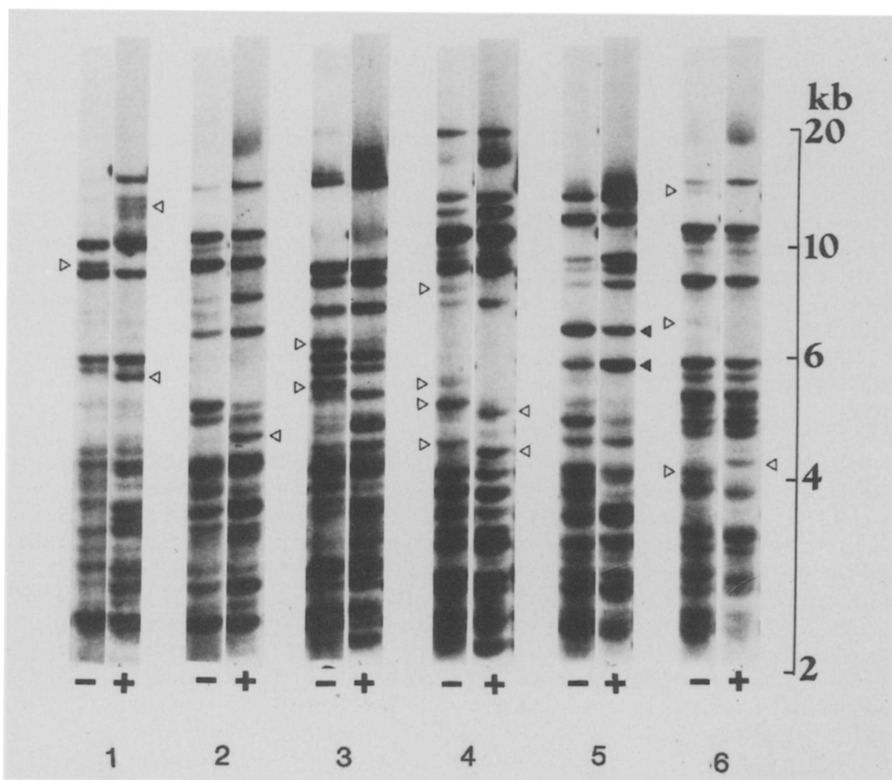

Figure 1. The effect of carrier DNA showing typical changes in banding patterns. Purified samples (1 to 6) of DNA, approximately µ1 g, were applied to the gel. Filters were hybridised in the absence of carrier DNA (-, hybridisation conditions after Reed and Mann (1985)). Following auto radiography the filters were stripped of probe and rehybridised in Denhardt's solution containing 0.5µg/ml carrier DNA and primer extended probe 33.15(+). Additional bands observed are denoted (▷); a reversal in intensity is denoted for 2 bands (▶).

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Table 3. Chance co-migration of bands in DNA fingerprints

DNA Fragment size in kb	Number of fragments per individual ± S.D.		Probability that fragment in A is present in B	
	+ carrier DNA ■	no carrier DNA □	+ carrier DNA ■	no carrier DNA □
10-20	1.3±1.0	2.1±1.2	0.07	0.16
6-10	3.8±1.3	4.2±1.6	0.2	0.28
4-6	5.9±1.3	5.3±1.4	0.26	0.27

■ As reported for 41 individuals by Gill et al (1987) using M13 sequencing primer extension in Denhardt's/carrier DNA.
□ Results for 112 individuals using oligolabelling of Ig5ls following the hybridisation protocol of Reed and Mann (1985). The mean probability that all fragments detected by probe Ig5ls in individual A are also present in individual B is given by: $0.16^{2.1}x0.28^{4.2}x0.27^{5.2}=9.8x10^{-8}$, compared with $2.5x10^{-8}$ previously reported for 33.15. Mean probability of a given band in A matching in individual B=0.26, compared with 0.22 reported for 33.15 with carrier DNA.

The detection of DNA fingerprints under conditions of low stringency requires that the conditions of the test are kept constant, small changes in the protocol, particularly the temperature of hybridisation, can cause changes in the band patterns. Furthermore if the temperature of the washes after hybridisation are allowed to rise above 61°C then the probe may be stripped from many of the most informative high molecular weight fragments. Thus it is essential that before comparisons are made between different hybridisations the controls must be carefully examined for any variance in the pattern. As a further precaution the filters can be stripped and rehybridised in the same hybridisation chamber.

