# Identity and Paternity Testing of Cattle: Application of a Deoxyribonucleic Acid Profiling Protocol

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## ABSTRACT

We have applied DNA profiling for identity and parentage studies of cattle using a standardized procedure based on synthetic micro- and minisatellite multilocus core probes in Southern blot hybridization assays. This protocol is useful for paternity analysis of cattle and for real case work (e.g., identity and paternity disputes).
(**Key words**: paternity testing, deoxyribonucleic acid fingerprinting, variable number of tandem repeat loci, core probes)

## INTRODUCTION

Deoxyribonucleic acid profiling using multilocus core probes (probes that simultaneously detect several variable number of tandem repeat loci) has been shown to be a powerful tool for identity and paternity testing of humans (8, 10), of cattle [(5, 6, 11); Trommelen et al., 1992, unpublished data], and of other organisms (3, 7, 9). Because of the high number of independent genetic loci that can be assayed by multilocus core probes, exclusion powers are high for identity and paternity testing, especially when a combination of core probes is applied.

We have recently applied a standardized protocol for DNA profiling of cattle based on the use of multilocus core probes (Trommelen et al., 1992, unpublished data). In this paper, we demonstrate the usefulness of this protocol for paternity analysis of cattle and in four real cases, including an identity case (case 1) and several paternity disputes (cases 2, 3, and 4). The results demonstrate that, by using only three core probes, these cases could be resolved more accurately than by protein analysis.

## MATERIALS AND METHODS

### Materials

The cattle used for the pedigree analysis and analyzed for the identity and paternity disputes were all Holstein-Friesian. Semen of the bulls was available in straws, and blood was collected in tubes coated with $Na_2$-EDTA.

### DNA Extraction

*Blood.* The erythrocytes were lysed by addition of 3 volumes of lysis buffer (155 m*M* $NH_4Cl$, 10 m*M* $KHCO_3$, and 1 m*M* $Na_2$-EDTA) and incubation on ice for 30 min. After centrifugation at 12,000 rpm (in an Eppendorf centrifuge; E. Merck, Darmstedt, Germany), the pellet containing the white blood cells was resuspended and incubated in 10 m*M* Tris·HCl (pH 8.0), 10 m*M* $Na_2$-EDTA, 100 m*M* NaCl, .5% SDS, and 500 $\mu$g/ml of proteinase K (E. Merck) at 65°C for 4 h under gentle shaking. The mixture was extracted once with Tris-saturated phenol and once with chloroform-

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isoamylalcohol (24:1, vol/vol) and subsequently precipitated in ethanol. The DNA was dissolved in 10 m*M* Tris·HCl (pH 8.0) and 1 m*M* $Na_2$-EDTA to a concentration of approximately .8 μg/μl. The yield of DNA from blood was usually 35 μg of DNA/ml of whole blood.

*Sperm.* The sperm cells were transferred to a test tube. After centrifugation at 12,000 rpm, the pellet containing the sperm cells was washed in 10 m*M* Tris·HCl (pH 8.0), 10 m*M* $Na_2$-EDTA, and 100 m*M* NaCl and then recentrifuged. The pellet containing the sperm cells was resuspended in 10 m*M* Tris·HCl (pH 8.0), 10 m*M* $Na_2$-EDTA, 100 m*M* NaCl, .5% SDS, 500 μg/ml of proteinase K, (E. Merck), and 6 mg/ml of dithiothreitol (E. Merck) and then incubated for 4 h at 65°C under gentle shaking. The mixture was then extracted with phenol and chloroform and precipitated in the same way that DNA was extracted from blood.

#### Agarose Gel Electrophoresis and Blotting

Restriction enzyme digestion was performed according to the manufacturer's instructions (Gibco BRL, Bethesda, MD). Seven micrograms of the restriction fragments were electrophoresed in 1.5% agarose gels in 1 × TAE (40 m*M* Tris-acetate, 34 m*M* Na-acetate, 1 m*M* $Na_2$-EDTA, pH 8.0) at a constant 70 V (220 mA) for 18 h with continuous buffer recirculation in a horizontal electrophoresis apparatus (type 2025; Gibco BRL). Thirty nanograms of the analytical marker (Promega Corp., Madison, WI) were applied. The gels were stained in 1 μg/ml of ethidium bromide for 20 min and photographed under UV light (302 nm) using a transilluminator (UVP products, San Gabriel, CA). The DNA in the gels was made single stranded by incubation for 60 min in .4 *M* NaOH plus .6 *M* NaCl (transfer buffer). The separation pattern was subsequently transferred to a nylon membrane (Zetaprobe; Bio-Rad, Hercules, CA) by vacuum blotting (Vacugene XL; Pharmacia LKB, Uppsala, Sweden) for 1 h in the denaturation solution.

