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BamHI polymorphism of locus D2S44 in a West German population as revealed by VNTR probe YNH24

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Summary. BamHI polymorphism at the VNTR locus D2S44 was investigated, concentrating on band frequencies, mutation rate and confirmation of Mendelian inheritance. In this series 39 restriction fragments showing frequencies less than 10% could clearly be distinguished. No mutations could be observed and the Mendelian character of inheritance is beyond reasonable doubt.

Key words: DNA polymorphism – VNTR probe YNH24 – Band frequency – Mutation rate

Zusammenfassung. Der locus-spezifische BamHI/YNH24-DNA-Polymorphismus wurde in einer westdeutschen Stichprobe ($n = 122$) untersucht mit dem Ziel, Daten über die Fragmenthäufigkeiten, Mutationsraten und Vererbungsweisen zu erhalten. 39 Restriktionsfragmente konnten zweifelsfrei voneinander unterschieden werden. Keines der Fragmente erreichte eine Häufigkeit von mehr als 10%. Die Restriktionsfragmente werden der autosomal kodominanten Vererbungsweise entsprechend vererbt. Hinweise auf eine Mutation liegen nicht vor.

Schlüsselwörter: DNA-Polymorphismus – VNTR-Sonde YNH24 – Restriktionsfragment-Häufigkeit – Mutationsrate

Introduction

Since the first report of a highly polymorphic locus in human DNA [26], a number of additional hypervariable loci have been discovered. Estimates derived from the frequency of recombinants in genomic libraries which detect hypervariable loci indicate that there may be at least 1500 highly variable loci [15]. A systematic approach to the isolation of additional hypervariable sequences has been described by Nakamura et al. [18]. From 372 clones analysed, 77 revealed a VNTR (variable number of tandem repeats) polymorphism and 18 detected loci with heterozygosities > 80%.

Increasing emphasis on the rights of children has led to improvement of the methods for establishing paternity or non-paternity. The history of forensic hemogenetics shows that research and science are closely related to practical work in the field of DNA technology [1–14, 16–19, 21–24]. It is accepted that DNA technology is becoming an essential tool for the improvement of forensic evidence. However, basic reliability and biostatistical standards established in conventional blood group tests should also be applied to the forensic use of DNA polymorphisms. The aim of this paper is to present family and population genetic data concerning the BamHI polymorphism of probe YNH24 in West Germany.

Materials and methods

The blood samples tested for this study were routinely examined in the following conventional blood group systems: ABO, MNSs, Rhesus, P, Kell, Duffy, Kidd, Lutheran, Colton, Hp, Gc, Gm, Km, C3, C6, Bf, Tf, PLG, Pi, FXIII B, A2HS, acP, PGM_1, AK, ADA, 6-PGD, GPT, EsD, GLO, PGP and HLA-ABC by means of standard techniques. Whether all or only some of these systems were tested depended on the problems involved in a given case.

Individuals tested. 1 Multi-member three-generation family (94 members); 91 Father-mother-child triplets; 17 Mother-child duplets; 122 Unrelated individuals.

DNA preparation and processing. High molecular weight DNA was isolated by a phenol-free procedure from 2–10 ml peripheral blood [17, 20]. DNA samples (5 µg) were digested with restriction enzyme BamHI (Pharmacia) overnight, according to the manufacturer's specifications (7 units/µg DNA). Fractionation of DNA was carried out by electrophoresis in a 0.7% agarose (Pharmacia) gel (20 cm long). Electrophoresis was performed at 30 V for about 36 h until the 2-kb fragment had migrated 13 cm. Following electrophoresis the DNA was transferred to Hybond-N membranes (Amersham).

