THE APPLICATION OF DNA-PRINT FOR IDENTIFICATION FROM FORENSIC BIOLOGICAL MATERIALS

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Abstract

DNA purified from more than 170 forensic samples was characterized by restriction fragment length polymorphism analysis. DNA isolated from dried blood stains, various tissues, and semen stains was digested with the restriction endonuclease Pst 1, size separated by agarose gel electrophoresis, transferred to nylon membrane and hybridized with DNA probes which recognize hypervariable regions in the human genome. The size of DNA fragments, reacting to these probes, was measured with a computer assisted digitizing system and a likelihood of identity calculated from databases containing the frequency of the alleles identified with each probe. The combined discrimination potential with 4 DNA probes that hybridize to hypervariable regions is greater than 10^8. The present report also summarizes some of our experience in the analysis of forensic biological evidence.

Introduction

Differences which occur at the DNA level can be visualized as restriction fragment length polymorphisms (RFLPs) and can be used as genetic markers for disease diagnosis (1) and identification (2,3). The use of a number of DNA probes which recognize single locus hypervariable regions allows the determination of a genotype (DNA-Print) characteristic for an individual (4). In cases where identification is necessary from forensic evidence, a DNA-Print can yield vital information as to biological origin. We present in this report results of the analysis of forensic samples and the power of this system to resolve issues of identity.

Materials and Methods

DNA Isolation. DNA was isolated from peripheral blood, dried blood stains, or semen stains as previously reported (2-4). Kidney, liver muscle, and brain tissues were minced and processed the same as peripheral blood. The quantity of DNA isolated was determined in a spectrophotometer at 260 nm and its integrity by electrophoresis in agarose gels. DNA (0.2-5.0 ug) was digested with the restriction endonuclease Pst 1 and size separated by electrophoresis on 0.9% agarose gels. Molecular weight standards composed of lambda bacteriophage and phi X174 DNAs cut with various restriction endonucleases were incorporated in each gel to allow size determination of the polymorphic DNA bands from the samples. After transfer to a nylon membrane, the blots were hybridized (2) to ^{32}P-labelled DNA probes (6) and exposed to X-ray film. If rehybridization of a filter to a different probe was necessary, the signal was removed by incubating 30 min. at 65^{o}C in 50% formamide, 2XSSCP (2), 1% SDS. Filters could be reused at least 6 times without significant loss in sensitivity. The resulting autoradiographs were analyzed with a computer assisted

Advances in Forensic Haemogenetics 2
Edited by W. R. Mayr

digitizing system as previously described (4).

DNA Probes and Data Analysis. The arbitrary DNA probes used for hybridization were derived from human genomic libraries. The following DNA probes recognize a single locus: pAC061, pAC225, pAC255, and pAC256. One DNA probe, pAC225 (derived from 29C1)(5) detects from one to six bands per person. The probe, pAC004, hybridizes to a 3.7 Kb Pst 1 Y-specific repeated DNA sequence and can be used to show the presence of male DNA. The probe pAC101 hybridizes to human specific repeated DNA fragments and can be used to confirm the human origin of the samples. The probe pAC267, derived from an E. coli ribosomal gene clone, kindly provided by Dr. Squires, Columbia University, was used to detect the presence of bacterial DNA. The properties of these probes are summarized in Table 1. The number of distinct alleles in the hypervariable loci varied from 30 to >100.

A database containing the frequency of the fragment sizes detected for each locus has been established using non-related American Black and Caucasian individuals. The allele frequency distribution of these polymorphic loci will be published elsewhere. For the calculations of identity, the allele frequency of a particular DNA fragment in the population was obtained using a mathematical expression incorporating band resolution and standard deviation (manuscript in preparation).

Results and Discussion

Following extraction of DNA from forensic samples, the amount and integrity of the DNA was determined by electrophoresis as described in Methods. If sufficient DNA was available for analysis, it was digested with Pst 1. About 0.1-0.5 micrograms of this digest was fractionated by electrophoresis on a gel and examined for completeness of digestion. The DNA was transferred to a nylon membrane and hybridized sequentially with the Y-specific probe (pAC004), the human specific probe (pAC101), and the bacterial specific probe (pAC267). The results of these hybridizations indicated whether the sample contained undegraded human male/female DNA, and the presence of contaminating bacterial DNA. The relative intensity of the hybridization signal with the human vs. bacterial probes gave a qualitative estimate of the amount of human specific DNA in the sample. Next, a diagnostic gel(s) was used to fractionate the DNA samples so as to maximize resolution in the 2-20 Kb DNA size range. Following transfer to a nylon membrane, the DNA from the diagnostic gel was hybridized to the ^{32}P-labelled probes that recognize hypervariable regions.