#### Probe Preparation and Labeling

The core probes used in this study (Table 1) consisted of double-stranded polymers of a chemically synthesized oligonucleotide corresponding to a particular core sequence [(13); Trommelen et al., 1992, unpublished data]. The probe consisted of a mixture of polymers of lengths ranging from 1 to 10 kb. Twenty nanograms of such mixtures were labeled by [$\alpha^{32}$P] deoxycytosine triphosphate by the random-primer oligolabeling method (4), using a random primer labeling kit (Gibco BRL). To visualize the size marker, 30 ng of a commercially available analytical marker (Promega Corp.) was labeled as described and used for hybridization. Length of the marker fragments is indicated in the figures.

TABLE 1. Micro- and minisatellite core probes used in this study.

| Name | Sequence[1] | Reference |
|---|---|---|
| . . . | $(CAC)_n$ | (1) |
| . . . | $(GACA)_n$ | (1) |
| 33.6 | $(AGGGCTGGAGG)_n$ | (10) |
| INS | $(ACAGGGGTGTGGGG)_n$ | (12) |
| HBV-3 | $(GGTGAAGCACAGGTG)_n$ | (12) |
| YNZ-22 | $(CTCTGGGTGTGGTGC)_n$ | (12) |
| HBV-1 | $(GGAGTTGGGGGAGGAG)_n$ | (12) |

[1]The n varies and indicates that the probe consists of polymers of the oligonucleotide sequence shown.

#### Hybridization and Autoradiography

The filters were prehybridized in 500 m*M* phosphate buffer, pH 7.2, 7% SDS, and 1 m*M* $Na_2$-EDTA for 10 min at 65°C. The single-stranded radioactive probe was added to the same hybridization solution, and incubation was continued for 2 h. Filters were washed three times in 2.5× saline sodium citrate plus .1% SDS at 65°C for 20 min. Autoradiography was performed by exposure to Kodak XAR film for 3 to 48 h at −80°C with intensifying screens (Kodak, Germany). Membranes were rehybridized after first removing the hybridized radioactive probe ("stripping") by placing the membrane in boiling .1× saline sodium citrate plus .1% SDS, letting the solution cool to room temperature (23°C) for 30 min, and rinsing the membrane in 2.5× saline sodium citrate. Membranes were checked for residual radioactivity by inspection with a monitor (Berthold type LB 122; Wildbad, Germany). If more than the background signal (> 30 cps) was measured, the stripping procedure was repeated until no residual activity could be measured.

### Pattern Interpretation and Paternity Index

*Pattern Interpretation.* Banding patterns were analyzed visually by at least two persons. Only unambiguous differences were used to compare banding patterns. By analyzing different cattle populations and pedigrees, we have shown that the probability that a variable band of similar electrophoretic mobility and autoradiographic intensity present in the DNA profile of one individual is also present in that of another individual can be conservatively estimated to be .45. This band-sharing probability is, in fact, heterogeneous, falling to <.15 for the largest variable fragments [(2); Trommelen et al., 1992, unpublished data]. The band-sharing probability has been determined to be the same for Holstein-Friesian and several other breeds. The band-sharing probability, therefore, represents a useful average but is not necessarily applicable to any given locus or population.

Occasionally, bands were observed that were detected by two or more core probes because of crosshybridization of the particular fragment to the different core probes. If such overlap was suspected for a particular band, this overlap was corrected for in the analysis by elimination from the comparison of all but one of the bands detected at that position in the gel by different core probes.

