

Fig. 1. Mendelian inheritance of the polymorphism in feline myosin regulatory light chain gene in an American shorthair family (family 1). Genotypes (AA and AB) are indicated.

for 30 s, 64 °C for 25 s, and 72 °C for 20 s. A final extension step at 72 °C for 10 min followed the last cycle. After adding 9 µl of loading dye (95% formamide, 10 mM NaOH, 0.25% bromphenol blue, 0.25% xylene cyanol FF), the samples were heated to 95 °C for 2 min then placed on ice for 5 min. Five microliters of each sample was electrophoresed on a 0.5C × MDE polyacrylamide gel (FMC, Rockland, ME) in 0.5C × TBE buffer for 8 h at 6–8 W. After drying, the gel was exposed to autoradiographic film for 24 h.

Sequence analysis: PCR products were isolated from a 1% agarose gel, purified with a Qiaquick column (Qiagen, Valencia, CA), TA cloned using a TOPO TA cloning kit (Invitrogen, Carlsbad, CA), and sequenced (Genbank accession number AF071561) using a T7 sequenase kit, version 2.0 (Amersham Pharmacia Biotech, Piscataway, NJ).

Polymorphism: Nineteen American shorthairs (family 1), nine Maine coons, two British shorthairs, and five mixed breed cats were genotyped. Two alleles (A and B) were identified by SSCP (Fig. 1). Allele B was found to have two single nucleotide polymorphisms (G > A, C > T), both in intron 5. A third single nucleotide polymorphism (A > G) was identified in exon 5 by nucleotide sequence analysis in Maine coon cats only. This polymorphism was not detected by SSCP.

Mendelian inheritance: Codominant Mendelian segregation for the two alleles was observed in an American shorthair family (family 1).

Chromosomal location: Unknown in the cat.

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References

1 Poetter K. *et al.* (1996) *Nat Genet* **13**, 63–9.

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Assignment of laminin B1 polypeptide (*LAMB1*) and 2,3 bisphosphoglycerate mutase (*BPGM*) to the physical and genetic maps of BTA4

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In order to assist with the alignment of BTA4 to HSA7, we screened a bovine BAC library² for clones containing laminin B1 polypeptide (*LAMB1*) and 2,3 bisphosphoglycerate mutase (*BPGM*) which map to HSA7 bands q31.1–q31.3 and q31–q34, respectively. Primers for the systematic PCR screening of the BAC library were designed from aligned sequences from human and mouse (accession numbers M61916 and M15525 for *LAMB1* and X13586 and X04327 for *BPGM*). Primers *LAMB1*F: TAT TTC CAG ATG TCT

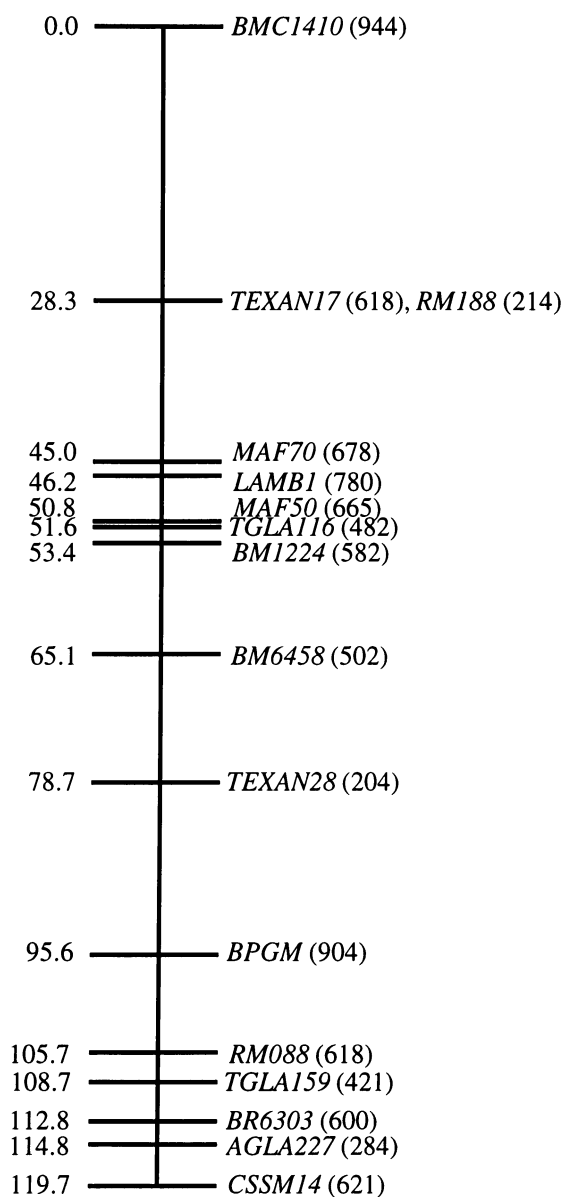


Fig. 1. Sex-average genetic map of bovine chromosome 4 oriented at the centromere. All loci are ordered with a likelihood ratio 1000 : 1 support for locus order vs. alternative orders. Number of informative meioses in parentheses.

