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# DNA FINGERPRINTING IN FOUR CATTLE BREEDS USING $(CAC)_5$ MICROSATELLITE PROBE

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**Abstract.** Genetic variations in four cattle breeds (Estonian Native cattle, Estonian Red cattle, Estonian Black-and-White cattle, and Bestuzhevskaya cattle from Russia) were estimated according to hypervariable hybridization patterns. Oligonucleotide $(CAC)_5$ probe was hybridized to cattle DNA digested with Bsu RI, and hypervariable hybridization patterns were obtained. Genetic variation within and between breeds was estimated. Also, a family analysis was carried out. Inside a breed the highest band sharing value was in Bestuzhevskaya breed (0.6) and in Estonian Native cattle (0.5). In Estonian Red cattle and in Estonian Black-and-White cattle the appropriate values were 0.45 and 0.32, respectively. This indicates that the Estonian Native cattle breed and the Bestuzhevskaya cattle breed display a comparatively limited genetic diversity, which may be caused by high inbreeding level in these local populations. Family analyses revealed Mendelian inheritance of DNA fingerprinting patterns obtained with $(CAC)_5$ probe. The inter-breed relationships, estimated by the inter-D value comparisons, may determine genetic distances between the four breeds investigated. We are of the opinion that the oligonucleotide $(CAC)_5$ probe is more suitable for individual identification, parentage testifying, and searching for hypervariable landmark markers in cattle than for distinguishing between different cattle breeds.

**Key words:** DNA fingerprinting, oligonucleotide $(CAC)_5$ probe, polymorphic markers, cattle.

## INTRODUCTION

During the recent years efforts for the development of a low resolution marker map of the bovine genome have become acute. The objective is to identify marker loci distributed across the genome, and to determine their physical and genetic relationship to one another (Georgoudis, 1993). Markers which are highly polymorphic in cattle will be identified and placed on a linkage map on the basis of the analysis of their segregation

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in different breeds and families. In perspective, such highly polymorphic markers and their probable linkage with Quantitative Trait Loci (QTL) could be used in Marker Assisted Selection (MAS), which will be a principally new approach in animal breeding. Still, MAS could be applied only in the future. At present, efforts are concentrated on searching for suitable highly polymorphic markers in cattle (Georgoudis, 1993).

In order to make the map to be of value in identifying QTLs, it is important that the markers show high levels of heterozygosity. The discovery of micro-and minisatellite sequences distributed all over the genome of the highest eukaryotes and showing high levels of polymorphism, might be a real contribution in identifying a large number of polymorphic markers needed for identifying QTLs (Kashi et al., 1990; Hundrieser et al., 1992; Kaukinen, Varvio, 1992).

As is well known, DNA fingerprinting has proved to be the most sensitive method for identifying individuals and for determining genetic relationships (Jeffreys et al., 1985a, b). The technique is based on simultaneous screening for polymorphism in several hypervariable regions known as minisatellites (Ellegren et al., 1992). Such hypervariable loci are regions which contain tandem repeats of short DNA segments. Variability arises from differences in the number of repeats (Kuhnlein et al., 1989). In humans, complex and highly diverse hybridization patterns have also been obtained with synthetic oligonucleotide probes consisting of simple repeats (Hundrieser et al., 1992, Ellegren et al., 1992). The triplet repeat probe $(CAS)_5$ or its complement $(GTG)_5$ has shown high informativeness on human material (Schäfer et al., 1988b).

In the present study, the synthetic oligonucleotide $(CAC)_5$ was evaluated as a probe for DNA fingerprinting in four cattle breeds. The aim was to find out the informativeness of this probe on cattle DNA.

## MATERIAL AND METHODS

**Animals.** The animals studied belonged to the following cattle breeds: (1) Estonian Black-and-White cattle (Kehtna Insemination Centre, Raplamaa, Estonia and Vanaküla farm, Harjumaa, Estonia); (2) Estonian Red cattle (Vändra farm, Pärnumaa, Estonia); (3) Estonian Native cattle (Pärivere Breeding Centre, Pärnumaa, Estonia), and (4) Bestuzhevskaya breed (Russia). For breed comparisons the DNA obtained from five randomly selected individuals from each breed, and for family analysis the DNA taken from three half-sib families (consisting of one bull, cows, daughters and granddaughters) was used.

**DNA methods.** Genomic DNA was prepared, with some modifications, from venous blood sampled in heparin according to Singner et al. (1988).

Samples of 5 μg DNA were digested with 25u Bsu RI (Hae III) in total volume of 100 μl. DNA was separated in alkaline conditions on 20 cm, 0.7% agarose gel, at 50 V for 20 h, and transferred by vacuum blotter to nylon filter (Hybond N).