*Paternity Index.* The chance that another unrelated bull is the father of a calf is calculated by the paternity index. The paternity index is corrected for overlap among different core probes, linked bands, and bands that are part of allelic pairs, of which the latter two represent about 10% of the total number of variant bands (Trommelen et al., 1992, unpublished data). The paternity index is calculated as follows: paternity index = $1/(.45)^n$, where n is the number of matching bands between the calf and the alleged parent. In a paternity case, maternal and nonmaternal bands are identified in the pattern of the calf. The nonmaternal bands represent the paternal bands and possible mutant bands: bands that have arisen de novo in the calf and are derived from neither the mother nor the father. From analysis of 40 offspring in pedigrees and 20 offspring in casework with four to six of the core probes used here [33.6, $(CAC)_n$, INS, HBV-1, HBV-3, and $(GACA)_n$], the fraction of mutant bands has been determined to be < .07% of the informative bands (Trommelen et al., 1992, unpublished data). Including observations from others for human DNA (8), we conclude nonpaternity in casework if more than two nonmatching, nonmaternal bands were observed in the candidate father.

As it is used here, the paternity index represents an odds ratio for a null hypothesis; each paternal-specific band was inherited from the putative sire (with a probability of 1 for each band), and an alternative hypothesis, each paternal-specific band is present in the putative sire completely by chance (with a .45 probability for each band). If, in particular cases, such as case 4, no maternal bands or bands that are common to the sire, dam, and calf can be identified in the DNA profiles from the calf, the probability that a paternal-specific band is present in the DNA profiling pattern is .45 (instead of 1). In that case, paternity can be concluded by comparison of band sharing between an unrelated calf and the sire and that between the calf in question and the sire.

