UNIVERSITA' DEGLI STUDI DI TORINO
Dipartimento di Scienze Veterinarie

Bovine serum amyloid A3: analysis of the genomic region

Soglia Dominga[1], Sartore Stefano[1], Maione Sandra[1], Pepe Elvira[1], Gallino Alice[1], Rasero Roberto[1], Sacchi Paola[1]

[1]Dipartimento di Scienze Veterinarie, Università degli Studi di Torino, Italy

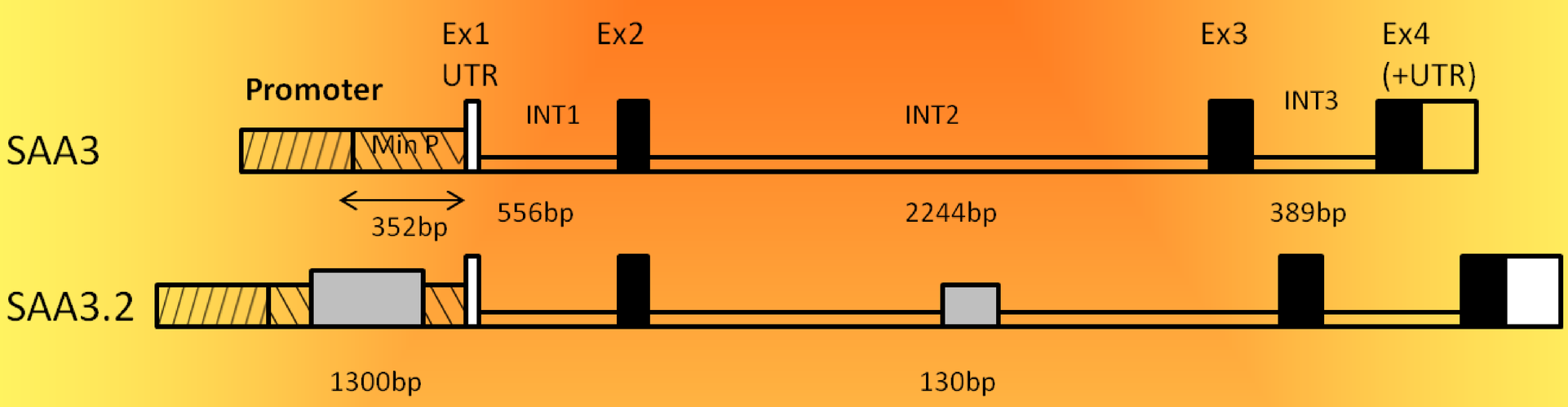
Corresponding author: dominga.soglia@unito.it, Dipartimento di Scienze Veterinarie, Università degli Studi di Torino, L.go Paolo Braccini 2, 10095 Grugliasco (Torino), Italy. Tel. +39 011 6709256

Serum Amyloid A belongs to a family of apolipoproteins considered one of the major vertebrate acute phase reactants. They are synthesized in response to infection, injury and inflammation. In mammals four genes, SAA1, SAA2, SAA3 and SAA4, have been described; in particular, in bovine mammary gland different products, proteins and mRNA, have been identified all deriving from SAA family genes, but the true identity of the involved genes is not yet clear.

The bovine Hap Map Consortium stated that a duplication on BTA29 resulted in two genes, SAA3 and M-SAA3.2, which are located about 80 kb apart. They encode protein isoforms with 96% amino acid sequence identity.

The aim of this study is to analyse the structure of the bovine SAA3 and M-SAA3.2 in order to distinguish their paralogous regions and to identify useful markers.

To identify the position of the SAA3 and M-SAA3.2 coding sequences, the bovine genome assembly version Bos taurus UMD 3.1.1 was consulted, accessible through NCBI data base. First, the obtained sequences were aligned to highlight differences and to calculate degree of homology using MEGA6 and BLAST. The sequences of the two genes show some differences: M-SAA3.2 presents two insertions of 210 and 1303 bp. The first maps in intron 2, whereas the latter disrupts the sequence of the minimal promoter identified by Larson et al. (2006). To confirm the results of the in silico survey, we performed an in vitro analysis of the two genes using specific sets of primers.



Stucture of SAA3 and SAA3.2 gene

In a second step, a region of 20kb on BTA29, containing *M-SAA3.2*, was identified. Using the DesignStudio web-based tool (Illumina), a project was designed for resequencing this region with MiSeq NGS approach. The DNA of 95 bulls was extracted, amplified with the custom assay and sequenced. 446 SNPs were identified. The SNPs with minor allele frequency >0.05 were 144: 109 mapped upstream from the promoter, 8 into the promoter, 20 in the ORF region, and 7 downstream.

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