Synthetic oligonucleotide $(CAC)_5$ was used as a probe, and was labelled with ($^{32}$P)-dATP in kinase reaction.

The filters were prehybridized at 60 °C for 2 h in 6× SSC, 5× Denhart, and 0.5% SDS. $^{32}$P-labelled probe was added to the prehybridization solution at a final activity of about 500,000 cpm/ml. Hybridization was performed for 1 h at 40 °C. After hybridization the filters were washed twice in 4× SSC, 0.1% SDS for 10 min, at 40 °C. The filters were exposed to Hyperfilm—MP (Amersham) for 5 days. Several exposures from every hybridization were analysed.

**Statistical analysis.** Band sharing values, genetic distances and genetic variability were calculated according to Jeffreys et al. (1985b) and Kuhnlein et al. (1989), followed by conventional probability estimates.

## RESULTS

**Hybridization pattern.** The $(CAC)_5$ probe detected several polymorphic fragments in bovine DNA digested with Bsu RI. In cattle DNA about 15—20 informative fingerprinting bands were revealed. Typical $(CAC)_5$ fingerprints are shown in Fig. 1. The obtained fingerprinting bands were distinct enough for being used for the following family analyses, as well as for inbreed and interbreed comparisons.

**Family analysis.** The inheritance of DNA fingerprinting patterns was tested in three half-sib families of Estonian Black-and-White cattle consisting of one bull, cows, daughters and grandmothers. Fingerprints from these families revealed Mendelian inheritance. All bands which could be scored reliably in each offspring could be traced back to either of the parents (Fig. 2).

**Genetic variation within a breed.** In order to estimate genetic difference between randomly selected individuals within a breed, comparisons of fingerprinting bands within breeds were carried out and the mean band frequencies were estimated. Different fingerprinting parameters are summarized in Table 1.

The highest band sharing ($x$) value was found within Estonian Native and Bestuzhevskaya breeds—0.5 and 0.6, respectively. Estonian Red cattle showed intermediate (0.45) band sharing value. The lowest value (0.32) was found in Estonian Black-and-White cattle population. An example of $(CAC)_5$ fingerprinting pattern inside Estonian Native cattle breed is presented in Fig. 3.

*Table 1*

**DNA fingerprinting data on four different cattle breeds using oligo$(CAC)_5$**

| Parameter | Estonian Native cattle | Estonian Red cattle | Estonian Black-and-White cattle | Bestuzhevskaya |
|---|---|---|---|---|
| $n$ | 10 | 6 | 9 | 6 |
| $Sy$ | 22 | 30 | 39 | 17 |
| $y$ | 11 | 15 | 21 | 10.3 |
| $x$ | 0.5 | 0.45 | 0.32 | 0.6 |
| $P$ | $1.3\times10^{-7}$ | $2.8\times10^{-10}$ | $6.8\times10^{-14}$ | $1.3\times10^{-5}$ |

$n$ — number of individuals investigated,
$Sy$ — total number of resolvable fragments,
$y$ — average number of resolvable fragments per individual,
$x$ — mean band frequency (Band Sharing),
$P$ — estimated probability that two randomly selected individuals would have the same fingerprint.

*Table 2*

**Comparisons between breeds in oligo(CAC)$_5$ fingerprinting**

| Breeds | BS | *D* | *n* |
|---|---|---|---|
| Within a breed | | | |
| EN | 0.5 | | 9 |
| ER | 0.45 | | 6 |
| EBW | 0.32 | | 10 |
| B | 0.6 | | 6 |
| Between breeds | | | |
| EBW/B | 0.37 | 1.14 | 4/4 |
| EBW/ER | 0.54 | 0.89 | 4/4 |
| EBW/EN | 0.42 | 1.078 | 4/4 |
| B/ER | 0.51 | 0.75 | 4/4 |
| B/EN | 0.52 | 1.44 | 4/4 |
| ER/EN | 0.6 | 1.19 | 4/4 |

BS — band sharing value,
*D* — genetic distance,
*n* — number of individuals,
EN — Estonian Native cattle,
ER — Estonian Red cattle,
EBW — Estonian Black-and-White cattle,
B — Bestuzhevskaya cattle.

**Interbreed genetic variability.** Comparison between breeds was carried out by running DNA of four randomly selected individuals from each breed on the same gel, facilitating the exact comparison of band position between breeds (Fig. 4). Data on comparisons within each breed and between breeds are summarized in Table 2.