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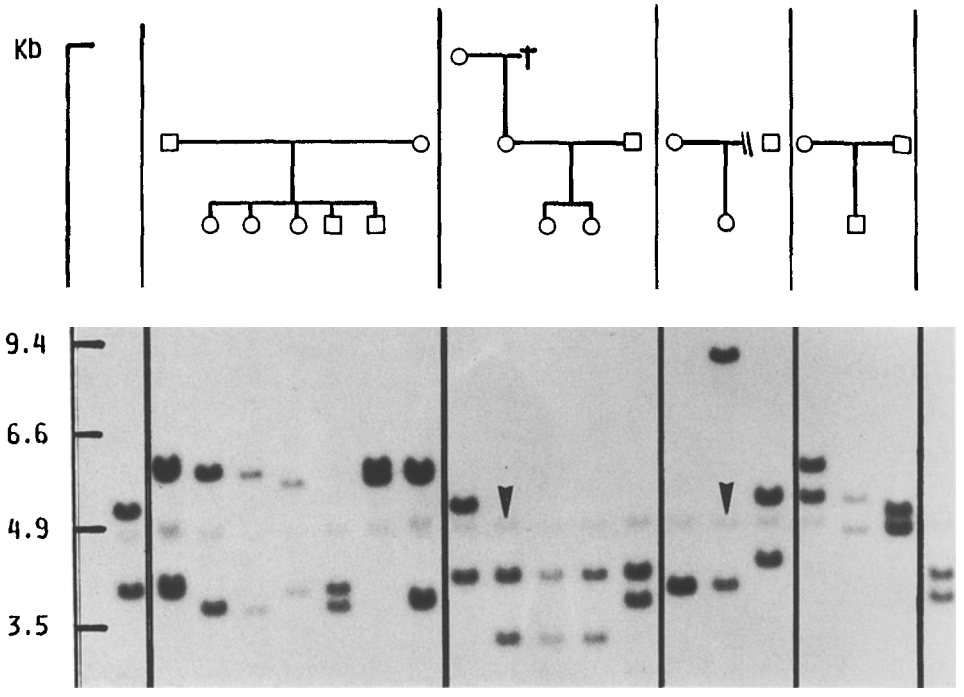


Fig. 1. BamHI/YNH24 polymorphism. Due to slightly reduced stringency conditions a constant extra fragment *(arrow)* becomes visible. The fragment is about 4.8 kb in size

