

# Rapid Communication: A Polymorphic Microsatellite in the Promoter Region of the Bovine *Calpastatin* Gene<sup>1</sup>

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*Marker Name.* CAST.

*Source and Description of Primers.* Primers were designed from a published sequence of the bovine calpastatin promoter region surrounding a cytosine-adenine (CA) repeat 1.4 kb upstream of the transcription start site (Cong et al., 1998), to amplify a product from genomic DNA of approximately 246 bp (primers CSTF5 and CSTR4) that was used for sequencing. A polymorphic 126 to 154 bp amplicon was produced with primers CSTF4 and CSTR4 for microsatellite analysis.

*Primer Sequences.* CSTF4: 5'-GTA AAG CCG CAC AAA ACA CAC CCA GG-3'; CSTF5: 5'-TTC AAC AGC CTC CTG AAA GGC AAT GG-3'; CSTR4: 5'-CCT GGA CCC TCT GGA TGA GGA AGC GG-3'.

*Method of Detection.* The PCR amplification of 50 ng of genomic DNA for cycle sequencing was performed for 35 cycles of 94°C for 30 s, 57°C for 45 s, and 72°C for 45 s, followed by a 5-min final extension at 72°C in an MJ (Watertown, MA) PTC-100 thermocycler. Sequencing was performed as in Geesink et al. (1998). For microsatellite analysis, a total of 25 ng of genomic DNA, 5 pmol of each primer, and .1 U of *Taq* polymerase were included in an 8- $\mu$ L reaction containing 1 $\times$  *Taq* buffer, 30  $\mu$ M of dTTP, dGTP, dCTP, and 15  $\mu$ M dATP, and .1  $\mu$ Ci of [ $\alpha$ -<sup>32</sup>P]ATP. The cycling profile was 1 min at 92°C, with 30 cycles of 30 s at 94°C, 1 min at 55°C, and 1 min at 72°C, followed by a final 5-min extension at 72°C.

*Description of Polymorphism.* The polymorphism was a (CA)<sub>n</sub> repeat that identified nine alleles in the MARC mapping population (Bishop et al., 1994) with fragment lengths of 126, 132, 136, 142, 146, 148, 150, 152, and

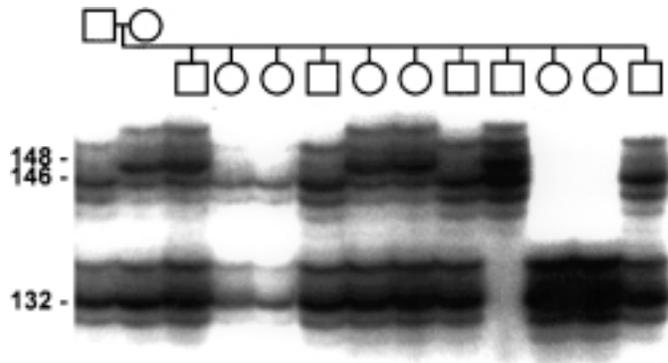


Figure 1. Autoradiograph showing pedigree analysis of bovine calpastatin (CA)<sub>n</sub> polymorphism from a 13-member Hereford backcross family. Numbering indicates base pairs of alleles.

154 bp (Figure 1). Heterozygosity in this population was 71%.

*Inheritance Pattern.* Codominant Mendelian inheritance of alleles was observed in the MARC reference pedigree.

*Frequency.* Frequency of alleles of the parents in the MARC reference pedigree representing 77 genomes (Bishop et al., 1994) was .013, .31, .013, .052, .49, .026, .065, .013, and .013 for fragments of 126, 132, 136, 142, 146, 148, 150, 152, and 154 bp, respectively.

*Chromosomal Location.* There were 218 informative meioses for the bovine calpastatin microsatellite, with two-point LOD scores ranging from 3.11 to 43.93 (Cri-Map, v2.4, Greene et al., 1990). Calpastatin has previously been mapped to bovine chromosome 7, relative position 117.8 cM (Bishop et al., 1993; Kappes et al., 1997), using an RFLP. Heterozygosity for this marker was 44% with 63 informative meioses. The microsatellite marker reported here maps to the same chromosomal location as the RFLP marker.

*Comments.* Calpastatin is a specific, endogenous inhibitor of the calcium-dependent neutral proteases (calpains) that plays a regulatory role in muscle proteolysis and postmortem meat tenderization (Koohmaraie et al., 1995). The addition of this highly informative microsatellite marker for calpastatin should be useful to determine whether calpastatin is linked to QTL involved in meat quality and muscle growth.

<sup>1</sup>Mention of a trade name, proprietary product, or specific equipment is necessary to report factually on the available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

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Received February 16, 1999.

Accepted May 12, 1999.

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**Key Words:** Calpastatin, Bovidae, Microsatellites, Chromosomes

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*J. Anim. Sci.* 1999. 77:3114–3115