There appeared a great overlap in the inbreed and interbreed BS-values, indicating that the individuals investigated could not be firmly assigned to any one breed on the basis of these DNA fingerprinting data.

## DISCUSSION

A probe suitable for DNA fingerprinting should distinguish individuals to such a degree that the probability of two randomly selected individuals showing identical fragment patterns is very low. In animals and birds, the most efficient minisatellite probes reveal probabilities for identity of $10^{-8}$—$10^{-9}$ (Ellegren et al., 1992). The genetic variability revealed by minisatellite probes in domestic species has in general been lower than that in outbreed natural populations of animal and bird species.

Taking into account the total number of resolvable fingerprinting fragments (17—39), and considering that the probability for identity ranges from $10^{-5}$ to $10^{-15}$ in the four breeds studied, it could be supposed that the oligo(CAC)$_5$ probe is extremely useful for identifying indivduals in cattle.

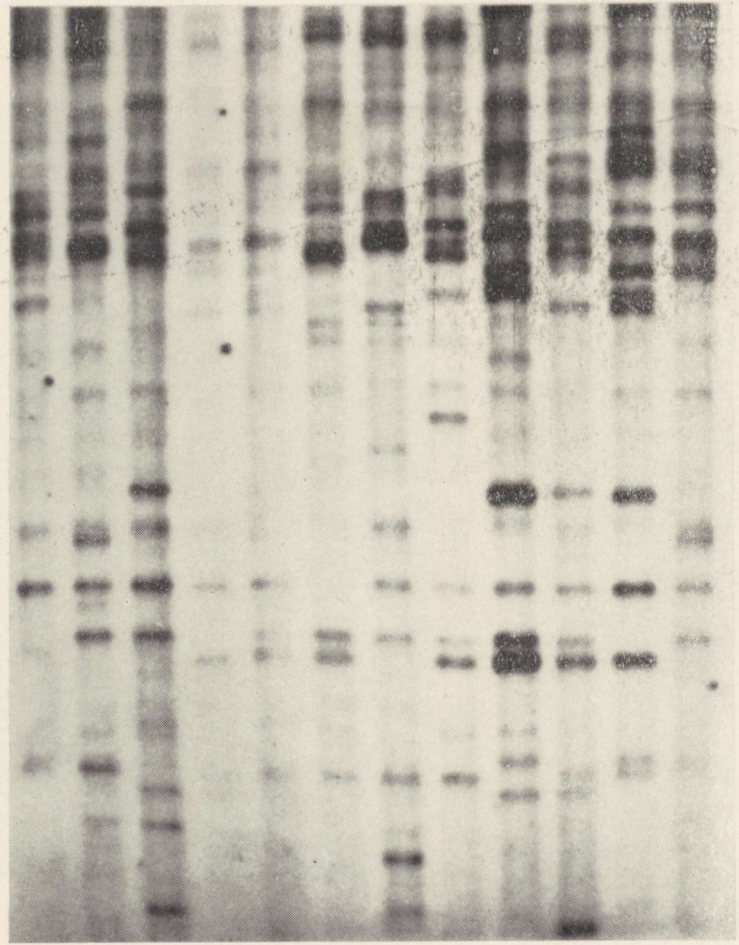

Fig. 1. Typical $(CAC)_5$ fingerprinting pattern on the DNA of Estonian Black-and-White cattle.

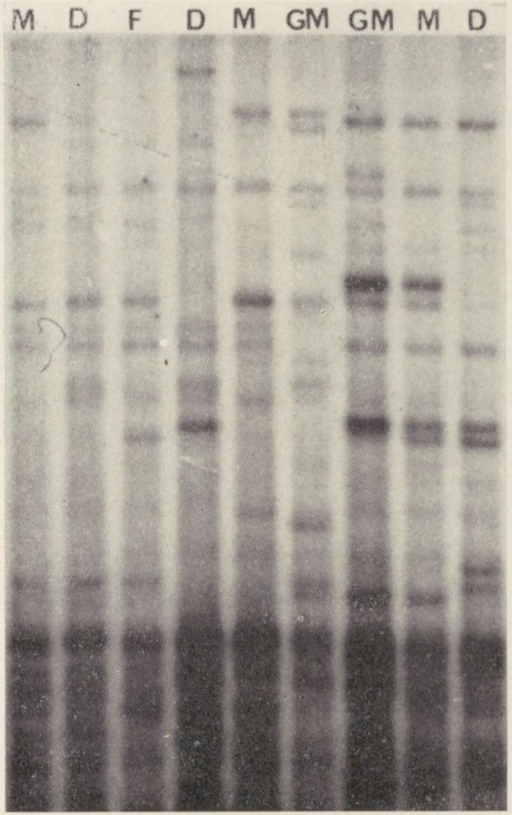


Fig. 2. DNA fingerprints from a half-sib family of Estonian Black-and-White cattle. F — father, M — mother, GM — grandmother, D — daughter.