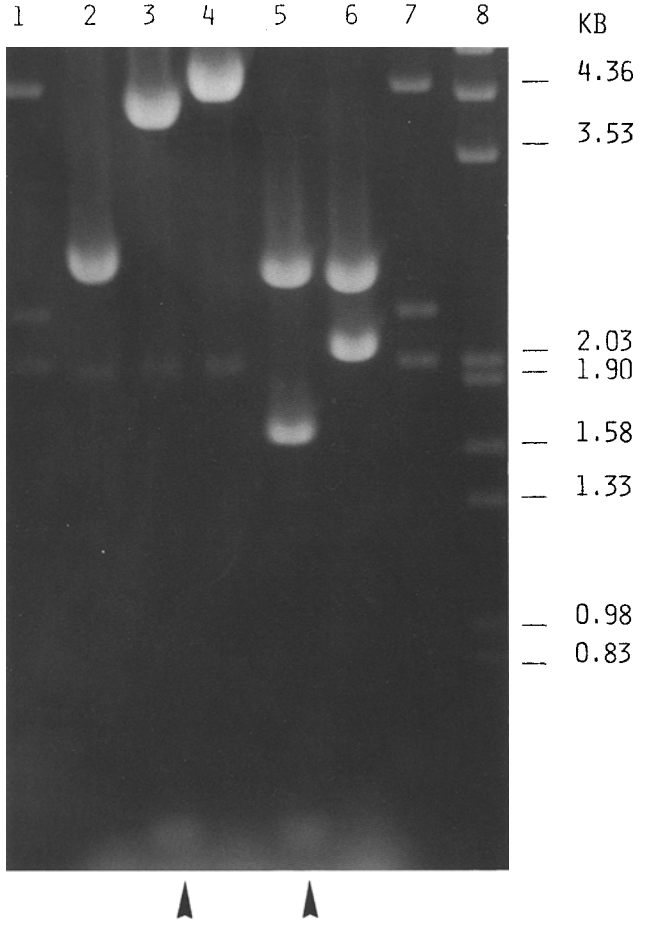


Fig. 2. Digestion experiments carried out to demonstrate that the insert has a BamHI restriction site. *Lane 1,* Lambda HindIII; *lane 2,* pYNH24 undigested (pUC 18-containing probe); *lane 3,* BamHI-digested pYNH24 (*arrow* indicates extra fragment); *lane 4,* EcoRI-digested pYNH24; *lane 5,* BamHI/PstI-digested pYNH24 (*arrow* indicates extra fragment); *lane 6,* EcoRI/HindIII-digested pYNH24; *lane 7,* Lambda HindIII; *lane 8,* Lambda EcoRI/HindIII

DNA hybridization. The probe YNH24 was labelled with ^{32}P by using an oligolabelling kit (Pharmacia) according to the manufacturer's recommendations. Following hybridization, filters were washed twice (2 × 10 min at room temperature) in 2 × SSC, 1.5% SDS buffer followed by a third washing step (1 × 15 min at 62°C) in 1 × SSC, 1.0% SDS buffer. Radioactive-labelled bands were visualized at −20°C as described elsewhere [10]. Once the results were available (time of exposure: 1–2 days) radioactive DNA probes could be stripped for further hybridizations.

Manual fragment size calculation. The size of the detected fragments was calculated by using Eco RI/HindIII- and Bst EII-digested Lambda DNA, a high molecular weight marker (BRL) and a 1-kb ladder (BRL) as external markers and manual calculation by means of a plotted curve of the DNA size standards [20]. The mobility of fragments was measured on the autoradiographs by two persons independently.

Calculation of the standard deviation. Genomic DNA from a laboratory member was used as the internal control. After digestion with HinfI, this DNA was hybridized simultaneously with probes YNH24 [18], MS1, MS31, MS43 [25], and G3 [24]. Because of heterozygosity at all loci involved, 10 different fragments ranging from 1.7 kb–10.8 kb were observed on at least 23 different autoradiographs, allowing multiple kilobase size measurements.

Source and description of probe YNH24. Probe YNH24 was kindly donated by Y. Nakamura (Howard Hughes Medical Institute, Salt Lake City, Utah) and is manufactured by subcloning a 2.0-kb MspI fragment from cosmid YNH24 isolated by HBV-2 oligonucleotide (GGAGTTGGGGGAGGAG) [18] into the AccI site of pUC18.

Investigation of probe YNH24. As Fig. 1 shows, BamHI/YNH24 polymorphism exhibits an extra and constant restriction fragment about 4.8 kb in size. Intensified washing procedures reduced the visibility of that band. Digestion studies were undertaken in order to clarify the origin of this constant fragment and to exclude the possibilities that the appearance of this band is due to either partial digestion of DNA samples and/or to contamination during DNA preparation. After BamHI digestion (even after a double digest with PstI and BamHI) of the vector an additional fragment of about 0.3 kb appeared (Fig. 2), indicating that the insert carries a BamHI recognition site. This constant fragment is regarded as a useful internal kilobase size indicator.

Table 1. Size and number of observed DNA fragments

Size (kb)	Number	Size (kb)	Number
2.6 ± 0.05	1	5.0	5
3.0 ± 0.1	2	5.1	22
3.2	1	5.2	18 (2)
3.3	3	5.3	8
3.4	4	5.4	7 (1)
3.5	7 (2)	5.5 ± 0.2	8 (1)
3.6	10 (2)	5.6	10 (1)
3.7	19	5.7	4
3.8	20 (1)	5.8	2
3.9	13 (3)	6.0	6
4.0 ± 0.15	6 (1)	6.1	2
4.1	9	6.2	1
4.2	3	6.3	1
4.3	12 (2)	6.4	2
4.4	3	6.6	1
4.5	4	6.8	1
4.7	7	6.9	1
4.8	8	7.7 ± 0.3	1
4.9	10	8.7	1
		8.8	1

