# Molecular Characterisation of the Mafriwal Dairy Cattle of Malaysia Using Microsatellite Markers

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**ABSTRACT :** The Mafriwal dairy cattle was developed to meet the demands of the Malaysian dairy Industry. Although there are reports on its production and reproductive performance, there has been no work on its molecular characterization. This study was conducted to characterize the Mafriwal dairy cattle using microsatellite markers. Fifty two microsatellite loci were analysed for forty Mafriwal dairy cows kept at Institut Haiwan Kluang, Malaysia. The study showed two microsatellite loci to be monomorphic. Allele frequencies for the polymorphic loci ranged from 0.01 to 0.31. Genotype frequencies ranged from 0.03 to 0.33. The mean overall heterozygosity was 0.79. All polymorphic microsatellite loci deviated significantly ($p<0.01$) from Hardy-Weinberg equilibrium. The Mafriwal dairy cattle showed high genetic variability despite being a nucleus herd and artificial insemination being practiced. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 10 : 1366-1368)***

**Key Words** : Mafriwal, Dairy Cattle, Microsatellite, Genotype, Gene Frequency

## INTRODUCTION

Various aspects of a population are useful in its characterisation, including morphological traits, production and reproductive performance, geographic distribution, origin and habitat. The genetic characterisation of populations, breeds and species allows the assessment of genetic variability, an important element in determining breeding strategies and genetic conservation programs. This is especially important in highly specialized livestock breeds since the latter are subjected to assisted reproductive techniques, such as artificial insemination, embryo transfer and selection. Molecular markers, such as RFLP and microsatellites have been widely used to access genetic variability since they provide information on every region of the genome analysis may be combined with polymerase chain reaction (PCR). These markers have been used to explain bovine domestication and migration patterns (Bradley et al., 1994; Loftus et al., 1994) and to characterise cattle populations (MacHugh et al., 1997; Kemenes et al., 1999).

The Mafriwal dairy cattle is a synthetic breed developed by Department of Veterinary Services Malaysia from crossbreeding of the Sahiwal and the Friesian, as a high milk producer with resistance to local diseases, to meet the demand of the Malaysian dairy industry. It has 50-75% Friesian genes. Although there are reports on the production and reproductive performance of this cattle, there is no report on its molecular characterization. The aim of the present study was to identify and evaluate polymorphic microsatellite loci for the Mafriwal dairy cattle of Malaysia.

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## MATERIALS AND METHODS

### Experimental material

Forty Mafriwal cows kept at Institut Haiwan Kluang, Johor, a farm belonging to the Department of Veterinary Services Malaysia, were used in this study. These animals were chosen randomly to represent the total population at the Institute, that served as one of the nucleus herds. DNA was extracted from the blood samples using the commercially available Qiagen Blood kit. Fifty-two microsatellite marker primer pairs, selected from the cattle genome database (http://sol.marc.usda.gov/genome/cattle/html/marker), were analyzed using PCR followed by agarose gel electrophoresis.

### Genotyping

The PCR reactions were carried out in a MJ Research Peltier Thermal cycler. PCR mix contained 200 ng of DNA, 1.5 mM $MgCl_2$, 200 μM of dNTP mix, 20 pm of each primer and 2.5 units of Taq DNA polymerase in a final volume of 20 μl. PCR amplification conditions were as follows : intial denaturation step at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 4 min, annealing for 1 min at 55-60°C, extension for 1 min at 72°C, and a final extension at 72°C for 10 min. Ten microlitre of the amplified product was electrophoresed with a standard 25 bp ladder on 4% metaphor agarose at 70 Volts for 2½ hours. Gels in 1X TBE buffer were stained with ethidium bromide and photographed using gel imaging system (Vilberlourmat).

