

# Rapid Communication: A Novel DNA Polymorphism of the Bovine Calpain Gene Detected by PCR-RFLP Analysis<sup>1</sup>

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**Polymorphism.** Two alleles of the bovine calpain II regulatory subunit gene were identified with PCR-RFLP analysis using the endonuclease *Hha*I.

**PCR Primer Sequences.** 5' primer: CCC CTC GCA CAC ATT ACT CCA AC; 3' primer: ATA CGG CCT GCC ACT TTT TGA TG.

**PCR Conditions.** 10 mM Tris, pH 8.3, 50 mM KCl, .1% Triton X-100, 1.5 mM MgCl<sub>2</sub>, 100 μM deoxynucleoside triphosphates, .4 μM each primer, 122 ng genomic DNA, and 2.5 units of *Taq* DNA polymerase in a final volume of 50 μL reaction. The PCR cycling conditions were 97°C 1.5 min for the first cycle and 94°C 1 min for rest of the cycles for denaturation, 57°C 1 min for annealing, and 72°C 2 min for polymerization, for a total of 35 cycles.

**PCR Product.** The above primers, designed based on the published bovine nucleotide sequence of cDNA (McClelland et al., 1989) encoding for the regulatory subunit of calpain II, successfully flanked the target sequences in PCR, generating a genomic DNA segment of about 1,800 bp, of which 288 bp were exon sequences, putatively including the fourth exon through the 5' end of the eighth exon.

**Method of Polymorphism Detection.** PCR product of 8 μL was digested with 1.0 μL 10× buffer 2 and 4 units of *Hha*I (New England Biolabs, MA) restriction endonuclease at 37°C for 2 h followed by 2% agarose gel electrophoresis.

**Description of the Polymorphism.** Analysis of the PCR products with restriction endonuclease *Hha*I (GCG'C) revealed three genotypes. One was a three-band pattern on 2% agarose gels, designated AA genotype; the second was a two-band pattern, designated BB genotype; and the heterozygous animals displayed a pattern with all four bands of approximately 1,520, 900, 620, and 280 bp in size (Figure 1).

**Frequencies.** The overall genotypic frequencies of 169 animals representing 13 bovine breeds were  $f(AA) = .35$ ,  $f(AB) = .40$ , and  $f(BB) = .25$ . Based on the samples analyzed, Angus (n = 10), Hereford (n = 68), and Wagyu (n = 10) were on the low frequency

side for the B allele (.05 to .26) compared to Brahman (n = 17), Brown Swiss (n = 9), Guernsey (n = 5), and Limousin (n = 9) breeds, which had high frequencies (.90 to .94) for the B allele.

**Comments.** Analyses with additional endonucleases of *Alu*I, *Dpn*I, *Hae*III, *Mbo*II, *Msp*I, *Pvu*II, and *Taq*I confirmed the minimum number of fragments predicted based on the 288-bp cDNA sequence, but no further genetic variation was detected among a sample of 25 cattle representing three breeds (Angus, Brown Swiss, and Hereford).

## Literature Cited

McClelland P., J. A. Lash, and D. R. Hathaway, 1989. Identification of major autolytic sites in the regulatory subunit of vascular calpain II. *J. Biol. Chem.* 264:17428.

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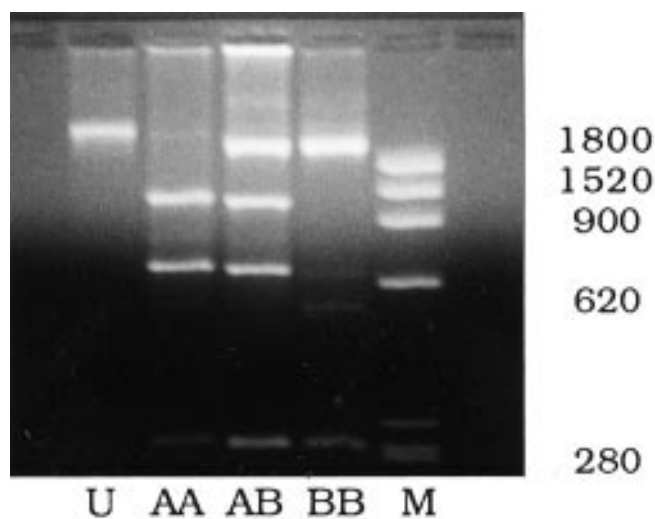


Figure 1. Bovine calpain gene *Hha*I DNA polymorphism separated on 2% agarose gel after restriction enzyme digestion. Estimated bp sizes of the restriction fragments are shown on the right. U and M on the bottom of the figure represent uncut PCR product and the DNA size marker (*Hae*III  $\phi$ x174), respectively.

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