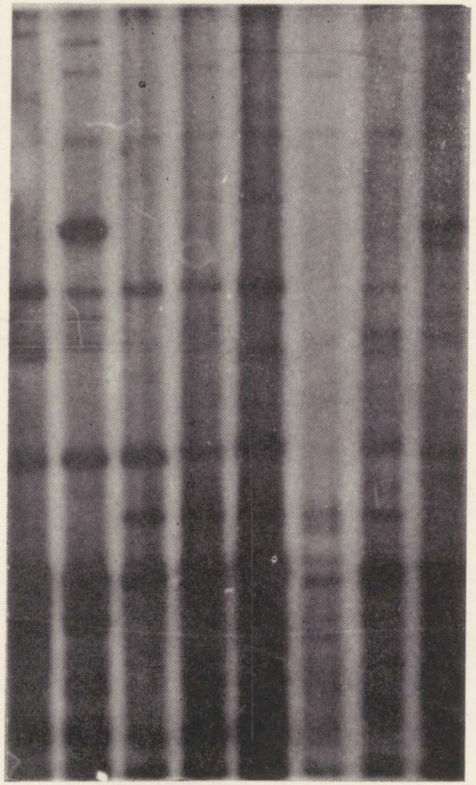

Fig. 3. DNA $(CAC)_5$ fingerprints of eight randomly selected individuals of Estonian Native cattle.

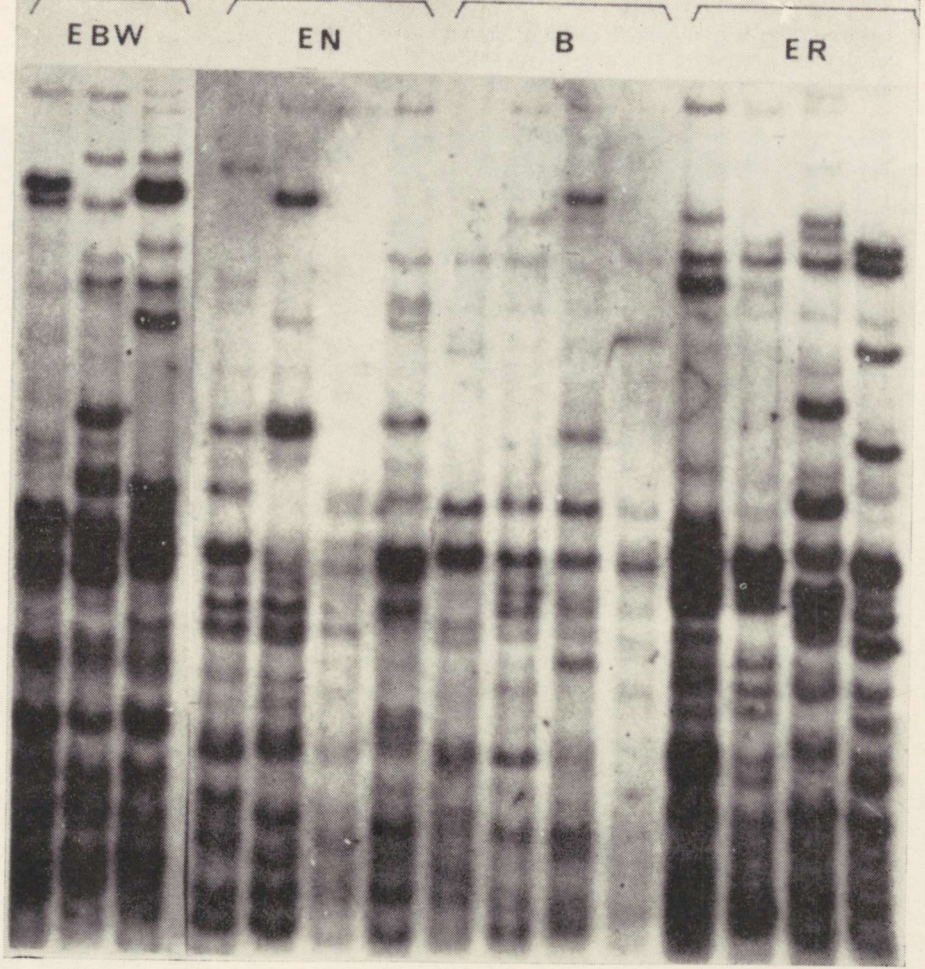


Fig. 4. DNA $(CAC)_5$ fingerprints of randomly selected individuals from Estonian Black-and-White (EBW), Estonian Native (EN), Bestuzhevskaya (B), and Estonian Red (ER) cattle.

