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1999

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Sonstegard, T. S. and Kappes, S. M., "Mapping of the SDHA Locus to Bovine Chromosome 20" (1999).

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Mapping of the *SDHA* locus to bovine chromosome 20

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Source/description: The bovine *SDHA* cDNA (succinate dehydrogenase flavoprotein subunit A) has been cloned and sequenced¹. From the published sequence (GenBank accession number [M60879](#)), primers [SDHA467](#) and 770 were designed to amplify across a potential splice site to find polymorphisms from an intron. The predicted product size from amplification of cDNA was 304 bp. Amplification of genomic DNA resulted in a 1202-bp product (GenBank accession number [AF139922](#)), which was sequenced to confirm proper amplification of *SDHA* alleles, intron size (898 bp) and splice donor site location (position 169 of [AF139922](#)). Exon and intron designations could not be determined, since all available *SDHA* sequences in GenBank are derived from cDNA. For polymorphism detection, a second primer pair ([SDHA613](#) and 657) was used to amplify a 943-bp fragment that spanned the intron.

Primer sequences:

SDHA467: 5'-GGA GCT GGA GAA TTA CGG C-3'

SDHA770: 5'-GTG TTC CTG GCC CTG ATG-3'

SDHA613: 5'-TGC TGC ACA CGT TGT ATG G-3'

SDHA657: 5'-AGC TGG TGT CAT AGC GCA G-3'

PCR and PCR-RFLP conditions: PCR amplifications were performed on a PTC-200 thermocycler (MJ Research, Watertown, MA) in a 12- μ l reaction containing 20 ng of genomic DNA, 50 m m KCl, 1.5 m m MgCl₂, 10 m m Tris-HCl (pH 9.0), 30 μ m each dNTP, 0.4 μ m of each primer, and 0.35 units of *Taq* DNA polymerase (Promega, Madison, WI). The profile for thermal cycling was, for 35 cycles: denaturation 94 °C, 15 s; annealing 62 °C (SDHA467 and 770) or 58 °C (SDHA613 and 657), 30 s; elongation 72 °C, 45 s. After amplification with SDHA613 and 657, 10 μ l of reaction mix containing NEBuffer 4 + BSA (final concentration 1 \times), and 1 U *Nla*III (New England BioLabs, Beverly, MA) was added to each sample before incubation at 37 °C for 1 h. Digested products were electrophoresed on a 3% agarose (1 \times TBE) gel. Monomorphic product sizes were 192, 27, 383, and 97 bp; and the polymorphic sizes were either 20 (not visible) and 224 or 244 bp.

Polymorphism: Sequence analysis of the *SDHA* 1202 bp products derived from 12 parental animals of the USDA MARC reference population² revealed single nucleotide polymorphisms (SNPs) at sense strand position 863 (GGCCC A/G TGTGC) and position 978 (TCCCT C/G TCCCC). Both SNPs were detected only in animals of *Bos taurus* \times *Bos indicus* descent.

Linkage analysis and chromosomal location: Genotypes from the reference population (76 informative meioses) were generated by PCR-RFLP detection of the A/G-862 SNP in the 943 bp *SDHA* product. Linkage analysis revealed that the *SDHA* locus maps 1.3 c m distal to *BMS521* (twopoint rec. freq. = 0.01, LOD 17.91). This result extends coverage of the linkage group 1.3 c m closer to the telomeric end of bovine chromosome 20 (BTA20). The human orthologue of *SDHA* is localized to HSA5p15, therefore the placement of bovine *SDHA* extends the syntenic conservation between BTA20 and HSA5q13.3-p14.3-5p15.

Acknowledgements: The authors express their appreciation to T. DeLuca for expert technical assistance. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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