CASE EXAMPLE

Within the vicinity of a small community two murders had been committed. In each case the victim, a schoolgirl, had been sexually assaulted. The murders were separated by three years, the first took place in 1983 the second 1986. A youth had been charged with murder with regard to the latter case, however, the police also suspected that he was involved in the murder that took place in 1983.

Conventional blood grouping tests: ABO, PGM and Lewis, had revealed that the semen recovered from both victims could have a common origin. However the frequency of the relevant groups was such that the semen could have come from at least 10% of the male population. (These tests were carried out

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at the Home Office Forensic Science Laboratory, Huntingdon, Cambridgeshire, UK.)

Samples were available from both cases: these comprised semen stained pubic hair from the 1983 victim and a semen stained skirt and vaginal swabs from the 1986 victim. Blood samples/blood-stains were also available from the accused and the two victims.

Preferential extraction of the seminal DNA was carried out and, together with purified DNA from the control bloods/blood stains, electrophoresis and Southern blotting was carried out as described above. The filter was pre-hybridised in Denhardt's/carrier DNA and then probed with 33.15, produced from a guanine rich template force cloned into M13 mp8.

From the results shown in Figure 2 it can be seen that the seminal profile from the 1983 victim (Lane 5) and the 1986 victim (Lanes 7, 8 and 10) are the same, virtually confirming that they had originated from the same individual (chance association of less than 5.8×10^{-8}). However these profiles are different from the profiles obtained from the suspect, Lanes 1 and 2. Also illustrated quite clearly by the figure is the effectiveness of the preferential extraction of semen which has provided an unambiguous answer even in the presence of vaginal material (See Lane 8, vaginal swab from victim 2 and Lane 9 blood sample from victim 2). There is no evidence of partial digests, as seen by the presence of a band at the molecular exclusion point, confirming the effectiveness of the dialysis procedure prior to restriction.

The likely explanation of these findings was that one man had had intercourse with the victims shortly before, or even after death, and that this man was also the murderer. The youth, who had been held in custody was released.

The use of genetic fingerprinting in this case has prevented an ill founded and costly murder trial. However since the murders took place within the vicinity of a small community and the police had exhausted the normal avenues of investigation, a screen of all men on a voluntary basis (approximately 4000), fulfilling certain criteria established by the police, was proposed. The survey was considered possible because of the combined efficacy of conventional tests and DNA fingerprinting. The conventional tests: ABO, PGM and Lewis, could be used to eliminate approximately 86% (Asec, PGM1+) of the men, the remainder would then need to be DNA fingerprinted. The screening is now underway each sample is being analysed in duplicate, over 1000 DNA fingerprinting tests are anticipated. The results of the screen will be available later this year. It is worthy of emphasis that DNA and conventional tests on semen, in particular, are not mutally exclusive even when the amount of material available for testing is limited. An extract should be made of the seminal staining and the sperm pelleted, all conventional

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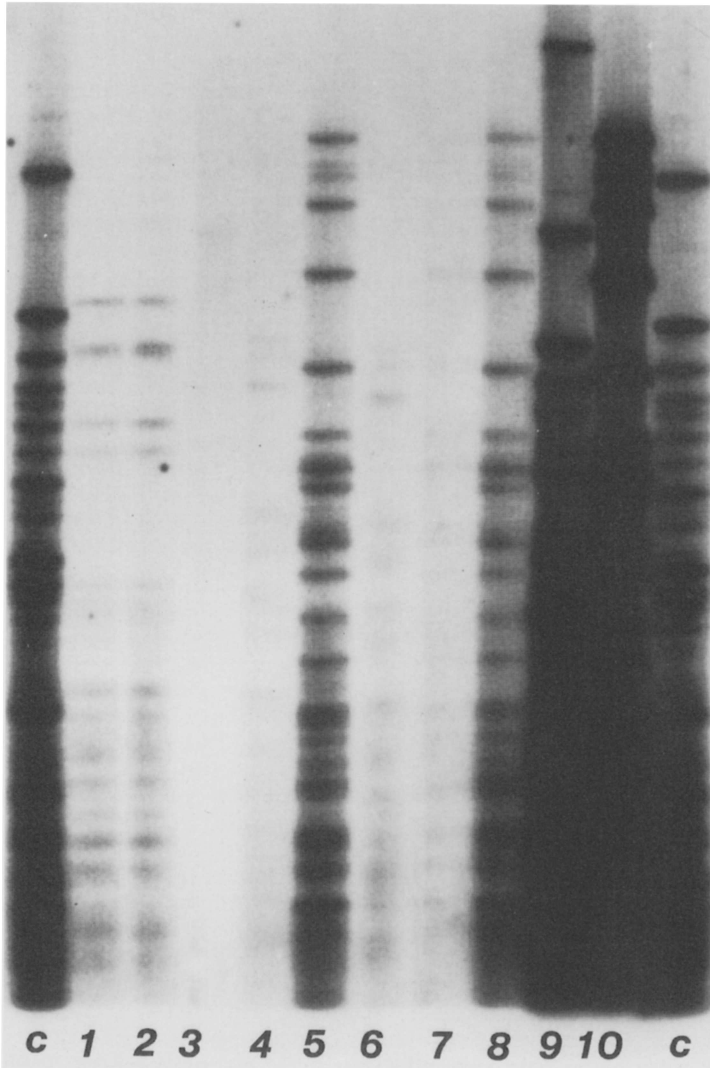

