Alternative genotyping method for the single nucleotide polymorphism A2959G (AF159246) of the bovine CAST gene

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Abstract – The objective of this work was to genotype the single nucleotide polymorphism (SNP) A2959G (AF159246) of bovine CAST gene by PCR–RFLP technique, and to report its use for the first time. For this, 147 Bos indicus and Bos taurus x Bos indicus animals were genotyped. The accuracy of the method was confirmed through the direct sequencing of PCR products of nine individuals. The lowest frequency of the meat tenderness favorable allele (A) in Bos indicus was confirmed. The use of PCR–RFLP for the genotyping of the bovine CAST gene SNP was shown to be robust and inexpensive, which will greatly facilitate its analysis by laboratories with basic structure.

Index terms: calpastatin gene, meat texture, PCR–RFLP, SNP.

Método alternativo de genotipagem do polimorfismo de nucleotídeo único A2959G (AF159246) do gene CAST bovino


Termos para indexação: gene da calpastatina, textura da carne, PCR–RFLP, SNP.
One-hundred forty-seven bovine, of which 46 were pure Nellore (*Bos indicus*), 41 Canchim (5/8 *Bos taurus* + 3/8 *Bos indicus*), 26 Rubia Gallega x Nellore crossbred (1/2 *Bos taurus* + 1/2 *Bos indicus*), 19 Brangus three-way cross (9/16 *Bos taurus* + 7/16 *Bos indicus*), and 15 Brown Swiss three-way cross (3/4 *Bos taurus* + 1/4 *Bos indicus*), were genotyped using forward 5’ AAT ATA TGC GCT TCC TGG TCT GTC CAG 3’ and reverse 5’ AAT ATA TTC TCC CCA CAG TGC CTG TAA 3’ primers (Morris et al., 2006), along with DdeI restriction endonuclease.

The amplification reactions were performed in a final volume of 25 µL containing 50 ng of DNA, 0.2 µM of each primer, 1x PCR buffer (20 mM Tris-HCl, pH 8.4 and 50 mM KCl), 1.5 mM MgCl₂, 0.24 mM of each dNTP, and 0.75 U Taq DNA polymerase. After initial denaturation at 95°C for 5 min, amplification was performed in 38 cycles at 95°C for 1 min, 54°C for 1 min, and 72°C for 2 min, followed by a final extension step at 72°C for 5 min. Twelve microliter aliquots of the amplification products were digested with 3 U of DdeI at 37°C for 4 hours. DNA fragments were separated on 2% agarose gel for 90 min and were visualized with bromide staining and exposure to ultraviolet light.

Based on the genotypes identified on gels, allele frequencies were calculated according to Weir (1996). Differences in allele frequencies of the polymorphism between genetic groups were determined by qui-square test ($\chi^2$) of SAS software (SAS Institute, 1999).

Amplified products of 269 bp were obtained which, when submitted to digestion, revealed the $A$ (137 and 132 bp) and $G$ allele (269 bp) (Figure 1). The accuracy of bovine CAST gene SNP A2959G (AF159246) genotyping by PCR–RFLP was confirmed through the direct sequencing of PCR products of nine individuals. DNA amplification failure was not observed in the studied genetic groups. The absence of null alleles with the used primers was important, since many markers described in the literature cannot be genotyped in certain populations, due to no amplification, which results in a problem for the marker assisted selection.

The $A$ allele frequencies found in the studied genetic groups – Nellore, Canchim, Rubia Gallega x Nellore, Brangus three-way cross, and Brown Swiss three-way cross – were 0.42, 0.70, 0.85, 0.84, and 0.73, respectively. Despite the small number of individuals of some genetic groups, a significant increase ($p<0.01$) of the $A$ allele frequency was observed in *Bos taurus* x *Bos indicus* animals. These results agree with Casas et al. (2006) who, despite the higher $A$ allele frequency observed in *Bos indicus* Brahman (0.72), also observed the highest $A$ allele frequency in animals *Bos taurus* (0.80) and *Bos taurus* x *Bos indicus* (0.83). Working exclusively with populations of *Bos taurus* breeds, Morris et al. (2006) found frequencies of the $A$ allele between 0.84 and 0.99. The frequency of the $A$ allele, favorable for the meat traits (Barendse, 2003), lower in *Bos indicus* than in *Bos taurus* x *Bos indicus* animals was expected, since, according to Wheeler et al. (1994), the *Bos indicus* breeds produce less tender meat, when compared to *Bos taurus* and *Bos taurus* x *Bos indicus* animals.

In conclusion, the use of the PCR–RFLP technique, for the genotyping of A2959G (AF159246) SNP of the bovine CAST gene, was shown to be robust and

Figure 1. Bovine CAST gene SNP A2959G (AF159246) genotyping by PCR–RFLP, using DdeI endonuclease. M: 100 bp molecular weight standard; ND: undigested product of 269 bp; AA: genotype characterized by the presence of 137 and 132 bp fragments; AG: heterozygous genotype characterized by 269, 137 and 132 bp fragments; GG: genotype characterized by 269 bp fragments. The 137 and 132 bp fragments presented as superimposed under electrophoresis conditions performed.
inexpensive, which will greatly facilitate analysis of this polymorphism through basic laboratory equipment and reagents, when compared to the mass spectrometry method.

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