We have analyzed 20 cases of sexual assault where the evidence examined included semen stains on undergarments, vaginal aspirates, and/or vaginal swabs. Microscopic examination of the samples showed the presence of sperms in 40% of cases. In these cases sufficient human specific DNA was available for analysis and this was confirmed by the presence of specific hybridization signal with the probes recognizing the hypervariable loci. In 2 of the 8 successful rape cases (Table 2) we could detect the presence of some bacterial DNA contamination in the DNA isolated from evidence material. However, the amount of bacterial DNA was small and did not interfere with the interpretation of the results. Because of the non-sterile nature of the samples, naturally occurring bacteria can propagate and its DNA will co-purify with

the human DNA. This can lead to false estimates of the amount of DNA in the sample. In the other 60% of cases no significant number of sperms were observed. DNA purification from those samples yielded degraded DNA of bacterial origin or no DNA. The discrimination potential of a combination of four DNA probes that hybridize to hypervariable regions, in American Blacks and Caucasians, is shown in Table 3. The calculations were performed using the formula described by Fisher (7). The values represent the average number of people discriminated with each probe.

The following are examples of some of these forensic cases:

Case 1 - Sexual Assault. A vaginal aspirate was recovered from a rape victim. DNA was isolated from this sample as well as from peripheral blood of both victim and suspect. The amount of high molecular weight DNA recovered from the semen sample was approximately 25 ug. Results indicated that, using 3 probes, the DNA-Print pattern seen in the evidence (figure 1A,B lane 2) matched that of the suspect (lane 3) and it was different to that of the victim (lane 1). The frequency of the matching pattern in a Caucasian population was calculated as 1 in 12,000,000.

Case 2 - Homicide. A car was found abandoned and fragments of brain tissue were found in the grill of the car. The owner of the car was reported missing, but no body was found. DNA was isolated from the brain tissue (>100 ug of DNA) and also from the peripheral blood of the alleged parents of the victim. The DNA samples were hybridized to 4 probes and in each case the pattern of bands from the evidence (figure 2A,B,C lane 2) was consistent with having been derived from the alleged parents (lanes 1 and 3). Statistical analysis indicated that it was 160,000 times more likely that the DNA isolated from the brain tissue might have been from the offspring of the two parents than from a random individual.

Case 3 - Product of conception in a rape case. DNA was isolated from an abortus recovered from a rape victim. Four individuals were tested, with 3 probes, as possible biological fathers. The results excluded three of the suspects and included a fourth one (figure 3A,B lane 6) with a probability of paternity of 99.9%.

Case 4 - Homicide. A 2 cm blood stain on cloth was recovered from a murder scene. Blood samples were provided from the victim and a suspect. The DNA-Print patterns with 3 probes indicated that the unknown stain (figure 4A,B lane 2) matched the pattern of the victim (lane 1) and it differed from that of the suspect (lane 3). The frequency of the matching pattern in the Black population was 1 in 150,000,000.

Table 1. DNA probes used for the analysis of Pst 1 digested DNA.

Probe	Specificity	Number of Alleles
pAC004	Y-specific	NP
pAC061	single locus	> 80
pAC225	single locus	>100
pAC255	single locus	> 50
pAC256	single locus	> 30
pAC101	human specific	NP
pAC267	bacteria specific	NP

NP: not polymorphic

Table 2. Summary of Forensic Cases.

CASE TYPE	NUMBER	SUCCESSFUL DNA	NO HUMAN DNA	DEGRADED
RAPE - ID	20	8	7	5
RAPE - PATERNITY	11	9	0	2
MATERNITY	3	2	0	1
PATERNITY - BODY ID	3	2	0	1
OTHER - STAINS	10	6	2	2

Table 3. Discrimination potential of 4 DNA probes used for identification.