CTT GAG GCA GA and LAMB1R: TTG TAG CTT GCC TGC CAG TTC AT were designed to amplify an 189-bp fragment of *LAMB1* and primers BPGMF: AAT AAG GAG AAC CGT TTT TGT AG and BPGMR: AGC AAT CCT TTC ATT CCA ATA GG to amplify a 473-bp fragment of *BPGM*. For both primer pairs, samples were denatured at 94 °C for 2 min and 35 PCR cycles were performed at 94 °C denaturing for 30 s, 55 °C annealing for 30 s and 72 °C extension for 30 s with final extension at 72 °C for 10 min. Sequencing the PCR products from BACs B65F6 and B154F3 revealed an 85% sequence identity to human *LAMB1* and from B3D3, B72D5 and B230A5 an 88% sequence identity to human *BPGM*. One BAC clone containing *LAMB1* was assigned to BTA4 band 22 and a clone containing *BPGM* to bands 32–33 by fluorescence *in situ* hybridization.

Polymorphic microsatellites were derived from the BACs harboring *LAMB1* and *BPGM* as previously described⁶. Primers LAMB1MF: GAA CTA GGT CAT GAT TGC TC and LAMB1MR: ATG CTG ATA TTG AGG ACT AT were designed to amplify a 153-bp product from a clone harboring *LAMB1* and BPGMMF: CAT TCT TGC CTG GAA AA and BPGMMR: GAC CCC TAA CCA AAG ATA CA a 150-bp product from a clone harboring *BPGM*. Annealing temperatures were 55 and 51 °C, respectively. Both microsatellites were scored in the Angleton pedigrees 6 and incorporated into the Texas A&M University genetic map of BTA4 using CRI-MAP³ according to standard procedures^{1,4}. Three alleles were detected for *LAMB1* (PIC = 0.540) and 10 alleles for *BPGM* (PIC = 0.753). The support for locus order was LOD > 3.0 for all loci. There was no evidence for a difference in the length of the male and female maps ($\chi^2_{16} = 24.94$; $P > 0.10$) and the sex-average BTA4 map length was 119.7 cm (Kosambi) with an average interval of 8.0 cm. The 16 marker, sex-average map along with the number of informative meioses for each marker is reported in Fig. 1. Locus order was consistent with the published bovine genetic maps^{1,4,5}. The locus *BPGM* maps 30.5 cm distal from *BM6458* and 10.1 cm proximal to *RM088* which anchors this gene to the interval containing the *OBES* gene⁴.

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References

- 1 Barendse W. *et al.* (1997) *Mamm Genome* **8**, 21–8.
- 2 Cai L. *et al.* (1995) *Genomics* **29**, 413–25.
- 3 Green P. *et al.* (1990) *Documentation for CRI-MAP, version 2.4*. Washington University School of Medicine, St Louis, MO.
- 4 Kappes S.M. *et al.* (1997) *Genome Res* **7**, 235–49.
- 5 Ma R.Z. *et al.* (1996) *J Hered* **87**, 261–71.
- 6 Taylor J.F. *et al.* (1998) *Anim Genet* **29**, 194–201.

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Interleukin-12 p35 encoding gene of cattle and sheep harbours a polymorphic T stretch in intron 4

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Source/description: Bioactive interleukin-12 (IL12) consists of two polypeptides, p35 and p40, encoded by the *IL12A* and *IL12B* genes, respectively. It plays an important role in the regulation of cellular immune defence¹. Animals with disrupted *IL12A* of the genetically

resistant inbred mouse strain 129/Sv/Ev become susceptible to infections with *Leishmania major*². This suggests that *IL12A* might be a potential candidate gene for resistance to infectious pathogens. To search for gene variants, primers for amplification of bovine *IL12A* fragments were derived from the bovine cDNA sequence³ (EMBL accession number U14416), considering the exon/intron borders of the murine gene as published by Tone *et al.*⁴ and Yoshimoto *et al.*⁵ Among these, PCR amplification with bovine primer pair A_{F/R} yields an amplicon containing a DNA fragment of 711 bp in cattle (EMBL accession number AJ271034) and a corresponding fragment of 714 bp in sheep (EMBL accession number AJ271035).