39 DNA fragments could be clearly distinguished. Numbers in brackets indicate how often they appeared as homozygotes. Incorrect measurements (± 1 mm) would lead to false kilobase size estimations ranging between 0.05 and 0.3 kb. This assumption is consistent with the experimental data reported in part 2 of "Results"

Results

1. Range of fragment sizes

In our series the BamHI polymorphism revealed restriction enzyme fragment sizes of between 2.6 and 8.8 kb. The fragments and their distributions are listed in Table 1.

2. kb-Size measurement: determination of the standard deviation

For practical reasons, the scale was subdivided into steps of 100 bp. As outlined in Methods, DNA from one of the laboratory staff was used as laboratory standard. Simultaneous hybridization of this DNA with five probes (YNH24, MS1, MS31, MS43, and G3) resulted in the demonstration of ten bands.

The measurements obtained with Lambda HindIII, Lambda EcoRI/HindIII, Lambda BstEII, and a 1-kb ladder are listed below

Total number of measurements	63	65	67	65	61
Minimal fragment size	10.00	9.25	7.60	5.90	5.30
Maximal fragment size	11.50	10.00	8.80	6.25	5.80
Mean fragment size	10.86	9.58	7.96	6.11	5.60
Standard deviation	0.29	0.16	0.21	0.09	0.11
Variance	0.08	0.03	0.04	0.01	0.01
Total number of measurements	67	24	23	59	40
Minimal fragment size	5.15	4.05	2.85	2.35	1.55
Maximal fragment size	5.80	4.30	3.10	2.7	1.85
Mean fragment size	5.35	4.20	2.98	2.50	1.70
Standard deviation	0.12	0.06	0.08	0.08	0.04
Variance	0.02	0.00	0.01	0.01	0.00