## RESULTS

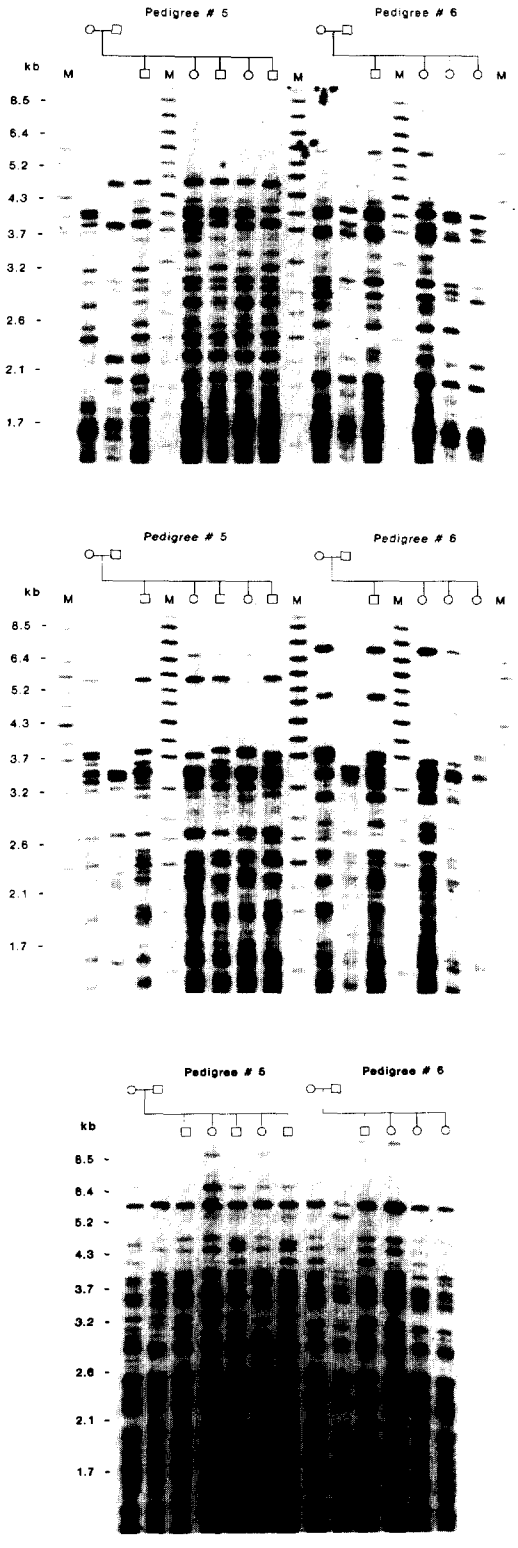
### Pedigree Analysis

Five two-generation pedigrees were analyzed by DNA profiling using six different synthetic multilocus core probes: 33.6, $(CAC)_n$, INS, HBV-1, HBV-3, and $(GACA)_n$ [(13); Trommelen et al., 1992, unpublished data]. The DNA profiles were obtained by subsequently rehybridizing the Southern blot containing separation patterns of *Hae*III-digested genomic DNA from members of each pedigree with each of the six core probes. Figure 1 shows the hybridization patterns of two of these pedigrees that were obtained after hybridization with core probes 33.6, $(CAC)_n$ and INS. In total, 24 calves were analyzed for paternity. The DNA profile of each calf is composed of variable bands and bands shared by both parents (common bands). Variable bands are derived from the sire or have arisen because of mutation (nonmaternal bands) or are derived from the dam (maternal bands). We have not observed any mutant bands in the pedigrees analyzed herein. The number of variant bands detected per calf differs per core probe (see Table 2). In these five pedigrees, we

TABLE 2. Number of maternal-(Mat) and paternal-(Pat) specific bands in individual offspring of five multisib pedigrees obtained with six different core probes. The calculated paternity index[1] for each of these offspring is also shown.

| | | 33.6 | | $(CAC)_n$ | | INS | | HBV-1 | | HBV-3 | | $(GACA)_n$ | | Total | | Paternity |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pedigree | Sib | Mat | Pat | Mat | Pat | Mat | Pat | Mat | Pat | Mat | Pat | Mat | Pat | Mat | Pat | index |
| 1 | 1 | 2 | 3 | 2 | 4 | 2 | 6 | 1 | 2 | 1 | 2 | 2 | 2 | 10 | 19 | $7.9 \times 10^5$ |
| | 2 | 2 | 3 | 2 | 5 | 2 | 5 | 1 | 1 | 1 | 2 | 2 | 4 | 10 | 20 | $1.7 \times 10^6$ |
| | 3 | 5 | 3 | 4 | 8 | 3 | 7 | 1 | 1 | 1 | 2 | 3 | 1 | 17 | 22 | $8.6 \times 10^6$ |
| | 4 | 1 | 4 | 1 | 6 | 4 | 6 | 1 | 1 | 1 | 2 | 2 | 3 | 10 | 22 | $8.6 \times 10^6$ |
| | 5 | 2 | 3 | 0 | 5 | 3 | 5 | 1 | 1 | 1 | 2 | 3 | 1 | 10 | 17 | $1.6 \times 10^5$ |
| 4 | 1 | 10 | 3 | 5 | 2 | 3 | 1 | 4 | 2 | 2 | 1 | 5 | 1 | 29 | 10 | 1320 |
| | 2 | 7 | 2 | 6 | 2 | 3 | 2 | 3 | 1 | 2 | 1 | 7 | 1 | 28 | 9 | 600 |
| | 3 | 10 | 1 | 5 | 3 | 4 | 2 | 3 | 1 | 2 | 1 | 5 | 1 | 29 | 9 | 600 |
| | 4 | 5 | 1 | 5 | 2 | 3 | 3 | 1 | 1 | 2 | 0 | 4 | 1 | 20 | 8 | 270 |
| 5 | 1 | 6 | 5 | 4 | 1 | 3 | 2 | 4 | 2 | 1 | 1 | 3 | 4 | 21 | 15 | $7.1 \times 10^4$ |
| | 2 | 7 | 5 | 3 | 2 | 3 | 2 | 4 | 4 | 2 | 1 | 4 | 4 | 23 | 18 | $3.5 \times 10^5$ |
| | 3 | 8 | 5 | 3 | 3 | 5 | 3 | 5 | 3 | 2 | 1 | 4 | 2 | 27 | 17 | $1.6 \times 10^5$ |
| | 4 | 7 | 5 | 3 | 3 | 2 | 5 | 5 | 3 | 3 | 1 | 4 | 3 | 24 | 20 | $1.7 \times 10^6$ |
| | 5 | 6 | 6 | 3 | 3 | 6 | 4 | 6 | 4 | 3 | 1 | 4 | 3 | 28 | 21 | $3.9 \times 10^6$ |
| 6 | 1 | 8 | 1 | 8 | 2 | 2 | 0 | 2 | 1 | 2 | 1 | 6 | 2 | 28 | 7 | 120 |
| | 2 | 7 | 2 | 6 | 1 | 2 | 1 | 2 | 2 | 3 | 0 | 5 | 3 | 25 | 9 | 600 |
| | 3 | 5 | 1 | 6 | 1 | 3 | 0 | 1 | 1 | 1 | 1 | 3 | 3 | 19 | 7 | 120 |
| | 4 | 4 | 2 | 5 | 1 | 2 | 1 | 3 | 0 | 1 | 0 | 6 | 2 | 21 | 6 | 120 |
| 7 | 1 | 3 | 3 | 6 | 5 | 2 | 3 | 2 | 2 | 1 | 3 | 3 | 2 | 17 | 18 | $3.5 \times 10^5$ |
| | 2 | 3 | 6 | 4 | 7 | 2 | 3 | 6 | 4 | 2 | 2 | 4 | 3 | 21 | 25 | $9.5 \times 10^7$ |
| | 3 | 3 | 5 | 6 | 3 | 2 | 0 | 5 | 2 | 2 | 3 | 4 | 3 | 22 | 16 | $7.2 \times 10^4$ |
| | 4 | 3 | 2 | 5 | 7 | 1 | 2 | 4 | 3 | 2 | 1 | 3 | 3 | 18 | 18 | $3.5 \times 10^5$ |
| | 5 | 3 | 4 | 4 | 5 | 3 | 2 | 3 | 3 | 2 | 2 | 3 | 3 | 18 | 19 | $7.9 \times 10^5$ |
| | 6 | 3 | 3 | 2 | 4 | 1 | 2 | 4 | 2 | 2 | 1 | 3 | 4 | 15 | 16 | $7.2 \times 10^4$ |

