# APPLICATION OF THE POLYMERASE CHAIN REACTION PROCEDURE FOR GENETIC EVALUATION IN CATTLE

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## INTRODUCTION

The main tool for the improvement of farm animals is genetic selection. The efficiency of selection depends on the accuracy of genetic evaluation. New molecular technologies are now available that can make possible an accurate identification of genotypes and widespread screening of animals in the population for marker traits related to quantitative characters. Among the new technologies the polymerase chain reaction (PCR) procedure of gene amplification represents a significant advance in the field of molecular biology for the analysis of DNA and RNA (White et al., 1989). This technique will play a prominent role in future application of molecular methods to animal breeding.

With the PCR technique a specific sequence of DNA can be amplified to several million fold in a matter of hours, even from the small amounts of DNA contained in a single cell. As a result of this exponential amplification, the presence or absence of a specific sequence in the genome can be determined. Sequences that are present can be analyzed for specific mutations or polymorphisms by the direct visualization of restriction fragment length polymorphisms (RFLP). Single base differences can be differentiated with allele specific oligonucleotide probes (ASO) or by direct sequencing of the amplified fragments.

We have developed methods to type the kappa-casein ($\kappa$-CN) (Medrano et al., 1990a), and beta-lactoglobulin ($\beta$-LG) genotypes at the DNA level (Medrano et al., 1990b), as well as a procedure for determining sex (X/Y) using PCR and RFLP analysis. Beta-casein ($\beta$-CN) genotypes have been typed by differential hybridization of nonradioactively labeled allele specific oligonucleotide probes on PCR amplified target DNA. This means that by taking a sample of blood, semen or any nucleated tissue of an animal, and extracting DNA, we can now type the milk protein genotype in males and females at any age and determine sex even at the embryonic stage.

## MATERIALS AND METHODS

In a typical PCR reaction .1 to .5 $\mu$g of DNA were used for amplification using the Perkin-Elmer Cetus thermocycler. The PCR conditions were as described by Medrano et al. (1990a,b). The samples were amplified for 30 cycles at 94°C for 45 sec (denaturation), 55°C for 1 min (annealing, Table 1) and 73°C for 1 min (extension). Twenty five microliters of the PCR reaction were digested with 25 units of a restriction enzyme (Table 1) and analyzed

by electrophoresis in 4 % ethidium bromide stained agarose gels (2% NuSieve GTG and 2% Ultrapure BioRad agarose). The DNA bands were visualized under ultra violet light.

Table 1: Annealing temperature for PCR, size of amplified fragment and restriction enzyme utilized for RFLP analysis.

| Gene | Annealing | Fragment | Enzyme/RFLP |
|---|---|---|---|
| κ-CN | 55 °C | 350 bp | HinfI |
| β-LG | 55 °C | 247 bp | HaeIII |
| X/Y | 57 °C | 440 bp | PstI |
| β-CN | 59 °C | 293 bp | - |

ASO probes specific for the beta-casein alleles were labeled with digoxigenin-11dUTP using terminal transferase (DNA tailing kit; Boehringer Mannheim Biochemicals). After PCR amplification dot blots prepared on Nytran membrane were hybridized with ASO probes. Color development was carried out as described in protocol for the nonradioactive DNA labeling and detection kit (Genius, Boehringer Mannheim Biochemicals).

## RESULTS

The results of PCR amplification and genotype classification in the systems described above are shown in figure 1.

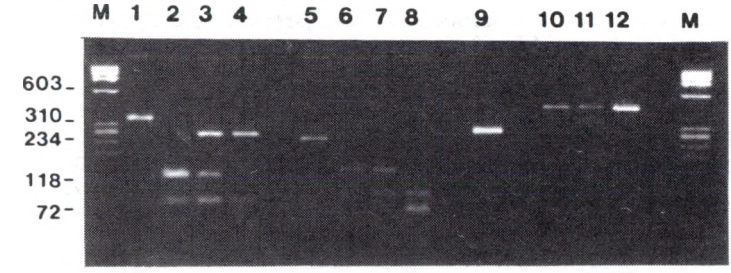


FIGURE 1: Ethidium bromide stained agarose gel separation of PCR amplified fragments, allele classification for κ-CN and β-LG and sex identification by RFLP analysis. Lane M, φX174 HaeIII marker; lane 1 κ-CN amplified 350 bp fragment; lanes 2, 3 & 4 κ-CN genotypes AA, AB and BB, respectively; lane 5 β-LG amplified 247 bp fragment; lanes 6, 7 & 8 β-LG genotypes AA, AB and BB, respectively; lane 9 β-CN amplified 293 bp fragment; lane 10 X/Y amplified 440 bp fragment; lanes 11 & 12 male and female DNA, respectively.

Efficient application of genetic selection for milk proteins in dairy herds requires information on sires. Using PCR, a sample of Holstein sires with high genetic merit for milk yield were analyzed for their κ-CN and β-LG genotype. Gene frequencies were consistent with those previously observed based on milk protein analyses (Table 2).

Table 2: Genotypic frequencies in Holstein sires.

| Milk Protein | No. of bulls | Genotype AA | AB | BB |
|---|---|---|---|---|
| κ-CN | (52) | 73% (38) | 23% (12) | 4% ( 2) |
| β-LG | (46) | 22% (10) | 37% (17) | 41% (19) |

## DISCUSSION

Several studies have indicated the association of the milk protein genotypes κ-CN BB, β-CN BB and β-LG BB, with important properties of milk affecting cheese yield and cheese quality (McClean et al., 1984; Schaar et al., 1985; Marziali et al., 1986). Traditionally, milk protein genotypes have been classified by milk protein gel electrophoresis. Use of this type of methodology was limited to lactating cows; determination of sire genotype required analysis of multiple dam/daughter pairs and an average of 6 years. Thus, genotype determination by gel electrophoresis analysis was not practical for dairy herd improvement. Direct DNA genotyping provides a significant advantage. However, traditional DNA analyzing techniques that have been used with milk proteins (Rando et al., 1988; Leveziel et al., 1988; Rogne et al., 1989, Damiani et al.,1989) are time consuming, technologically demanding, and frequently require the use of radioactive materials which makes them impractical for widespread use. The recent development of the PCR technique has helped overcome the limitations of traditional DNA analysis.

An important objective in a breeding program is to improve the efficiency of genetic selection by an accurate identification of superior genotypes and a reduction of the generation interval. The combination of the typing technique at the DNA level described in this report in combination with new methods in reproductive biology will allow this improvement in efficiency of selection. If superior quality of milk can be associated with a specific allele such as the κ-CN B, the genotypes could be classified in sires, heifers and cows. Young sires could be genotyped and pre-screened before they are progeny tested for other production traits. High producing cows carrying the desirable genotype could be superovulated, mated to a specific sire, the embryos split, genotyped and sexed, such that only specifically selected embryos would be transferred to recipient cows.

Allelic diagnosis can be significantly simplified using PCR amplification and RFLP analysis. With PCR large numbers of individuals can be easily analyzed for allelic variation including promoter regions involved in the control of quantitative variation. Because PCR is so specific it is possible to amplify segments of the genome containing allelic variations that can be identified by RFLP's. The large number of copies of a specific DNA fragment generated by PCR make it possible to identify the variants without use of labeled probes. Also, since DNA can be isolated from any tissue, including sperm, the method described here can be applied to breeding programs that rely on artificial insemination.

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