Figure 2. A case example: the association of two murders where the victims had also been sexually assaulted. The suspects fingerprints, Lanes 1 and 2, do not match the profile obtained from semen staining the pubic hair of victim 1, Lane 5, nor does it match the profiles obtained from semen staining a vaginal swab, Lane 8, and skirt, Lane 10, from victim 2. However the semen profiles obtained from the victims are identical. Note that the profile given by the blood sample from victim 2, Lane 9, demonstrates that the female contribution to the putative mixture of body fluids on the vaginal swab, Lane 8, and also perhaps the skirt, Lane 10, has been successfully eliminated.

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tests can then be carried out on the supernatant without jeopardising any subsequent DNA tests.

Prior to DNA fingerprinting at the Central Research Establishment tests were carried out on material from this case by Dr A J Jeffreys (Personal Communication). He used locus specific probes to exclude the suspect and suggested that the same individual could have been responsible for the 2 murders (frequency of chance association was calculated as 1 in 10,000). The advantage of using probes for the locus specific analysis of hypervariable regions is that the band pattern is uncomplicated, typically two bands. Population studies can reveal the heterozygosity of the locus and the common alleles can be readily determined. In contrast the simultaneous analysis of many highly polymorphic loci gives complex band patterns and the chance association of random individuals is made by statistical estimate.

A particularly useful role of locus specific probes could be, therefore, the screening of large numbers of samples.

ALTERNATIVE HYPERVARIABLE PROBES

The probes described by Jeffreys et al (1985b) were derived from tandemly repeated sequence within an intron of the myoglobin gene on chromosome 11. It is probable, therefore, that probes derived from other HVRs may also prove to be suitable candidates for DNA fingerprint analysis. Several HVRs are associated with the alpha-globin gene cluster on chromosome 16 (Higgs et al 1981; Proudfoot et al 1982; Goodbourn et al 1983; Jarman et al 1986). The alpha-globin 3' HVR, described by Higgs et al (1981) is a tandem repeat array of 17 base pairs, the number of repeats varies considerably between alleles. However no relationship is believed to exist between the core sequence of this HVR and the core sequence of the minisatellite probes of Jeffreys (Jarman et al 1986).

If a HVR probe to a tandem repeat array, like the 3' alpha-globin region, can be used to hybridise to autosomal loci throughout the human genome it may provide useful extra/alternative information to that derived from the Jeffreys probes. The minisatellites identified by the 3' alpha-globin probe should be largely independent of those identified by for example 33.15 (Jeffreys, 1985a) because of the difference in core sequence. Using RNA transcripts derived from this probe, DNA fingerprints have been obtained under hybridisation conditions previously described by Church and Gilbert (1984). We have examined the relationship between band patterns derived from both unrelated and related individuals.

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Method

DNA was extracted as for 33.15, however for the family studies, in particular, the conditions of electrophoresis were modified: a 0.85% agarose gel was used and the samples run until the 4kb marker was 3cm from the end of the gel.