Cattle family analyses, according to $(CAC)_5$ fingerprints, indicate that, as it has been shown before with other probes for other species (Kuhnlein et al., 1989), that the DNA fingerprinting patterns are inherited as stable genetic traits. This makes it possible to use them for family screening (inherited diseases) and for paternity testing.

By analysing allelic frequency in individual hypervariable loci, population structure can be efficiently monitored. In some recent studies also the DNA fingerprinting technique has been used for examining genetic variation on the population level, either interspecifically or intraspecifically (Kuhnlein et al., 1989; Ellegren et al., 1992).

In the present study, the highest band-sharing probability occurred in Estonian Native and Bestuzhevskaya breeds (0.5 and 0.6, resp.). In Estonian Red cattle it was 0.45 and in Estonian Black-and-White cattle only 0.32. So, the Bestuzhevskaya breed shows the highest degree of genetic homogeneity within the breed.

These results indicate that individuals of Estonian Native cattle and Bestuzhevskaya breed are genetically more similar than those of the two other breeds studied. Very probably this is the result of a high level of inbreeding in these two populations (there are approximately 500 individuals in Estonian Native cattle population all in all), whereas Estonian Red cattle and Estonian Black-and-White cattle populations possess more genetic variability within a breed.

In domestic animals it should be of great interest to use DNA fingerprints for breed affiliation. The DNA fingerprinting oligo $(CAC)_5$ system, used by us, fails to distinguish between these four breeds by fingerprinting patterns only. However, the inter-D value comparison (Table 2) clearly revealed genetic distances between different breeds, being the highest between Estonian Native cattle and Bestuzhevskaya breeds. This appears to be quite logical, as these two breeds are of local importance and are not used in broader selection programmes. It can be concluded that hypervariable DNA polymorphism with oligo $(CAC)_5$ might be suitable for investigating breed affiliation. Moreover, hypervariable DNA polymorphism in this case enables to use oligo $(CAC)_5$ as a probe for screening new landmark markers in size-selected plasmid library from Bsu RI fragments of Estonian Native cattle DNA. This could be of interest, as local land races might have specific features of adaptation, being potential sources of new valuable gene combinations (Kaukinen, Varvio, 1992).

Summarizing the results of the present investigation it could be said that the probe $(CAC)_5$ is a suitable tool for screening cattle's genetic material and seeking for highly polymorphic marker loci.

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## MIKROSATELLIITSE SONDI OLIGO$(CAC)_5$ KASUTAMINE DNA «FINGERPRINTIDE» SAAMISEKS NELJA VEISETÕU PUHUL

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On uuritud mikrosatelliitse sondi $(CAC)_5$ kasutusvõimalusi genoomsete «fingerprintide» saamiseks veise DNA-l ning näidatud, et nimetatud sond on efektiivne isendite individuaalseks eristamiseks ja isaduse tuvastamiseks. Oligo$(CAC)_5$ «fingerprintide» võrdlusel selgunud geneetiline kaugus uuritud veisetõugude vahel ei võimalda siiski eristada tõuge omavahel. Nimetatud sondi saab edukalt kasutada hüpervariabiilsete, tõuspetsiifiliste ja produktiivsete omadustega seotud geneetiliste markerite otsimiseks veisel.

## ИСПОЛЬЗОВАНИЕ ОЛИГО$(CAC)_5$ МИКРОСАТЕЛЛИТНОЙ ПРОБЫ ПРИ ФИНГЕРПРИНТЕ ДНК ЧЕТЫРЕХ ПОРОД КРУПНОГО РОГАТОГО СКОТА

Терье РАУДСЕПП, Тамара ЛУШНИКОВА, Николай КОРОХОВ, Александр СМИРНОВ

Исследованы возможности использования олигонуклеотида $(CAC)_5$ в качестве зонда для идентификации ДНК крупного рогатого скота с помощью метода геномного фингерпринта. Показано, что данный зонд имеет высокую информативность для целей идентификации индивидуумов и установления отцовства. Значения генетических расстояний, полученные при сравнении олиго$(CAC)_5$ фингерпринта у четырех пород, не достаточно дискретны в случае полной идентификации пород, но в целом исследованный зонд может быть успешно применен для поисков гипервариабельных породоспецифических маркеров генов продуктивности.