[1]Paternity index = $1/(.45)^n$, where n is the total number of paternal-specific bands. The paternity index is corrected for overlap, linkage, and allelism of bands.




could detect, on average, 0 to 8 nonmaternal bands per probe when individual sib-mother pairs were analyzed, depending on the probe used. This number was corrected for overlap. The largest number of nonmaternal bands was detected with probe 33.6 (see Table 2) although $(CAC)_n$ and INS were almost as effective. The paternity index (i.e., the odds that the bull is the true sire relative to the probability of a random match) in these pedigrees, using only the core probe 33.6, varies between 120:1 and 2:1. When $(CAC)_n$ is included as a second probe, these numbers increased very rapidly (varying between 32,000:1 and 5:1). When six core probes were applied, the paternity index was as high as $9.5 \times 10^7$:1 in some cases and 120:1 in the worst case (see Table 2).

### Case 1

Black calves were born from a red cow, which was supposed to have been inseminated with semen from a red bull. The artificial insemination center was therefore thought to have delivered a different semen sample, although name and number on the semen straws agreed with that recorded as the inseminating bull. We compared different semen and blood samples from this particular bull, obtained on the same day that the sample was taken for artificial insemination.

Figure 2 shows DNA profiles of *Hae*III-digested DNA from the blood of this bull (denoted X) and DNA profiles from four different semen samples purportedly taken from this bull on the day in question (denoted 1 to 4). The probe used was $(CAC)_n$. Semen samples 1 and 3 share the same profile, which differs from that of the blood sample in having four extra bands. Semen sample 2 has a DNA profile that differs from all other samples. Semen sample 4 and the blood sample have identical profiles. These results demonstrate that semen samples of different origins (in total, three different bulls) were mixed in one

Figure 1. The DNA profiles from two pedigrees, obtained with core probes 33.6 (top), $(CAC)_n$ (middle), and INS (bottom) after rehybridization of a single membrane. Restriction enzyme used is *Hae*III. M = Marker lane.

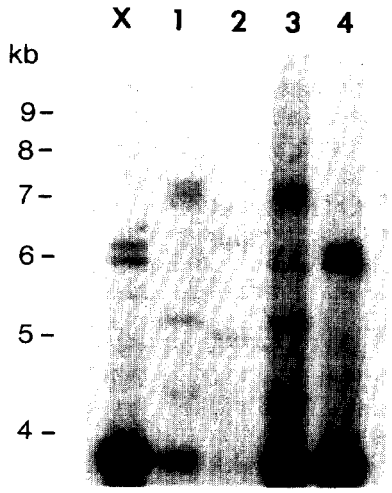



Figure 2. The DNA profiles from *Hae*III-digested blood DNA of bull X (X) and DNA profiles from different semen samples taken on the same day (lanes 1 to 4) with probe $(CAC)_n$. The DNA profile in lane 4 is identical to that of the blood (X). Samples 1 and 3 have the same profile, which is different from that of the blood (X). Four extra bands can be observed in samples 1 and 3 compared with bands in the blood sample (X). Sample 2 has a DNA profile different from all other samples.

