# PARENTAGE CONTROL IN CATTLE BY GENOTYPING MICROSATELLITES

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**Summary.** *Microsatellites are highly polymorphic, readily detectable markers that offer great promise for paternity testing and individual characterization in humans and domestic animals. We present here our experiences with a system consisting of 6 microsatellite loci, as a new approach to parentage control in cattle. The microsatellite polymorphisms were visualized by automated fluorescence detection on the Applied Biosystems 373 DNA Sequencer with 672 Genescan Analysis software, and allowed us to solve all tested bovine paternity cases not solveable by conventional blood typing, and only partially solveable by multilocus DNA fingerprinting (pV47). We propose microsatellite genotyping as the sole method for parentage control in cattle.*

## Introduction

Blood typing for parentage control has been offered as a service to breeders and herdbooks in many laboratories throughout the world since about 1950. Parentage control is carried out to verify the relationship of animals for pedigree registration, to determine the father in cases of multiple insemination or when mixed semen was used, and in animals originating from embryo transfer. Correct parentage among breeding stock is a prerequisite for an efficient breeding program.

Most questions of parentage in cattle can be solved adequately by current conventional testing: in our lab (Berne) conventional blood typing consists of the determination of blood group antigens, serum proteins and red cell enzymes; cases which are not conclusive in the routine analysis are completed, if possible, by the determination of bovine leucocyte antigens (BoLA). Nevertheless, in a few cases, the application of the mentioned polymorphisms does not allow a clear conclusion and should be extended by utilising other genetic marker systems.

Recently, a new type of genetic polymorphism in the area of DNA profiling (Easteal et al. 1991), based upon microsatellites, (i.e., repetitive 1 - 5 base pair motifs) has been described in several species, including cattle. These polymorphisms are due to the variable number of repeat units and can be visualized using the polymerase chain reaction (PCR) and subsequent polyacrylamide electrophoresis. Microsatellite genotyping can be automated by fluorescence-based fragment sizing, e.g., on the Applied Biosystems 373 DNA Sequencer equipped with 672 Genescan Analysis software (Ziegle et al. 1992). We now present a system of 6 polymorphic microsatellites of the $(CA)_n$ type that allowed us to solve 25 parentage control cases which could not be solved by conventional typing, and for which even DNA fingerprinting was not in every case conclusive.

## Material and Methods

Blood and semen samples were obtained from routine paternity cases not solveable by conventional blood typing and only partially solveable by DNA-fingerprinting. DNA extraction was carried out as described by Dolf et al. (1992). Six polymorphic (number of alleles $\geq 6$) microsatellite loci located on 6 different chromosomes were chosen (Table 1). PCR amplification was performed using a Perkin Elmer DNA Thermal Cycler in two multiplex reactions, each containing the primer pairs for three loci. The primers were synthesized and labelled at their 5'-ends (one of each pair) by a commercial oligonucleotide synthesizing service with the fluorochromes as indicated in Table 1. 50-100 ng of template DNA were amplified in 25 µl standard PCR reactions containing 2.5 units Taq polymerase (Boehringer Mannheim) and 0.5 - 0.8 µl of each fluorescent-dye-labelled forward and each unlabelled reverse primer (20 µM). Samples were subjected to 28 cycles of denaturation at 94° C for 30 sec, annealing for 30 sec at the temperature indicated in Table 1, and extension at 72° C for 1 min. The last polymerization step was extended to 7 min. The co-amplified PCR products were diluted 1:4 . To 1 µl of the mixture (combined 1:1 from the two diluted multiplex reactions) 4 µl of formamide and 0.5 µl of the Genescan 2500 Rox internal lane standard (Applied Biosystems) were added. Each mixture was heated at 95° C for 4 min, chilled on ice and applied to one lane of 6% polyacrylamide gel containing 8.3 M urea installed in a 373 DNA Sequencer of Applied Biosystems. The fluorescent signals were collected and analysed on a Macintosh computer equipped with the Genescan 672 software (Applied Biosystems). The fragment size was automatically calculated based on the internal lane standard, as described by Ziegle et al. (1992).

Blood group determination, BoLA-typing, and protein electrophoresis were carried out using standard procedures, and DNA fingerprinting (probe pV47) was as described by Dolf et al. (1992).

**Table 1.** Characteristics of the microsatellite loci and PCR reactions

| PCR system [AT[a)]] | Locus | Chromosome | Microsatellite designation | No. of alleles | Size range (base-pairs) | Labelling dye | Reference[b)] |
|---|---|---|---|---|---|---|---|
| 1 [58°C] | BOLADRBP1 | 23 | MHCII | 7 | 208-226 | FAM | 1 |
| | DU17S2 | U17 | ETH121 | 7 | 173-211 | TAMRA | 2 |
| | D21S4 | 21 | ETH131 | 8 | 141-163 | JOE | 2 |
| 2 [65°C] | D5S3 | 5 | ETH10 | 6 | 210-224 | JOE | 3 |
| | DU2S1 | U2 | ETH225 | 6 | 141-159 | TAMRA | 2 |
| | D19S2 | 19 | ETH3 | 6 | 114-126 | FAM | 3 |

a) Annealing temperature. b) 1, Creighton et al. (1992) 2, Steffen et al. (1993); 3, Solinas et al. (1993).