The labelled RNA transcripts of the probe to 3' HVR were produced using a commercial SP6 labelling kit (Amersham Cat. No. RPN 1506). After removal of the DNA template the probe was purified by G50 Sephadex spun column (Maniatis et al 1982), followed by phenol/chloroform extraction and alcohol precipitation in the presence of 6M ammonium acetate.

The probe was hybridised to the filters at 65°C overnight using the method of Church and Gilbert (1984). Autoradiography was carried out as for 33.15.

Chance association

DNA fingerprints from 40 unrelated individuals were examined (Figure 3). On average 22 bands were visualised in the range 4-23kb per individual, this compares favourably with

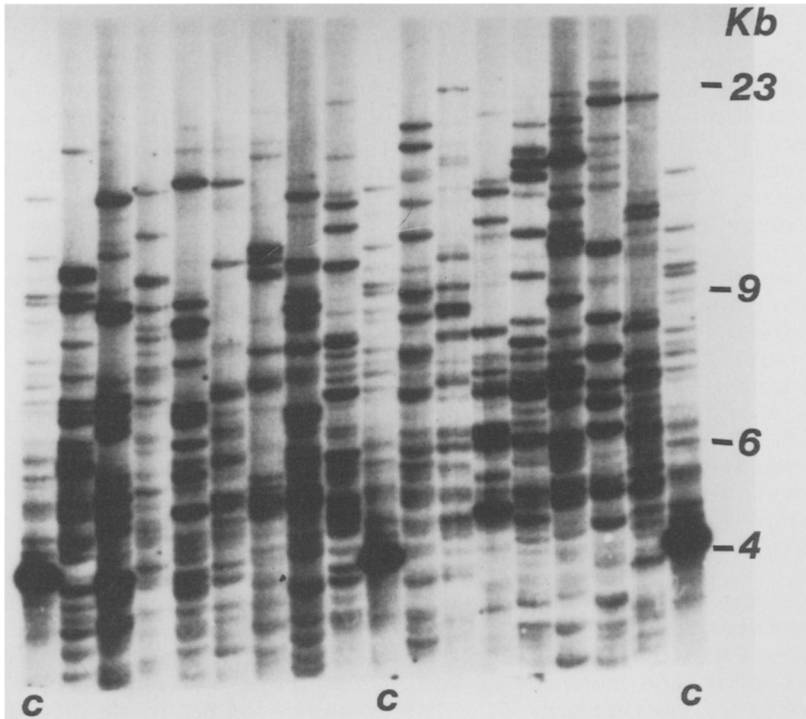

Figure 3 DNA fingerprints from 15 unrelated individuals. The control sample is denoted by (c).

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an average of 15 for 33.15 (Jeffreys et al 1986). The patterns produced were highly variable as reflected by the band sharing statistics and probabilities of individuality given in Table 4.

Table 4. Similarities of DNA fingerprints between random pairs of individuals for 3' alpha-globin

DNA fragments size (kb)	No. of fragments per individual (SD)	Probability that fragment A is present in B
10-23	5.25(±1.7)	0.16
6-10	6.8 (±1.92)	0.2
4-6	9.97(±1.44)	0.31

Mean probability that all fragments detected in an individual A are also present in B is given by:

$$0.16^{5.25} \times 0.2^{6.8} \times 0.31^{9.97} = 7.00 \times 10^{-15}$$

Segregation analysis

The segregation of individual DNA bands was investigated by examining the inheritance of fragments in a large sibship comprising 12 children (Figures 4 and 5). It was possible to score 25 paternal bands and 22 maternal bands using autoradiographs of different intensities. The transmission of scored bands is given in Table 5, fragments of less than 4kb were not scored.

The segregation data were analysed according to the method of Jeffreys et al (1986) in which segregations were compared against the expected binomial distribution. Within the bands there were two which were present in all of the children, as these were probably from homozygous loci they were eliminated from the transmission frequency calculations. Heterozygous paternal and maternal bands were transmitted to approximately 44% of the progeny. The number of children receiving each fragment was, therefore, consistent with a 1:1 segregation and approximated to the expected binomial distribution.

There was no evidence of linkage indicating that the DNA fragments are randomly distributed throughout the genome. However there were 3 allelic pairs. Presumably the second alleles of the remaining loci were smaller than 4kb and, therefore, not observed. The absence of linkage and the small amount of allelism would suggest that this probe can be used for paternity studies and analysis of linkage with genetic disease.