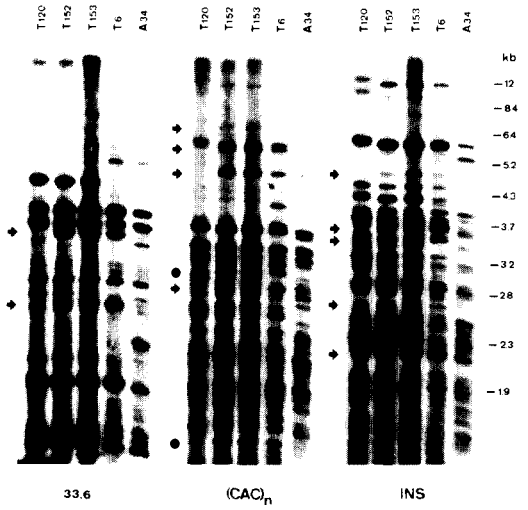



Figure 3. The DNA profiles of the dam T120, calves T152 and T153, and putative sires T6 and A34 obtained with core probes 33.6 , $(CAC)_n$, and INS after rehybridization of a single membrane. The restriction enzyme used is *Hae*III. Arrows indicate nonmaternal bands in both calves.

batch of semen of the bull in question. One sample (sample 4) is an actual semen sample of bull X. Samples 1 and 3 are also semen samples from bull X but apparently were mixed with semen from another bull, which explains the additional bands in these profiles. Sample 2 is semen from yet a third bull.

### Case 2

The identity was questioned of the sire of identical twin calves, T152 and T153, both genetic daughters of the dam T120. Protein studies had left only two possibilities, T6 and A34. Figure 3 shows DNA profiles of *Hae*III-digested DNA from T120, T152, T153, T6, and A34 obtained with 33.6, $(CAC)_n$ and INS (see Table 1) (5, 11). The two analyses showed that T152 and T153 had identical DNA profiles, indicating that they are indeed identical twin sisters. In the DNA profiles of T152 and T153, 16 of the total of 27 informative bands were also present in the DNA profiles of the mother T120 and represent the maternal bands. Only 4 of the remaining 13 nonmaternal bands were present in the DNA profiles of A34. This high number of nonmatching, nonmaternal bands excludes A34 from being the biological father of T152 and T153. However, all 13 nonmaternal bands in these calves were present in the DNA profiles of T6. The actual number of bands used for the calculation of the paternity index was corrected for 10% of the bands showing linkage or allelism and for overlap. The probability then that T6, by chance rather than relation, has the 12 nonmaternal bands is $(.45)^{12} = 1 \times 10^{-4}$ or an odds ratio of 14,000:1 that this is the true sire. Because the paternity dispute is only between two candidate sires, T6 can therefore be assumed beyond reasonable doubt to be the biological father of T152 and T153.

### Case 3

In paternity case 3, protein studies had left only two possibilities, N or SK, to be the sire of SB, son of dam SP175. Figure 4 shows DNA profiles of *Hae*III-digested DNA from SB, N, SK, and SP175 obtained with $(CAC)_n$

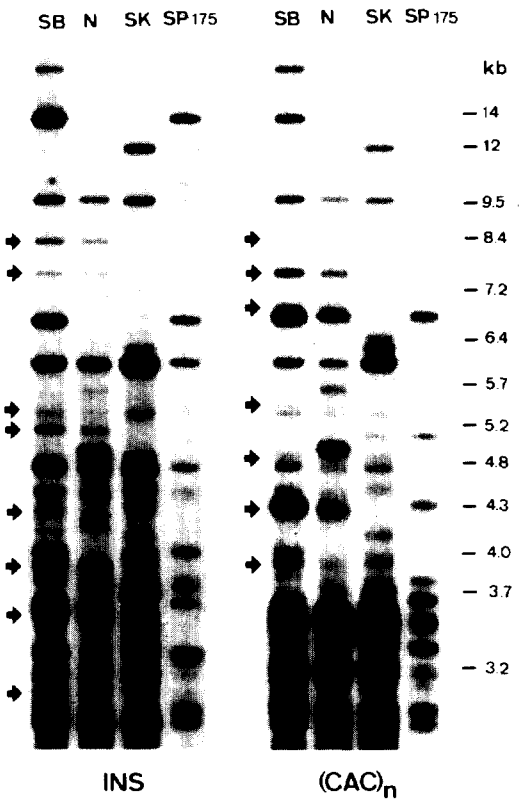



Figure 4. The DNA profiles from SB, putative sires N and SK, and dam SP175 obtained with core probes $(CAC)_n$ and INS after rehybridization of a single membrane. The restriction enzyme used is *Hae*III. Arrows indicate nonmaternal bands in SB.