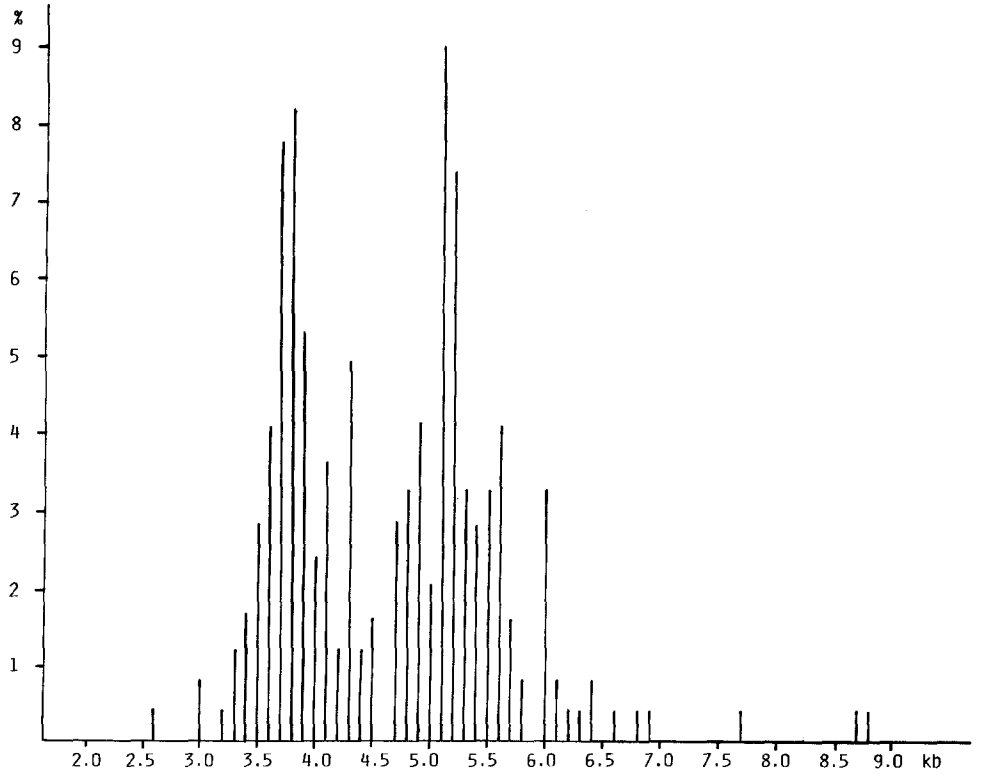


Fig. 3. Fragment size distribution of BamHI RFLP detected by probe YNH24 in 122 unrelated individuals

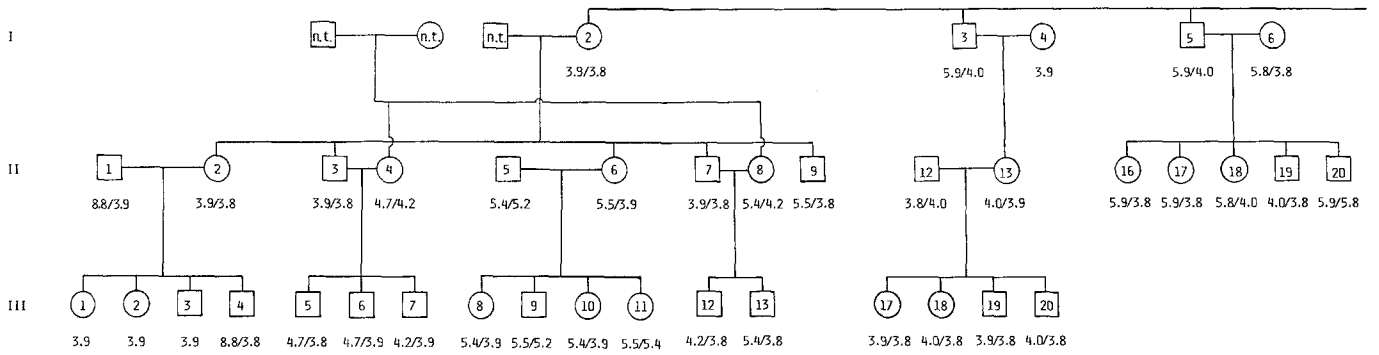


Fig. 4. Pedigree of a multi-member three-generation family

Table 2. Numbers of observed "phenotypes" (Nominations are given by commencing with the largest fragment)

Size (kb)	*n*	Size (kb)	*n*	Size (kb)	*n*	Size (kb)	*n*
*3.5	2	4.8/4.7	1	5.2/4.1	1	5.6/4.9	2
3.6/3.0	1	4.9/3.4	1	5.2/4.3	1	5.6/5.3	1
3.7/3.4	2	4.9/3.5	1	5.2/4.8	1	*5.6	1
*3.7	2	4.9/3.8	1	5.2/4.9	1	5.7/3.3	1
3.8/3.3	1	4.9/4.0	1	*5.2	2	5.7/4.5	1
3.8/3.6	2	4.9/4.7	1	5.3/3.0	1	5.7/4.9	1
3.8/3.7	2	5.0/3.6	1	5.3/3.2	1	5.8/3.8	1
*3.8	1	5.0/4.1	1	5.3/5.1	3	5.8/5.2	1
3.9/3.7	1	5.0/4.5	1	5.3/5.2	2	6.0/3.6	1
3.9/3.8	1	5.0/4.7	1	5.4/3.3	1	6.0/4.3	1
*3.9	3	5.0/4.8	1	5.4/4.2	1	6.0/4.7	1
4.0/3.8	2	5.1/3.4	1	5.4/4.3	1	6.0/4.8	1
*4.0	1	5.1/3.6	3	5.4/5.2	1	6.0/5.4	1
4.1/3.5	1	5.1/3.7	1	*5.4	1	6.0/5.5	1
4.1/3.8	3	5.1/3.8	3	5.5/3.8	1	6.1/5.7	1
4.2/4.1	1	5.1/3.9	1	5.5/3.9	1	6.3/5.1	1
4.3/3.6	1	5.1/4.1	2	5.5/5.2	1	6.4/5.1	1
4.3/3.7	1	5.1/4.5	1	5.5/5.3	1	6.4/6.1	1
4.3/3.9	1	5.1/4.8	2	*5.5	1	6.6/6.2	1
*4.3	2	*5.1	1	5.6/2.6	1	6.8/5.5	1
4.4/3.7	3	5.2/3.6	1	5.6/3.7	1	6.9/3.9	1
4.7/3.5	1	5.2/3.7	3	5.6/4.3	1	7.7/4.3	1
4.7/4.2	1	5.2/3.8	1	5.6/4.7	1	8.7/4.9	1
4.8/4.5	1	5.2/4.0	1	5.6/4.8	1	8.8/3.9	1