A_{F/R} amplicons from cattle and sheep include exon sequences that are homologous to exons 4–7 of the murine gene⁴. The exon sequences of bovine A_{F/R} amplicon confirm the previously published bovine *IL12A* cDNA sequence (Fig. 1A)³. The four exons are interrupted by three introns, displaying conventional GT–AG splice junctions. Lengths of exons and introns and sequence identity between cattle and sheep are shown in Fig. 1B. Codon borders are identical to the exon borders in the analysed region. The derived amino acid sequences of bovine and ovine *IL12A* exons are demonstrated in Fig. 1C. Intron 4 of *IL12A* harbours a highly polymorphic T stretch in cattle and sheep, providing a source of DNA variants at this locus. Primer pair B_{F/R} was designed for testing allelic frequencies.

Primer sequences:

A _{F/R}	5' AATTTTACTCCTGCACCTTCTGAG 3'	(cDNA, pos.185) ³
	5' CTCCACGTGGTACATCTTCAA 3'	(cDNA, pos.397) ³
B _{F/R}	5' AAATTAGTATCTTTTGTATGTGTG 3'	(upper primer)
	5' TCATAATATGAGCTGAAACCTT 3'	(lower primer)

PCR conditions and sequencing. Amplification of bovine/ovine *IL12A* exon 4–7: Amplifications of cattle and sheep DNA were performed with the bovine primer pair A_{F/R}. PCR reactions were performed in a final volume of 25 µl containing 40 ng genomic DNA, 15 pmol of each primer and 0.25 U *Taq* DNA polymerase in 10 mM Tris–HCl, pH 9.0, 50 mM KCl, 1.5 mM MgCl₂, 0.1% (v/v) Triton X100, 0.2 mg/ml BSA and 200 µM of each dNTP. After hot start, the first cycle was 4 min (94 °C); 1 min (57.1 °C for cattle, 52.1 °C for sheep); 4 min (70 °C) followed by 30 cycles of 1 min (94 °C); 1 min (57.1 °C for cattle, 52.1 °C for sheep); 4 min (70 °C) and one cycle of 1 min (94 °C); 1 min (57.1 °C for cattle, 52.1 °C for sheep); 7 min (70 °C) using the TRIO ThermoblockTM with heated lid (Biometra, Göttingen, Germany).

Genotyping: For genotyping, the primer pair B_{F/R} was used in cattle and sheep. Labelling of the lower primer (B_R) was performed in a reaction volume per PCR reaction of 0.7 µl containing 50 mM Tris–HCl, pH 7.5, 7 mM MgCl₂ and 1 mM DTT, 10 pmol primer, 0.25 U *T4* polynucleotide kinase and 0.5 µCi [γ -³²P]ATP for 40 min at 37 °C. The enzyme was inactivated by heating the samples for 10 min at 65 °C. PCR reactions were performed in 10 µl containing 60 ng genomic DNA, 10 pmol of each primer and 0.125 U *Taq* DNA polymerase in 10 mM Tris–HCl, pH 9.0, 50 mM KCl, 1.5 mM MgCl₂, 0.1% (v/v) Triton X100, 0.2 mg/ml BSA and 200 µM of each dNTP. After hot start, the first cycle was 4 min (94 °C); 1 min (53 °C); 2 min (70 °C) followed by 30 cycles of 1 min (94 °C); 1 min (53 °C); 2 min (70 °C) using the Gene Amp PCR System 9600 (Perkin Elmer, Norwalk, CT, USA). DNA fragments were separated on denaturing 6% polyacrylamide gels and detected by autoradiography.

Sequencing: DNA sequences of bovine and ovine A_{F/R} amplicons and lengths of intron 4 T stretches were determined by direct sequencing of PCR fragments derived from homozygous animals. For sequencing, DNA fragments were purified with the High PureTM PCR Product Purification Kit (Roche, Mannheim, Germany). Sequencing was performed using the ABI PRISMTM Big DyeTM Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, Weiterstadt, Germany) and the ABI PRISMTM 310 Genetic Analyzer (PE Applied Biosystems).