and INS (see Table 1) (5, 11). Only informative bands were taken into consideration (i.e., bands that were not present in all of the four individuals). The analysis showed that, from a total of 39 bands, SB shared 24 bands with SP175. These bands represent the maternal bands, and the remaining 15 bands represent the nonmaternal bands. Three of these bands (of approximately 8.4, 7.6, and 3.9 kb) were excluded from the comparison because of overlap among the probes. Only 5 of the 12 nonmaternal bands were present in the DNA profiles of SK. This high number of nonmatching, nonmaternal bands excludes SK from being the biological father of SB. However, all 12 nonmaternal bands were present in the DNA profiles of N, which does not exclude N from being the biological father of SB. The probability, then, that N by chance rather than relation, has the 11 paternal bands is $(.45)^{11} = 2 \times 10^{-4}$, corresponding to an odds ratio of 6500:1. (The actual number of bands used for the calculation of the paternity index was corrected for 10% of the bands showing linkage or allelism.) Because the paternity dispute is only between two candidate sires, N can therefore be assumed beyond reasonable doubt to be the biological father of SB.

### Case 4

In Case 4, a more unusual paternity case, it was questioned whether two calves, Lotje 1 and Lotje 2, both daughters of the same dam (Lotje) had the same sire; phenotypic characteristics suggested that they were probably not full sisters. Protein studies had previously indicated Briarwood Commodore to be the sire of Lotje 2 and had left for Lotje 1 only two possible fathers: Briarwood Commodore or Cherry Lane Starbright. Blood material was available from Lotje 1 and Lotje 2, and sperm material was available only from Cherry Lane Starbright. Because no material was available from Briarwood Commodore, only the DNA profiles from Lotje 1, Lotje 2, and Cherry Lane Starbright could be compared. If Lotje 1 had been the daughter of Cherry Lane Starbright, the profiles of Lotje 1 and Cherry Lane Starbright would have had a higher number of shared bands compared with the number of bands shared by Lotje 2 and Cherry Lane Starbright, who have no relation. In this case, no true paternal bands can be identified in Lotje 1; thus, paternity assignment is then based on the ratios of odds corresponding to band-sharing probabilities of Lotje 1 with Cherry Lane Starbright and Lotje 2 with Cherry Lane Starbright. The latter two are known to be unrelated.

Figure 5 shows DNA profiles of *Hae*III-digested DNA from Lotje 1, Lotje 2, and Cherry Lane Starbright obtained with 33.6, $(CAC)_n$, and YNZ-22 (see Table 1). Only informative bands (i.e., bands that were only present in one or two of the three individuals shown here) were taken into consideration. The three probes showed that Lotje 1 shared 14 bands with Cherry Lane Starbright. Also, the actual number of bands used for the calculation of the paternity index was corrected for 10% of the bands showing linkage or allelism.

