# Genetic Characteristics of Five Microsatellite Markers Associated with Milk Production Traits in Crossbred Dairy Cattle of Kerala

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**Abstract**

Marker Assisted Selection (MAS), which is the process of using the results of DNA testing to assist in the selection of individuals to become parents of the next generation by combining the genotypes and the expected progeny differences of the bulls. In the present study, all the five markers were highly informative. The highest PIC value was obtained for the microsatellite marker ILSTS096 (0.865), followed by BL41 (0.849), BM4305 (0.846), HUJII77 (0.842) and BM1508 (0.630). The highest direct count heterozygosity was observed for the microsatellite marker ILSTS096 (0.877), followed by BL41 (0.862), BM4305 (0.861), HUJII77 (0.851) and BM1508 (0.683). The highest unbiased heterozygosity of 0.880 was observed for the microsatellite marker ILSTS096, followed by BL41 (0.865), BM4305 (0.864), HUJII77 (0.854) and BM1508 (0.686).

**Keywords:** Microsatellite, Milk Production, Dairy Cattle, Kerala, Genetic Characteristic

## Introduction

Mammalian genome contains large amount of repetitive DNA sequences, an increasing number of which are being identified as stretches of tandem repeat units. The repeat units ranging in size from two to six base pairs form stretches of DNA referred to as short tandem repeats or microsatellites. Detection of allelic variations at loci influencing economically important traits of cattle has become feasible due to the construction of bovine linkage maps, mostly composed of microsatellites.

## Material and Methods

**DNA samples:** Blood was used as a source of DNA from crossbred cows maintained at the University Livestock Farm, Mannuthy and Cattle Breeding Farm, Thumburmuzhi. DNA samples from 217 genetically unrelated animals were used for the study. DNA was extracted by modifications in the phenol-chloroform protocol (Andersson *et al.,* 1986).

**Microsatellite markers:** Five markers viz. ILSTS096, HUJII77, BL41, BM1508 and BM4305 were chosen for the study (Table 1). For visualizing the PCR products by autoradiography, forward primer of each marker was radio-labelled at the 5' end with ?32P-ATP. The reaction was carried out with the DNA endlabeling Kit 1 (Genei).

Each PCR cycle had an initial denaturation at 94°C for three minutes followed by 35 cycles each consisting of denaturation at 94°C for one minute, annealing at 55°C-58°C according to the primer sets used for one minute and extension at 72°C for one minute. This was followed by a final extension for five minutes at 72°C. The samples were then cooled down to 4°C and stored at -20°C. To determine the allele size of markers, comparison with a sequencing ladder is necessary. Single stranded M13 phage DNA was sequenced using the DNA Sequencing Kit Version 2.0. The radio-labeled PCR products were fractionated using 6 per cent denaturing polyacrylamide gels. 3.5 µl of formamide loading buffer was added to the PCR products, mixed well, denatured at 95°C for 5 minutes and cooled immediately on ice. 3.5-4 µl each of this mixture was loaded into each well. Sequenced products of M13 DNA were loaded in 4 wells (G, A, T, C). The gels were electrophoresed at 35 W for 1.5-3 hours according to the PCR product size, maintaining a temperature around 45-50°C. After electrophoresis, the gel was dried in a gel drier at 82°C and set for autoradiography. The number of alleles for each marker was counted and their size was determined by comparing with M13 sequencing ladder. The G, A, T and C sequences were read from the bottom to the top in the order. The allele sizes were determined corresponding to the G, A, T and C bands. The allele frequency was worked out.

**Statistical Analysis**

**Direct Count Heterozygosity:** The usefulness of a marker depends on its heterozygosity. Heterozygosity was calculated by the method of Ott (1992).

$$H = 1 - \sum_{i=1}^{k} Pi2$$

Where, Pi is the frequency of ith allele at a locus.

**Unbiased Heterozygosity:** The unbiased heterozygosity was calculated.( Pandey *et al.,*2002).

$$H = [2n/(2n-1)][1- \sum_{i=1}^{k} (Pi2)]$$

Where Pi is the frequency of ith allele and n is the number of observations.

**Polymorphic Information Content (PIC):** PIC values for the markers were calculated as

$$PIC = 1 - [\sum_{i=1}^{n} Pi2] - [\sum_{i=1}^{n-l} \sum_{j=i-1} 2 Pi2 Pj2]$$

Where Pi and Pj are the frequencies of ith and jth alleles respectively (Botstein *et al*., 1980).