* Assumed homozygosity

3. Fragment size distribution of the BamHI RFLP detected by probe YNH24

Figure 3 illustrates the obviously unequal distribution of fragments. There are two clusters, around approximately 3.8 kb and approximately 5.1 kb. Fragments of almost the same size, which were initially featured on *different* membranes, were re-investigated together on one membrane. This procedure made it evident that restriction fragments that at first sight appeared almost identical were in fact not identical (data not shown). This in turn means that the estimation of fragment size distribution is correct and that there are no restriction fragments with a frequency in excess of 10%. With respect to biostatistical evaluation (probability of paternity), this deserves to be emphasized.

4. Heterozygosity

Of 122 individuals tested, 17 showed a one-fragment pattern, indicating either homozygosity or the existence of an undetected very small fragment (under 1.3 kb). This in turn corresponds to a heterozygosity rate of about 86%. Table 2 reviews the occurrence of fragments patterns ("phenotypes").

5. Multi-member family

Codominant autosomal inheritance of allele-like DNA fragments is obvious. Exceptions from the genetic pathway were not observed (Fig. 4).

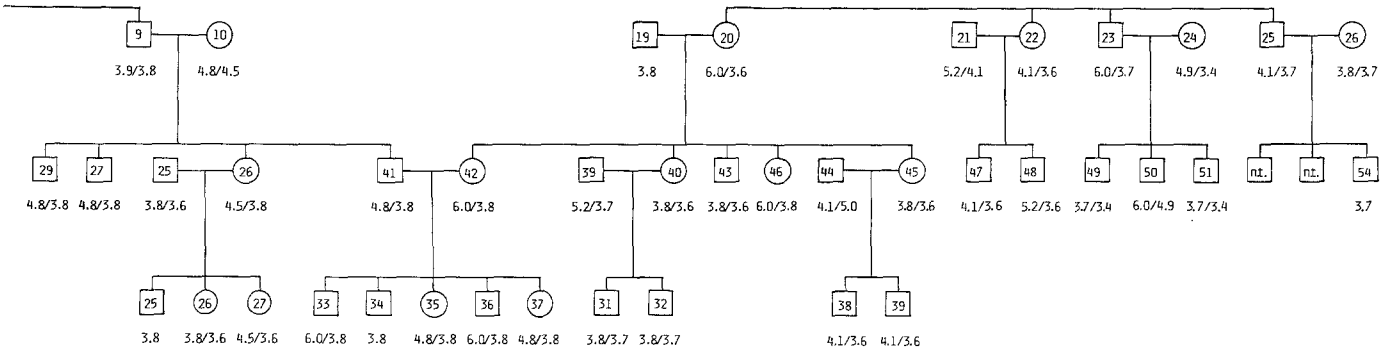
6. Paternity cases

Of 108 paternity cases reviewed, all were solved beyond reasonable doubt by means of conventional blood grouping, revealing an inclusion in 91 cases and exclusions in 17. BamHI/YNH24 polymorphism confirmed the serogenetic conclusions with no exceptions.

