Cloning and partial nucleotide sequence of the genomic bovine β-lactoglobulin gene

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β-lactoglobulin is a whey protein which appears to bind retinol and is found in the milk of animals including bovine species, sheep, deer, dogs, and pigs but not in humans (1). The gene coding for ovine β-lactoglobulin was first isolated from a cDNA library by (2). More recently, the genomic ovine β-lactoglobulin gene has been cloned and the nucleotide sequence reported (3, 4). Several genes coding for the bovine β-lactoglobulin A from a cDNA bank constructed from poly(A+)mRNA isolated from the bovine mamary gland have been reported (5–7) and a recombinant protein expressed in *Escherichia coli* (8).

The bovine β-lactoglobulin cDNA was used to probe a charon 28 genomic bovine library (9). From a number of putative clones, a single candidate was selected and characterized using seven exon specific synthetic oligonucleotide probes. A restriction map and the position of exons I through VII are presented in Figure 1. The *Hin*dIII, *Eco*RI and all but one *Bam*HI restriction sites within the region corresponding to these exons are conserved between the ovine and bovine β-lactoglobulin genes (Figure 1).

The nucleotide sequence for the regions 5′ of exon I and 3′ of exon VII were determined (TaqTrack, Promega) using end labelled primers specific for the distal ends of the published cDNA sequence (Figure 2). A total of 272 nt from the 5′ end of exon I and the nontranscribed region was obtained and shown to be 90.8% homologous to the ovine sequence. The absence of two amino acids in the signal sequence of the bovine β-lactoglobulin gene as compared to the ovine signal sequence agrees with the initial reports on the complete cDNA sequence (7). The 3′ end of the bovine β-lactoglobulin is only slightly less conserved (89%) with respect to the ovine sequence.

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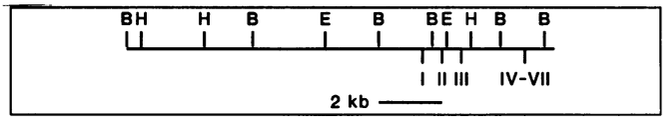

Figure 1. Restriction map of λBLG-13. E—*Eco*RI, B—*Bam*HI, H—*Hin*dIII. Positions of exons I–VII were determined using unique oligonucleotide probes.

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                10        20        30        40        50
                 |         |         |         |         |
  1 GCTTGGCCTCGAATGGAAGAAGGCCTCCTATTGTCCTTCGTAGAGGAAGC
 51 AACCCCAGGGCCCAAGGATAGGCCAGGGGGGATTCGGGGAACCGCGTGGC
101 TGGGGCGCGGCCCGGGCTGGCTGGCTGGCCCTCCTCCTGTATAAGGCCCC
151 GAGCCCGCTGTCTCAGCCCTCCACTCCCTGCAGAGCTCAGAAGCGTGACC
201 CCAGCTGCAGCCATGAAGTGCCTCCTGCTTGCCCTGGCCCTCACCTGTGG
                MetLysCysLeuLeuLeuAlaLeuAlaLeuThrCysGly
251 CGCCCAGGCCCTCATCGTCACC
     AlaGlnAlaLEUIleValThr
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Figure 2a. Nucleotide sequence of the 5′ end of the bovine β-lactoglobulin gene. Amino acid translation is for the region coding for the signal sequence.

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                10        20        30        40        50
                 |         |         |         |         |
  1 ATAAACCTGTGCTCTCCCTTCTGAAGTCTTTCCTGGATGATGGGCCGGGG
 51 GTGGAGAAGGCCCGGGACAGGGTGGGGAGTGGTCTGGCTCAGAAGGAATG
101 AATGGTAGGGCTGGGATCCAGGGCGTCTGCATTACAGTCTTGTGACATCT
151 GGGGCCCACACACATCACTACGCTCTT
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Figure 2b. Nucleotide sequence of the 3′ end of the bovine β-lactoglobulin gene.

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