**Results and Discussion**

Details on the estimation of the allele sizes based on the electrophoretograms and the calculated fragment sizes, the estimation of allele frequencies in the Swiss cattle population, and a study of the variation of the fragment sizes in different gel runs will be presented elsewhere (Glowatzki-Mullis et al., manuscript in preparation). However, in general run to run variations of the fragment size were minimal, which is very important when the results from newly analysed samples have to be compared to stored data. Table 2 summarizes the results from conventional blood typing, DNA fingerprinting and microsatellite genotyping in 25 parentage control cases. All cases were solved using the genotypes of the 6 microsatellite loci. Therefore, the set of microsatellites used in this study is an extremely powerful system for parentage control in cattle. Multiplexing of the PCR and the automated fragment analysis make this system very efficient and attractive for routine parentage control in cattle. In terms of time to perform the test (and this without the use of radioactivity), power of the results, costs and possibilities of computerization, it seems that in the area of DNA profiling microsatellite genotyping is the most promising approach. A PCR-based system also has other advantages over the conventional blood typing and minisatellite fingerprinting. Only minute amounts of DNA are required for PCR-based analyses. As opposed to the DNA fingerprints, the microsatellites represent a closed system, allowing consideration of both alleles and therefore providing complete genotype information for each locus. DNA-based typing is not affected by the age of the animals, in contrast to certain conventional systems. DNA can be extracted from blood, semen and other tissues and can be stored for practically unlimited periods of time, thus allowing the setup of DNA banks. Another disadvantage of conventional typing over microsatellite genotyping is the diversity of methods that need to be mastered. Furthermore, blood group testing depends on the production and maintenance of a panel of test sera. Based on the above findings and considerations, the blood typing service at the Institute for Animal Breeding in Berne, which handles about 3000 bovine parentage control cases a year, intends to gradually replace conventional blood typing with microsatellite genotyping.

**References**

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**Table 2.** The results from conventional blood typing, DNA fingerprinting and microsatellite genotyping in 25 parentage control cases. In cases **01 - 18**, the cow was subject to two AI (artificial inseminations) within 24 days with semen from two bulls; in cases **19 - 22**, parentage of a father or mother had to be confirmed; in case **25**, 4 bulls were indicated as possible fathers; in two cases (**23** and **24**) one parent (the mother) was not available for typing. ["-" indicates exclusion, "+" indicates, that the questionable animal(s) cannot be excluded from being the parent. The number in parentheses indicates the number of microsatellite loci leading to an exclusion].

| Case no. | Conventional blood typing | DNA-fingerprinting | Microsatellite genotyping |
|---|---|---|---|
| 01 | bull1 + bull2 + | bull1 + bull2 + | bull1 -(1) bull2 + |
| 02 | bull1 + bull2 + | bull1 + bull2 - | bull1 + bull2 -(1) |
| 03 | bull1 + bull2 + | bull1 - bull2 + | bull1 -(2) bull2 + |
| 04 | bull1 + bull2 + | bull1 - bull2 + | bull1 -(2) bull2 + |
| 05 | bull1 + bull2 + | bull1 - bull2 + | bull1 -(2) bull2 + |
| 06 | bull1 + bull2 + | bull1 - bull2 +(?) | bull1 -(3) bull2 + |
| 07 | bull1 + bull2 + | bull1 - bull2 + | bull1 -(3) bull2 + |
| 08 | bull1 + bull2 + | bull1 - bull2 + | bull1 -(3) bull2 + |
| 09 | bull1 + bull2 + | bull1 - bull2 + | bull1 -(3) bull2 + |
| 10 | bull1 + bull2 + | bull1 - bull2 + | bull1 -(3) bull2 + |
| 11 | bull1 + bull2 + | bull1 - bull2 +(?) | bull1 -(3) bull2 + |
| 12 a) | bull1 + bull2 + | bull1 + bull2 + | bull1 -(3) bull2 + |
| 13 | bull1 + bull2 + | bull1 - bull2 + | bull1 -(3) bull2 + |
| 14 | bull1 + bull2 + | bull1 - bull2 + | bull1 -(4) bull2 + |
| 15 | bull1 + bull2 + | bull1 + bull2 + | bull1 -(4) bull2 + |
| 16 | bull1 + bull2 + | bull1 - bull2 + | bull1 -(5) bull2 + |
| 17 | bull1 + bull2 + | bull1 - bull2 + | bull1 -(5) bull2 + |
| 18 | bull1 + bull2 + | bull1 - bull2 + | bull1 -(6) bull2 + |
| 19 | bull -(?) (chimerism ?) | bull - | bull -(1) |
| 20 | bull +(?) (chimerism ?) | not typed | bull + |
| 21 | bull - (?) | not typed | bull -(5) |
| 22 | mother - (disputed) | not conclusive | mother -(2) |
| 23 | bull1 - bull2 +(?) | bull1 -(?) bull2 +(?) | bull1 -(2) bull2 + |
| 24 | bull - (disputed) | not typed | bull -(2) |
| 25 | bull1 +(?) bull2 - bull3 - bull4 - | bull1 + bull2 - bull3 - bull4 - | bull1 + bull2 -(4) bull3 -(3) bull4 -(3) |

a) Bull2 is the son of bull1. The same case also showed probable allele-non-amplification (Koorey et al., 1993) in the mother and in the calf. Non-maternity appeared to be a very unlikely explanation considering the constellation at 7 other microsatellite loci (2 more were investigated). Furthermore, the fingerprints of the calf and the cow were very similar.