Discussion

The usefulness of single-locus DNA polymorphisms in forensic hemogenetics depends critically on the reliability of the band frequencies used. It is extremely desirable that data from different laboratories can be pooled and exchanged. By using polymorphisms generating fragment sizes smaller than approximately 7 kb this demand can be fulfilled [5], and to this end the BamHI/YNH24 combination can be used. Larger fragments are exceptional (see Fig. 3).

The mutation rate of DNA polymorphisms is also often regarded as a critical point. Like the interference of silent alleles in blood group systems, the occurrence of spontaneous mutations at DNA mini-satellite loci has to be taken into account. Thus, it is essential that, in



cases in which a parenthood is excluded, this be confirmed by more than one VNTR locus. For example, given two genetic systems, each having a mutation frequency of 0.2%, the probability that *both* systems would produce a false exclusion in the same individual is 2 in 1000000 cases [2].

Family studies have shown that the mutation rate of some VNTR loci may be as high as 2–5% [14] (Henke et al., unpublished). However, similar studies performed on locus D2S44 [2] have not revealed any meiotic recombination in the approximately 1000 gametes examined. A review of our 108 paternity cases has revealed neither a false-negative nor a false-positive result. This might be attributed to chance because of the small number of cases.

Confirmation of paternity may also be important in attempts to diagnose genetic diseases. Because probe YNH24 shows polymorphisms with a number of different restriction enzymes, e.g. HinfI, HaeIII, TaqI, MspI, PstI [5, 18] it can easily be used for this purpose.