	DNA probes				
	pAC061	pAC225	pAC255	pAC256	
Ethnic group					Total
Black	143	350	244	62	7.6×10^8
Caucasian	267	350	154	51	7.3×10^8

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Figure Legends.

Figure 1. DNA-Print pattern from a sexual assault case. Lane 1, DNA isolated from the victim. Lane 2, DNA isolated from the evidence, vaginal aspirate. Lane 3, DNA isolated from the suspect. M indicates size marker lanes and numbers along the side represent sizes in kilobases. (A). Results with pAC255 and pAC256. (B). Results with pAC225.
Figure 2. DNA-Print pattern from a homicide. Lanes 1 and 3, DNA isolated from putative mother and father respectively. Lane 2, DNA isolated from the evidence, brain tissue. (A). Results with probes pAC225 and pAC255. (B). Results with probe pAC061. (C). Results with probe pAC256.
Figure 3. DNA-Print pattern from a product of conception rape case. Lane 1, DNA from the rape victim. Lane 2, evidence (abortus material). Lanes 3-6 suspects 1 to 4 respectively. (A). Results with probes pAC255 and pAC256. (B). Results with probe pAC225.
Figure 4. DNA-Print results from blood stains. Lanes 1, DNA isolated from the homicide victim. Lane 2, DNA isolated from unknown blood stain. Lane 3, DNA isolated from the suspect. (A). Results with probe pAC225. (B). Results with probes pAC225 and pAC256.

Figure 1.

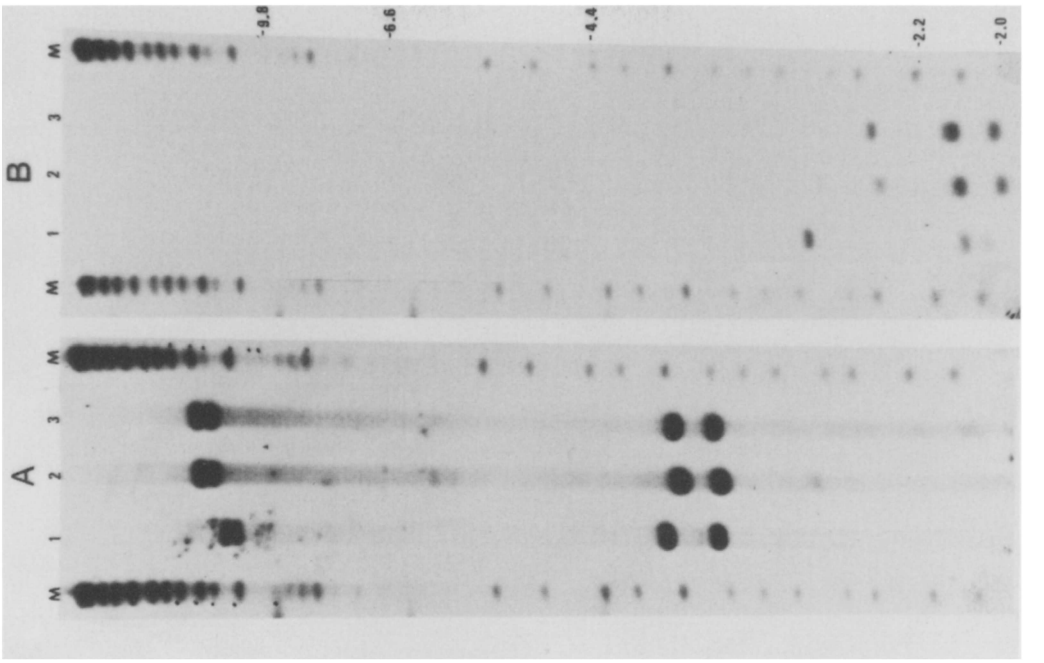

Figure 2.