### Statistical analysis

Genotype frequencies, allele frequencies and

**Table 1.** Summary of variation at 50 microsatellite loci in the Mafriwal Dairy cattle

| Locus | Na | Ne | Allele size range | No of genotypes | Ho | He | $\chi^2$ | DF |
|---|---|---|---|---|---|---|---|---|
| HUJ614 | 7 | 5.57 | 173-236 | 12 | 0.62 | 0.83 | 72.60 | 21 |
| BM1819 | 6 | 4.60 | 107-158 | 13 | 0.52 | 0.79 | 51.80 | 15 |
| HUJV177 | 6 | 5.79 | 180-235 | 12 | 0.40 | 0.83 | 86.20 | 15 |
| BMS678 | 5 | 3.68 | 93-160 | 10 | 0.45 | 0.73 | 45.94 | 10 |
| CSSM5 | 7 | 5.67 | 105-150 | 15 | 0.57 | 0.83 | 54.65 | 21 |
| CSSM19 | 7 | 5.77 | 130-184 | 15 | 0.65 | 0.83 | 57.17 | 21 |
| BM4208 | 6 | 5.42 | 154-200 | 13 | 0.57 | 0.82 | 53.85 | 15 |
| HUJV174 | 7 | 6.55 | 123-181 | 18 | 0.52 | 0.85 | 68.29 | 21 |
| BM1290 | 7 | 5.97 | 103-152 | 16 | 0.65 | 0.84 | 85.67 | 21 |
| BM2639 | 8 | 6.77 | 111-197 | 18 | 0.65 | 0.86 | 97.60 | 28 |
| BRN | 7 | 6.13 | 214-300 | 16 | 0.75 | 0.84 | 44.81 | 21 |
| BMS1331 | 7 | 5.47 | 130-177 | 16 | 0.45 | 0.82 | 67.71 | 21 |
| UWCA26 | 6 | 3.11 | 111-170 | 12 | 0.60 | 0.68 | 26.80 | 15 |
| BMS1318 | 6 | 4.79 | 111-173 | 12 | 0.47 | 0.80 | 70.61 | 15 |
| HEL5 | 5 | 4.10 | 143-185 | 10 | 0.50 | 0.76 | 36.89 | 10 |
| MB002 | 6 | 5.08 | 146-275 | 12 | 0.35 | 0.81 | 87.13 | 15 |
| RM372 | 6 | 5.15 | 117-182 | 14 | 0.52 | 0.81 | 55.04 | 15 |
| BM1329 | 4 | 3.62 | 146-180 | 9 | 0.42 | 0.73 | 30.17 | 6 |
| AGLA29 | 5 | 4.28 | 145-195 | 9 | 0.55 | 0.77 | 42.53 | 10 |
| UWCA20 | 6 | 4.57 | 65-111 | 14 | 0.70 | 0.79 | 27.27 | 15 |
| BMS1716 | 5 | 4.59 | 175-126 | 8 | 0.57 | 0.79 | 77.89 | 10 |
| BM6425 | 6 | 5.24 | 165-215 | 16 | 0.42 | 0.81 | 53.36 | 15 |
| BMS4028 | 7 | 4.42 | 101-154 | 14 | 0.50 | 0.78 | 55.76 | 21 |
| BMS1316 | 5 | 2.89 | 101-136 | 10 | 0.52 | 0.66 | 29.79 | 10 |
| BM2078 | 5 | 3.47 | 93-140 | 10 | 0.65 | 0.72 | 21.04 | 10 |
| BM1742 | 7 | 5.57 | 152-220 | 14 | 0.42 | 0.83 | 75.07 | 21 |
| BMS1126 | 7 | 5.51 | 137-185 | 16 | 0.47 | 0.82 | 66.24 | 21 |
| BMS2057 | 6 | 5.59 | 76-122 | 16 | 0.57 | 0.83 | 46.19 | 15 |
| CSRM60 | 8 | 5.69 | 80-132 | 17 | 0.70 | 0.83 | 66.85 | 28 |
| BM2113 | 7 | 6.07 | 123-180 | 15 | 0.60 | 0.84 | 51.88 | 21 |
| TGLA122 | 6 | 5.14 | 125-165 | 14 | 0.50 | 0.81 | 62.26 | 15 |
| TGLA126 | 6 | 4.97 | 107-150 | 12 | 0.65 | 0.80 | 48.13 | 15 |
| RM209 | 9 | 6.25 | 160-182 | 20 | 0.70 | 0.85 | 43.32 | 36 |
| BMC1222 | 10 | 6.55 | 194-300 | 17 | 0.57 | 0.85 | 134.29 | 45 |
| RM188 | 9 | 6.68 | 115-173 | 17 | 0.50 | 0.86 | 104.12 | 36 |
| BMS922 | 4 | 3.15 | 64-82 | 8 | 0.20 | 0.69 | 72.77 | 6 |
| BMS4001 | 6 | 4.27 | 102-133 | 9 | 0.72 | 0.77 | 71.35 | 15 |
| BMS3507 | 10 | 7.28 | 161-231 | 19 | 0.40 | 0.87 | 145.71 | 45 |
| TGLA337 | 6 | 4.91 | 97-123 | 11 | 0.35 | 0.80 | 97.53 | 15 |
| BM415 | 7 | 5.63 | 146-200 | 20 | 0.67 | 0.83 | 39.59 | 21 |
| HMH1R | 7 | 4.95 | 128-180 | 11 | 0.37 | 0.80 | 88.30 | 21 |
| TGLA227 | 6 | 4.55 | 73-113 | 12 | 0.42 | 0.79 | 67.92 | 15 |
| SPS115 | 8 | 6.80 | 235-296 | 17 | 0.47 | 0.86 | 100.40 | 28 |
| TGLA170 | 7 | 4.89 | 85-125 | 14 | 0.40 | 0.80 | 91.46 | 21 |
| BM143 | 7 | 5.00 | 85-143 | 17 | 0.55 | 0.81 | 40.30 | 21 |
| ETH152 | 7 | 4.95 | 194-250 | 12 | 0.67 | 0.80 | 57.38 | 21 |
| BMS896 | 7 | 5.76 | 111-171 | 12 | 0.52 | 0.83 | 94.81 | 21 |
| BM1824 | 7 | 5.10 | 171-232 | 13 | 0.32 | 0.81 | 92.28 | 21 |
| AFR227 | 5 | 3.68 | 83-118 | 11 | 0.32 | 0.73 | 66.59 | 10 |
| BM1314 | 7 | 6.42 | 130-196 | 16 | 0.60 | 0.85 | 64.11 | 21 |
| Mean | 65.60 | 5.16 | - | - | 0.52 | 0.80 | - | - |
| St. Dev | 1.28 | 1.02 | - | - | 0.12 | 0.04 | - | - |

