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## New polymorphisms for the BoLA-DRB3 upstream regulatory region

### Key words:

BoLA-DRB3; polymorphism; upstream regulatory region

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**Abstract:** Two new alleles, named BoLA-DRB3-P\*06 and BoLA-DRB3-P\*07, have been identified for the upstream regulatory region of the *BoLA-DRB3* gene. The 228-bp nucleotide sequences of the promoter comprising the W, X, Y, CAAT and TATA regulatory boxes were analysed. The BoLA-DRB3-P\*06 (AY858800) exhibits one insertion between the W and X boxes, and one transition between the X and Y boxes. On the other hand, the BoLA-DRB3-P\*07 (AY714592) showed one insertion in the X box.

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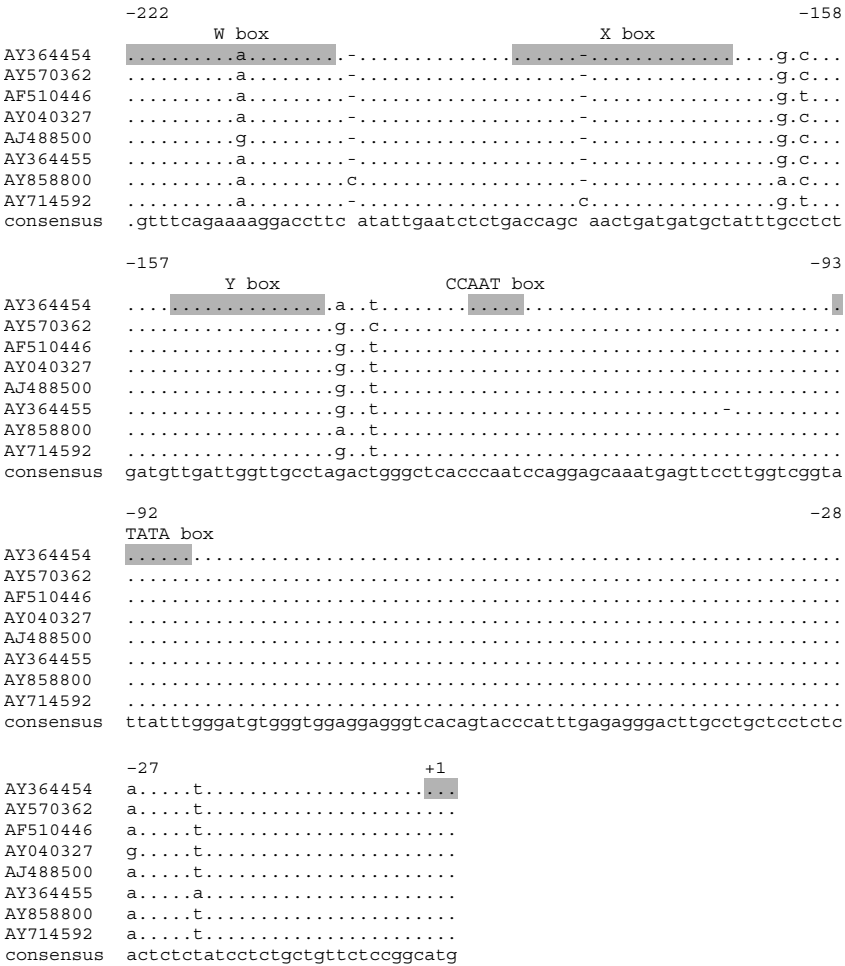
The bovine lymphocyte antigen (BoLA) system is encoded by the major histocompatibility complex in cattle. The upstream regulatory region (URR) of the *BoLA-DRB3* gene is located upstream of the transcription initiation site and is composed of highly conserved sequence motifs that include, from 5' to 3', the W, X, Y, CCAAT and TATA boxes. In this study, we describe two additional sequences for the URR of the *BoLA-DRB3* gene. The two new alleles, named BoLA-DRB3-P\*06 and BoLA-DRB3-P\*07, have been submitted to the GenBank nucleotide sequence database and have been assigned the accession numbers AY858800 and AY714592, respectively. We used a new interim nomenclature system, suggested by the BoLA Nomenclature Group, that shows the 'locus-promoter\*allele' and allows other authors to add new variants in the future.)

The URRs were amplified, as previously described by Ripoli et al. (1). First, the new variants were detected by the PCR-SSCP method, and then PCR products were cloned into pCR2.1-TOPO (TOPO TA Cloning, Invitrogen Life Technologies, Carlsbad, CA, USA). Three clones of each variant were sequenced with an Applied Biosystems 377 automated sequencer, using ABI PRISM ready reaction dye-terminator and T7 universal primer (BioResource Center, Cornell University, Ithaca, NY, USA). The clones corresponding to each variant exhibited 100% sequence similarity to each other.

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**Fig. 1.** Alignment of the nucleotide sequences of upstream regulatory regions of AF510446, AY364454, AY364455, AY570362, AY040327, AY858800, AY714592 and AJ488500. The A of ATG is designated as +1. Dots indicate nucleotide identity to the consensus sequence and dashes (-) represent gaps to achieve the best alignment.

The URR-BoLA-DRB3 variants reported in this study, the BoLA-DRB3-P\*01 (AF510446), BoLA-DRB3-P\*02 (AY364454), BoLA-DRB3-P\*03 (AY364455) and BoLA-DRB3-P\*04 (AY570362), variants reported by Ripoli et al. (1), the BoLA-DRB3-P\*05 (AY040327) variant reported by Ripoli et al. (2) and the AJ488500 variant reported by Russell et al. (3) were aligned (Fig. 1). The

alignment showed that the BoLA-DRB3-P\*06 allele (AY858800) exhibited one insertion between the W and X boxes, and one transition between the X and Y boxes, whereas the BoLA-DRB3-P\*07 variant (AY714592) showed one insertion in the X box. The functional role of these polymorphisms needs to be analysed and confirmed by means of gene expression assays.

References

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