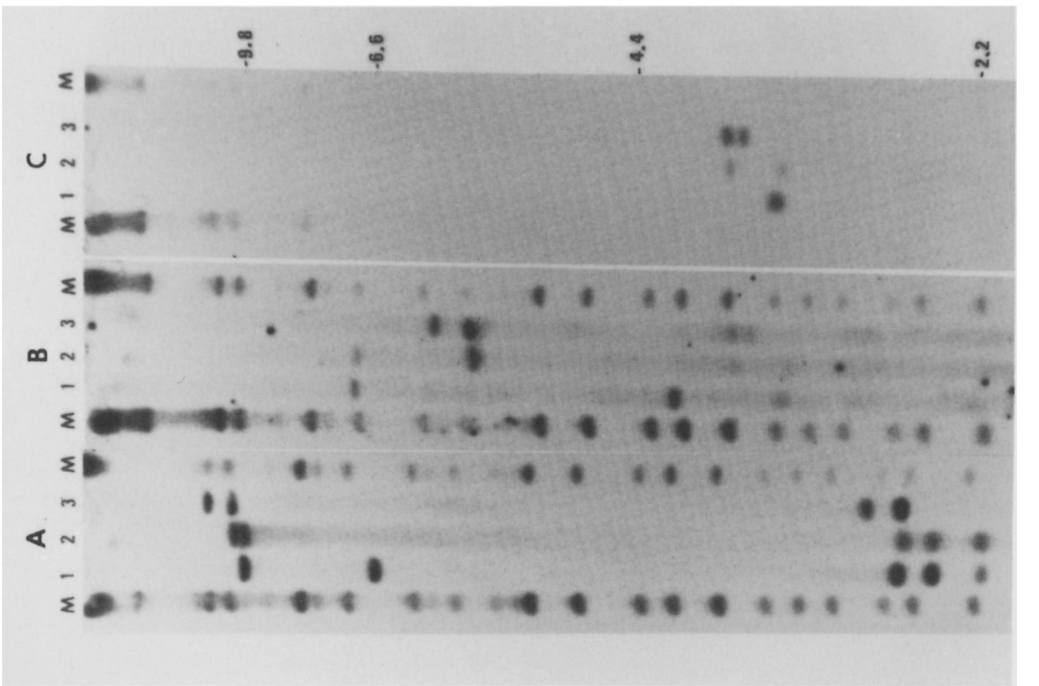

Figure 3.

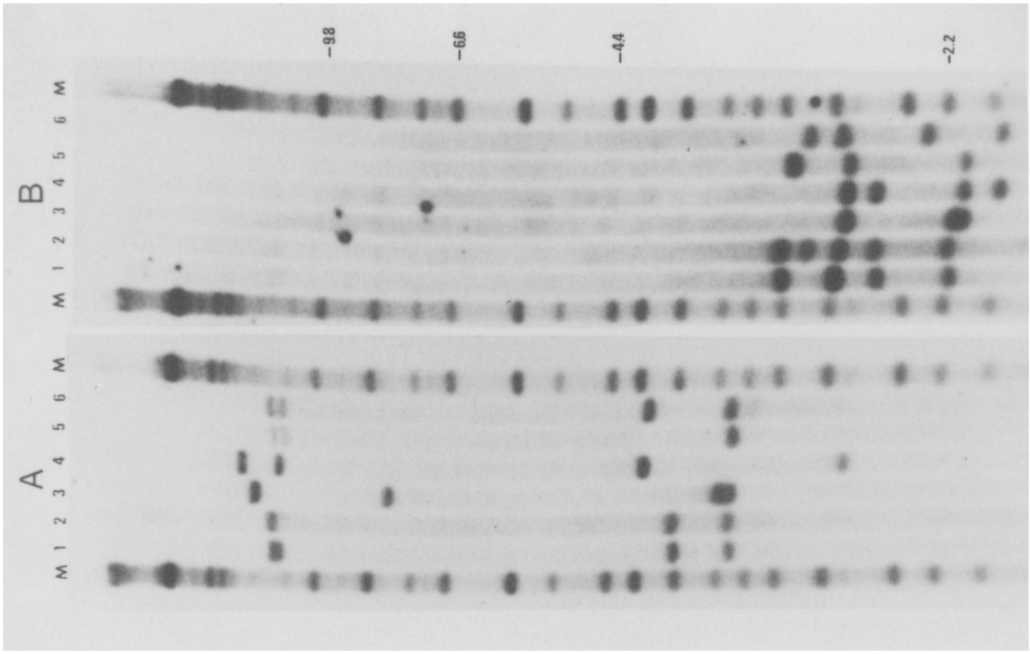

Figure 4.