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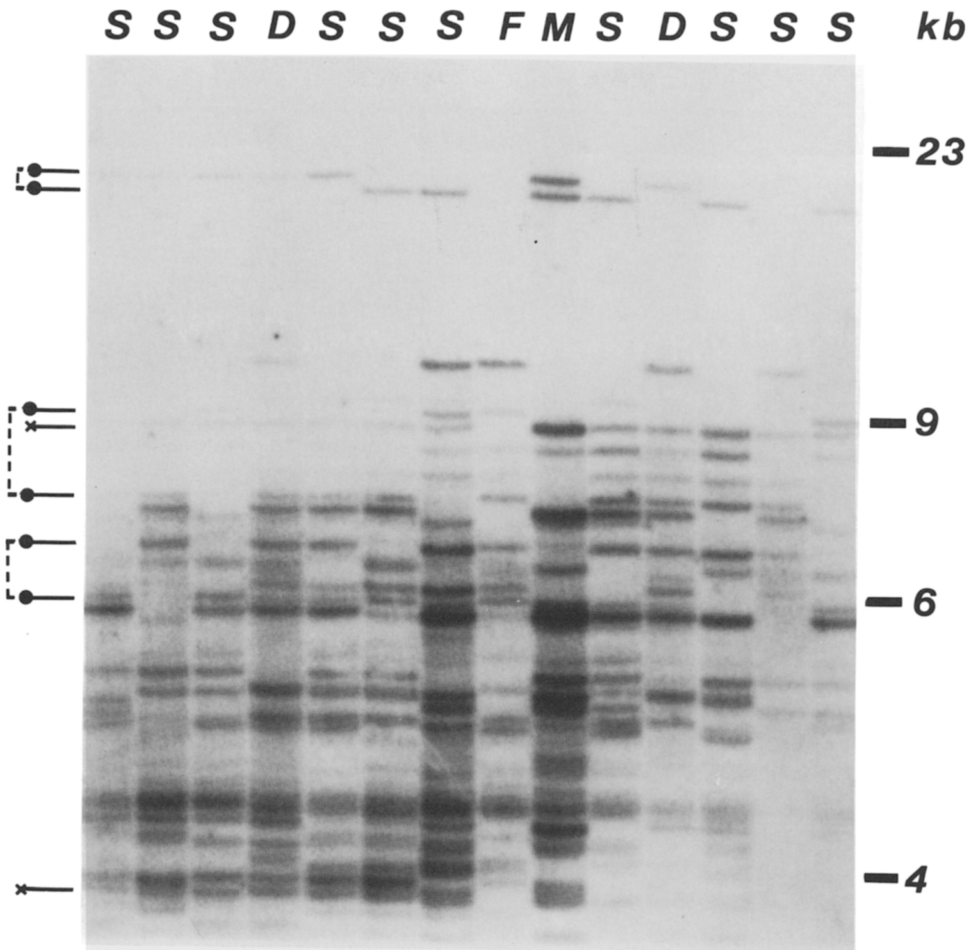

Figure 5. Short exposure autorad of a large sibship, see Figure 4 Legend.