[1] Note: $\chi^2$: chi square values; DF: degrees of freedom. Na: number of observed alleles. Ne: number of effective alleles. Ho: observed heterozygosity. He: expected heterozygosity.

heterozygosity (H) were estimated using the PopGene software. Chi-square goodness of fit was used to test for conformity with Hardy-Weinberg equilibrium.

## RESULTS AND DISCUSSION

The microsatellite screening revealed 50 loci to be

polymorphic while two loci. TGLA 53 and TGLA116, were monomorphic. Summary of genetic variation for the microsatellite loci is shown in Table 1. The observed number of alleles per locus ranged from 4 to 8, which was relatively similar to the results of Luciana et al. (2003), who reported observed numbers of alleles ranging from 3 to 12 for four microsatellite loci in the Aberdeen Angus cattle. The allele frequencies for the 50 microsatellite loci ranged from 0.02 to 0.52. The mean number of alleles per locus was 6.23 with a mode of 7. The effective number of alleles per locus ranged from 2.89 to 7.28 with a mean of 5.16. Despite the small sample size used in this study the value is similar to that of Bishop et al. (1994), who reported a mean number of 6.8 alleles per locus in a survey of 468 microsatellite markers in 206 *Bos taurus*/ *Bos indicus* animals comprising of 22 reference families. The number of genotypes per locus ranged from 8 to 20. The genotypic frequencies ranged from 0.03 to 0.38. The number of genotypes being high could be due to number of alleles being high in the population. This may also indicate the existence of heterozygous genotypes in this population. Allele frequencies being low in this study could be explained by the fact that the number of alleles is high and the sample size being low.

The observed heterozygosity per locus ranged from 0.20 (BMS922) to 0.75 (BRN), with a mean of 0.52 and modes 0.42 and 0.65. Wright's fixation index showed eight loci to be 50% heterozygote deficit. The Mafriwal dairy cattle is a synthetic breed developed with intense use of artificial insemination and, to a small extend, embryo transfer. These could result in decreased genetic variability (Luciana et al., 2003), however, the values of heterozygosity per locus do not indicate such reduction. The Mafriwal dairy cattle showed high variability with respect to microsatellite loci despite the small herd size. This may be partly attributed to the fact that Mafriwal comprise of animals also with 50-75% Friesian genes and to the continual crossing among these animals of different levels of Friesian genes. Significant ($p<0.05$) deviations from Hardy-Weinberg equilibrium (HWE) were observed for all polymorphic loci studied. If a population deviates significantly from HWE at a number of independent loci it suggests that the population may be subjected to migration from an external source, or selection as a result of natural factors or by man, or is perhaps undergoing non random mating (Falconer et al. 1996; Machugh et al., 1997). The Mafriwal animals are kept for breeding purpose and the herd kept at Institut Haiwan Kluang is a nucleus herd. Therefore, the animals of this herd are subjected to selection. Furthermore, they are also bred using frozen semen not only from bulls at the Institute but also from bulls kept at other farms; these were chosen based on progeny testing. These practices may explain the deviation of Hardy-Weinberg equilibrium in addition to these factors. the presence of null alleles, which distort the genotypic proportion, could also contribute to this (Callen et al., 1993).

In conclusion, genetic marker analysis showed that the genetic variability among the Mafriwal dairy cattle was high. Since Mafriwal is a composite breed its gene pool would be constantly changing. Hence, the performance and genetic variability of these animals need to be monitored. The results of this study will serve as a guide for future monitoring genetic variability in Mafriwal population.

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