**Results and Discussion**

The selected microsatellite markers were typed on genetically unrelated animals to find out allelic number, allelic size, allelic frequency, PIC, direct count heterozygosity and unbiased heterozygosity. Twelve alleles with a size ranging from 188-212 bp were detected for ILSTS096 locus. The highest frequency of 0.191 was observed for the alleles 198 and 200. HUJII77 had thirteen alleles with a size range of 193-221 bp. The highest frequency was observed for the allele 209. Thirteen alleles were detected with a size range of 232-258 bp. One peculiarity noted for this marker was 44 per cent animals in the genetically unrelated population were homozygous. BM1508 had seven alleles with a size range of 103-115 bp. Number of alleles observed for BM1508 was lowest and the size range was the smallest among the markers studied. Twelve alleles were detected with a size range of 146-168 bp for BM4305.

The level of informativeness of the microsatellite markers can be measured as PIC. PIC was calculated by the method of Botstein *et al*. (1980). According to Botstein *et al.* (1980) a marker is highly informative if its PIC is greater than 0.5. In the present study, all the five markers were highly informative. The highest PIC value was obtained for the microsatellite marker ILSTS096 (0.865), followed by BL41 (0.849), BM4305 (0.846), HUJII77 (0.842) and BM1508 (0.630). The highest direct count heterozygosity was observed for the microsatellite marker ILSTS096 (0.877), followed by BL41 (0.862), BM4305 (0.861), HUJII77 (0.851) and BM1508 (0.683). The highest unbiased hetero-zygosity of 0.880 was observed for the microsatellite marker ILSTS096, followed by BL41 (0.865), BM4305 (0.864), HUJII77 (0.854) and BM1508 (0.686).

**Conclusion**

A recent application of molecular technology in dairy cattle breeding is the identification of the regions of the DNA affecting the production traits. According to Botstein *et al*. (1980) a marker is highly informative if its PIC is greater than 0.5. In the present study, all the five markers were highly informative. The highest PIC value was obtained for the microsatellite marker ILSTS096 (0.865), followed by BL41 (0.849), BM4305 (0.846), HUJII77 (0.842) and BM1508 (0.630). Hence these markers can be used for Marker Assisted Selection (MAS), which is the process of using the results of DNA testing to assist in the selection of individuals to become parents of the next generation by combining the genotypes and the expected progeny differences of the bulls. MAS enables the selection of the superior genotypes based on the allelic information of the marker loci.

**Table-1. Microsatellite Markers**

| Marker | Location | Associated traits |
|---|---|---|
| ILSTS096 | BTA3 | milk yield, fat yield, fat percentage, protein yield and protein percentage (Heyen *et al.*, 1999; Rodriguez-Zas *et al*.,2002) |
| HUJII77 | BTA3 | milk yield and protein percentage(Heyen *et al.* ,1999; Rodriguez-Zas *et al*.,2002) |
| BL41 | BTA3 | milk yield, fat percentage and protein percentage (Heyen *et al.* ,1999; Rodriguez-Zas *et al*.,2002) |
| BM1508 | BTA14 | milk fat percentage (Heyen *et al.* 1999) |
| BM4305 | BTA14 | milk protein percentage and milk yield (Ashwell *et al.*,1998; Heyen *et al.* 1999) |

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## Book Review


**Welfare aspects of the long distance transportation of animals**
**Veterinaria Italiana, Volume 44 (1), January - March 2008.**
Publisher: Instituto Zooprofilattico Sperimentale dell' Abruzzo e del Molise "G. Caporale" in Teramo, Italy. Pages : 289.

The long distance of transport of animals is not only increasing significantly but is also changing in nature. Statistics show that the value of world trade in live animals soared from US$ 8.7 billion in 2000 to US$ 12.1 billion in 2005. This enormous figure does not include zoo animals, wildlife and illicit traffic.

What has made circumstances different in the 21st century is the nature of transport (land, sea, and air), the volume of traffic and a public awareness of welfare issues with demands that animals be treated humanely and in accordance with best contemporary practices. This increased volume of transport creates an unprecedented risk for disseminating infectious diseases, including those that may affect people.

This issue of Veterinaria Italiana deals with livestock transportation. It is not a collation of scientific articles on the subject per se. Rather, its uniqueness drives from the fact that it provides advice and guidance on practical measures to improve approaches based on analyses of the science. Policy makers, risk analysis, regulators, quality managers, engineers and educators will gain greatly from this edition, which will serve as a key reference document on the subject.

This book includes contributions from 53 of the world's top specialists in this field in a total of 27 peer-reviewed papers presented in 8 chapters (history, the views of civil society organisations, development of public policy, quality management and the various animal species, future directions for quality management, design and engineering of infrastructures, transport safety, training, education and outreach).

I hope that this book is very useful.

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