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References

1. Baird M, Balazs I, Giusti A; Miyazaki L, Nicholas L, Wexler K, Kanter E, Glassberg J, Allen F, Rubinstein P, Sussmann L (1986) Allele frequency distribution of two highly polymorphic DNA sequences in three ethnic groups and its application to the determination of paternity. Am J Hum Genet 39:489–501
2. Balazs I, Baird M, Clyne M, Meade E (1989) Human population genetic studies of five hypervariable DNA loci. Am J Hum Genet 44:182–190
3. Dykes DD (1987) Parentage testing using restriction fragment length polymorphisms (RFLPs). In: Lasky LC, Edwards-Moulds JM (eds) Clinical application of genetic engineering. AABB, Arlington, pp 59–85
4. Dykes DD (1988) The use of biotinylated DNA probes in parentage testing: non-isotopic labeling and non-toxic extraction. Electrophoresis 9:359–368
5. Eede PP van, Henke L, Henke J, Lange GG de (1990) Size calculation of fragments detected by probe YNH24 and restriction enzyme HaeIII by comparison between two laboratories: the generation of fragment size frequencies. Forensic Sci Int (in press)
6. Fowler SJ, Gill P, Werrett DJ, Higgs OR (1988) Individual specific DNA fingerprints from a hypervariable region probe: alpha-globin 3'HVR. Hum Genet 79:142–146
7. Harumoto T, Suzuki K, Matsui K, Ito S, Matsuo Y, Miyazaki T, Matsumoto H (1988) Allelic frequency of polymorphic sequences in Japanese and its application to the paternity testing. In: Mayr W (ed) Advances in forensic haemogenetics, vol 2. Springer, Berlin Heidelberg New York, pp 373–376
8. Helminen P, Ehnholm C, Lokki M-L, Jeffreys AJ, Peltonen L (1988) Application of DNA "fingerprints" to paternity determinations. Lancet I:574–576
9. Henke J, Henke L (1988) 'DNA-Fingerprinting' – Anwendung der Gentechnik in der forensischen Hämogenetik. BTF-Biotech Forum 5:115–118
10. Henke J, Henke L, Cleef S (1988) Comparison of different X-ray films for ^{32}P autoradiography using various intensifying screens at −20°C and −70°C. J Clin Chem Clin Biochem 26: 467–468
11. Higgs DR, Goodbourn SEY, Wainscoat JS, Clegg JB, Weatherall DJ (1981) Highly variable regions flank the human alpha-globin genes. Nucleic Acids Res 9:4213–4214
12. Higgs DR, Wainscoat JS, Flint J, Hill AVS, Thein SL, Nicholls RD, Teal H, Ayyub H, Peto TEA, Falusi AG, Jarman AP, Clegg JB, Weatherall DJ (1986) Analysis of the human alpha-globin gene cluster reveals a highly informative locus. Proc Natl Acad Sci USA 83:5165–5169
13. Jeffreys AJ, Brookfield JFY, Semeonoff R (1985) Positive identification of an immigration test-case using human DNA fingerprints. Nature 317:818–819
14. Jeffreys AJ, Royle NJ, Wilson V, Wong Z (1988) Spontaneous mutation rates to new length alleles at tandem-repetitive hypervariable loci in human DNA. Nature 332:278–281
15. Knowlton RG, Brown VA, Braman JC, Barker D, Schumm JW, Murray C, Takvorian T, Ritz J, Donnis-Keller H (1986) Use of highly polymorphic DNA probes for genotype analysis following bone marrow transplantation. Blood 68:378–385
16. Liebhardt E, Teifel-Greding J, Schuller E, Tutsch-Bauer E, Josephi E (1988) RFLP als zusätzliche Systeme in der Abstammungsbegutachtung. In: Mayr W (ed) Advances in forensic haemogenetics, vol 2. Springer, Berlin Heidelberg New York, pp 364–367
17. Miller S, Dykes D, Polesky H (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:1215
18. Nakamura Y, Leppert M, O'Connell P, Wolff R, Holm T, Culver M, Martin C, Fujimoto E, Kumlin E, White R (1987) Variable number of tandem repeat (VNTR) markers for human gene mapping. Science 235:1616–1622
19. Rittner C, Schacker U, Mattern R, Müntefering H, Rittner G, Schneider PM (1989) Vaterschaftsnachweis an 10 bis 11 Wochen alten Foeten nach Abort mit Hilfe der Minisatellitsonde MZ 1.3. Haima 4:9–12

20. Rodriguez R, Tait R (1983) Recombinant DNA techniques: an introduction. Benjamin-Cummings, London Amsterdam Don Mills Sydney Tokyo
21. Schäfer R, Zischler H, Epplen JT (1988) $(CAC)_5$, a very informative oligonucleotide probe for DNA fingerprinting. Nucleic Acids Res 16:5196
22. Thomson JA, Hoar DI, Lincoln PJ, Phillips CP, Tynan K, Watts PH, Wood NJ (1988) An evaluation of the use of DNA probe hMF1 in cases of disputed paternity by comparison with a range of well established blood group polymorphisms. In: Mayr W (ed) Advances in forensic haemogenetics, vol 2. Springer, Berlin Heidelberg New York, pp 368–372
23. Weber W, Olek K (1988) Ein Vergleich von 25 Blutgruppensystemen mit polymorphen DNA-Markern in der Vaterschaftsbegutachtung. In: Mayr W (ed) Advances in forensic haemogenetics, vol 2. Springer, Berlin Heidelberg New York, pp 359–362
24. Wong Z, Wilson V, Jeffreys AJ, Thein SL (1986) Cloning a selected fragment from a human DNA "fingerprint": isolation of an extremely polymorphic minisatellite. Nucleic Acid Res 14:4605–4616
25. Wong Z, Wilson V, Patel I, Povey S, Jeffreys AJ (1987) Characterization of a panel of highly variable minisatellites cloned from human DNA. Ann Hum Genet 51:269–288
26. Wyman AR, White R (1980) A highly polymorphic locus in human DNA. Proc Natl Acad Sci USA 83:5165–5169