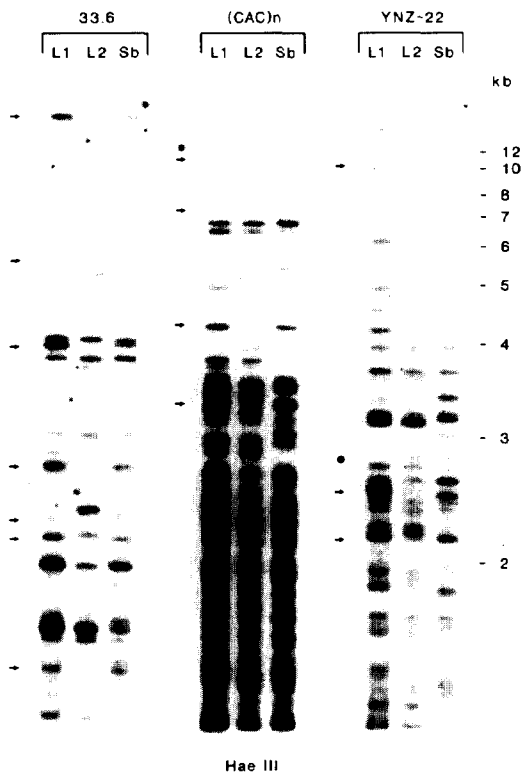



Figure 5. The DNA profiles from Lotje 1 (L1), Lotje 2 (L2), and Starbright (Sb) obtained with core probes 33.6, $(CAC)_n$, and YNZ-22 after rehybridization of a single membrane. The restriction enzyme used is *Hae*III. For three probes 14 bands (indicated by arrows) were shared by Lotje 1 and Cherry Lane Starbright. Bands shared by Lotje 2 and Cherry Lane Starbright are indicated by an asterisk.

The probability, then, that Cherry Lane Starbright, by chance rather than by relation, shares 13 bands with Lotje 1 is $(.45)^{13} = 3.1 \times 10^{-5}$, representing an odds ratio of 32,000:1 that this number of shared bands is based on relation rather than chance if these bands could be recognized as paternal-specific bands. Because this relationship is not the case, correction is necessary, and band sharing with an unrelated calf and the sire is compared. Lotje 2 shares only 2 bands with Cherry Lane Starbright, and the probability that this result is due to chance rather than relation is $(.45)^2 = .20$, corresponding to an odds ratio of 5:1. This number is used to correct the odds calculated for Cherry Lane Starbright being the sire of Lotje 1. Therefore, the odds that Cherry Lane Starbright is the true sire of Lotje 1 (i.e., by relation rather than by chance) are (32,000/5):1 = 6500:1. Therefore Cherry Lane Starbright can be assumed beyond reasonable doubt to be the biological father of Lotje 1. The relatively high degree of band sharing between Lotje 1 and Lotje 2 (13 bands) does not preclude them from being half-sisters. This deviation from the expected value of 25% bands shared by half-sisters might be due to the high level of inbreeding in the cattle population in general, as is expressed in the 45% band sharing that we found.

## DISCUSSION

We have designed a DNA profiling protocol based on using synthetic multilocus core probes (Trommelen et al., 1992, unpublished data). We show herein its application for identity and paternity analyses of cattle. High degrees of certainty can be obtained with this identification technique, and, if necessary, degree of certainty can be even higher when more core probes are applied. From the analysis of five pedigrees, in the optimal case, a paternity index (representing the odds ratio of bands being present from inheritance relative to bands being present completely by chance) of $9.5 \times 10^7$:1 can be obtained when six core probes are applied. The average paternity index of each pedigree differed among the five pedigrees analyzed; the sibs of pedigree 4 and 6 all have a very low paternity index, which is due to the low number of paternal-specific bands that were present in these two pedigrees. How frequently this phenomenon will be encountered remains to be established by more extensive population studies and casework. However, if these two pedigrees are omitted, an average paternity index of $7.7 \times 10^6$:1 is found in cattle DNA profiling. In general, with this DNA-profiling protocol, 3 to 4 multilocus core probes will probably be sufficient to obtain a paternity index of 50,000:1 or more. We present DNA-profiling casework in which, using two to three core probes, paternity disputes were resolved beyond the power of protein studies, which had left only two candidate sires. In more difficult cases (e.g., two related putative fathers, related putative parents, or larger populations of candidate sires to be analyzed), more probes have to be applied to

obtain the same degree of certainty. Although the results obtained with locus-specific variable number of tandem repeat probes may be easier to interpret [e.g., (5)], more of these probes have to be applied to obtain the same degree of certainty because only one paternal band is obtained per probe.

## CONCLUSIONS

The advantage of DNA profiling is that tissues other than blood can be used for identity and parentage testing; thus, semen can be tested for contamination with other semen, as in case 1. In addition, identification is possible for live calves at birth by testing tissue samples or blood. When the test is applied routinely, its cost will not differ much from protein polymorphism analysis, namely, approximately ECU 50 to 100 ($75 to 150). The test can also be combined with other tests that involve DNA. Thus, after an identification test of a cow, the same DNA can be used for a genotyping test to determine the presence of gene variants of economic interest.

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