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Mapping of the *ucp1* locus to bovine chromosome 17

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Source/description: The bovine *UCP1* cDNA (mitochondrial uncoupling protein 1) has been cloned and sequenced[1.] From this published sequence (GenBank accession number X14064), two primer pair combinations (UCP1–53/141 and UCP1–53/275) were designed to amplify across intron 1. The predicted product sizes from amplification of cDNA were 89 and 223 bp, respectively. Amplification of genomic DNA resulted in 832 and 966 bp products (GenBank accession number AF139921), respectively, both of which were sequenced to confirm proper amplification of *UCP1* alleles, intron size (743 bp), and splice donor site location (position 27 of AF139921)._*Primer Sequences*:

UCP1–53: 5′-CTG GAC ACC GCC AAA GTC-3′

UCP1–141: 5′-ATG ATT GTT CCC AGG ACA CC-3′

UCP1–275: 5′-CTT TCC CTG TGG TGA AGA ACT C-3′

PCR and PCR-RFLP conditions: PCR amplifications were performed on a PTC-200 thermocycler (MJ Research, Watertown, MA) in a 12-µl reaction containing 20 ng of genomic DNA, 50 m m KCl, 1 ·5 m m $MgCl_2$, 10 m m Tris–HCl (pH 9 ·0), 30 µm each dNTP, 0 ·4 µm of each primer, and 0 ·35 units of *Taq* DNA polymerase (Promega, Madison, WI). The profile for thermal cycling for both primer combinations was, for 35 cycles: denaturation 94 °C, 15 s; annealing 58 °C, 15 s; elongation 72 °C, 30 s. After amplification, 10 µl of reaction mix containing NEBuffer 3 (final concentration 1x) and 3 U *Dde*I (New England BioLabs, Beverly, MA) was added to each sample before incubation at 37 °C for 1 h. Digested products were resolved on a 5% denaturing acrylamide gel and visualized by autoradiography of the dried gel. Digestion of the 832 bp product (generated from primers UCP1–53 and 141) gave monomorphic

product sizes of 130, 140, 171, and 203 bp; and the polymorphic sizes of either 56 and 132 or 188 bp.

Polymorphism: Sequence analysis of the *UCP1*966 bp product derived from 12 parental animals of the USDA MARC reference map population[2] revealed eight single nucleotide polymorphisms (SNPs) within intron 1. Sense strand positions of the SNPs were C/T-186 (base types detected-nt position in 966 bp product), C/G-232, C/G-248, C/T-360, C/G-415, A/G-545, A/G-555, and C/T-588.

Linkage analysis and chromosomal location: Genotypes from the reference population (54 informative meioses) were generated by PCR-RFLP detection of the T/C-186 SNP in the 832 bp *UCP1* product. Linkage analysis positioned the *UCP1* locus (23 ·5 c m) between *EAF* (twopoint rec. freq. = 0 ·05, LOD 7 ·16) and *BMS2780* (two point rec. freq. = 0 ·03, LOD 9 ·13) on bovine chromosome 17 (BTA17). This addition of *UCP1* to the bovine genetic map reveals new regions of conserved synteny between BTA17 and MMU8. The human and swine orthologues of *UCP1* are localized to HSA4q28-q31 and SSC8q21, respectively. Therefore the placement of bovine *UCP1* agrees with conservation of synteny between BTA17q12-q23, HSA4q25-q31, and SSC8.

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