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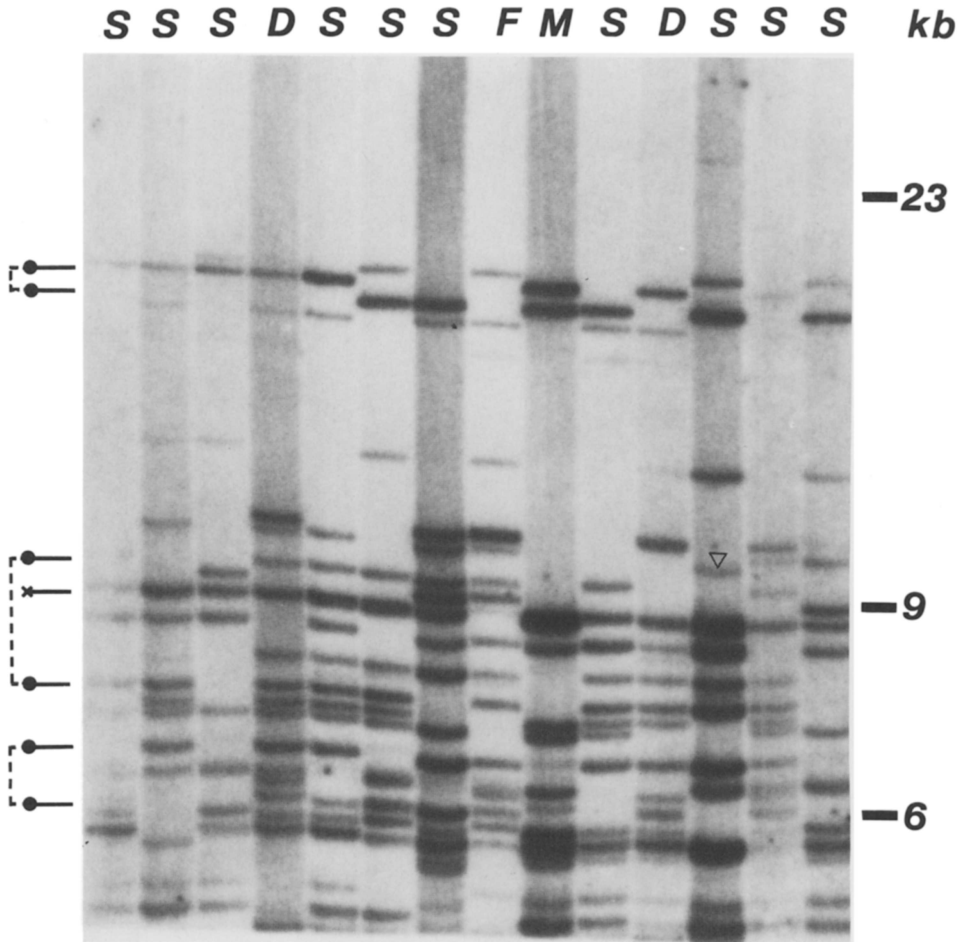

Figure 4. A sibship of 12 children showing the Mendelian inheritance of multiple DNA fragments. Allelic pairs are indicated by the dotted lines, possible homozygous loci are denoted by (*). Figure 5 is a short exposure autorad revealing fragments between 4kb and 23kb whereas this figure shows improved resolution to the 6 to 23kb range. F=Father, M=Mother, S=Son, D=Daughter,▽= Mutation to a new length allele.

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Table 5. Segregation of DNA fragments in a large sibship

Transmission of to Number children	Father Single fragment Obs	Father Single fragment Exp	Father Pair fragments Obs	Father Pair fragments Exp	Mother Single fragment Obs	Mother Single fragment Exp	Mother Pair fragments Obs	Mother Pair fragments Exp
0	0	0	(0)	0	0	0	(0)	0
1	0	0.1	2	1	0	0.1	0	1
2	1	0.4	3	3	2	0.4	2	3
3	3	1.3	11	11	1	1.1	14	10
4	4	3	17	25	6	2.7	16	23
5	6	4.8	38	41	4	4.3	32	38
6	5	5.6	44	44	2	5.0	41	43
7	3	4.8	43	41	4	4.3	39	38
8	2	3	33	25	2	2.7	28	23
9	1	1.3	13	11	1	1.1	11	10
10	0	0.4	5	3	0	0.4	7	3
11	0	0.1	1	1	0	0.1	0	1
12	0	0	0	0	(0)	0	0	0

(0) Homozygous and allelic bands removed for transmission analysis Transmission frequencies:

Father's fragments: 44.3%±3.1%
Mother's fragments: 44.0%±3.4%

A mutation was observed in this family (Figure 4), two further mutations have been observed in other family studies. Pooling the data gives a mutation rate of approximately 1 in 750, derived from 3 observed mutations in 2202 meioses, comparable to that of Jeffreys et al (1985). It may be postulated, in agreement with the linkage analysis above, that linkage disequilibrium is unlikely to exist in probes which have significant mutation rates. Thus the estimate of random association, even in the presence of linkage, would remain unaffected preserving the value of the test for criminal work where discrimination between individuals is paramount.

Finally it should be remembered that the Tm of RNA-DNA hybrids is significantly greater than that of DNA-DNA hybrids and, therefore, hybridisation at 65°C involving RNA probes would presumably be at 'low' stringency.

The full protocols for the use of these HVR probes are available, requests should be addressed to DJW. It is hoped that the probe 3' alpha-globin will form the basis of an international collaborative study for paternity and criminal work.

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