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(Article begins on next page)

Variability of bovine serum amyloid A3 and somatic cell score

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Using a target re-sequencing approach, we identified all SNPs in a region of 20kb of BTA 29 containing the SAA3.2 gene, a candidate gene for mastitis resistance in Italian Holstein cows. A TruSeq Custom Amplicon Assay was designed to resequence 12 kb upstream of the promoter and 1 kb downstream of the 3'-UTR (Miseq NGS technology). The DNA of 95 bulls was extracted, amplified with the custom assay and sequenced. Animals were chosen using a selective genotyping approach according to their somatic cell score breeding value—SCS(EBV). An individual average coverage threshold of 20X was considered, resulting in 52 high and 33 low EBV(SCS) individuals retained. A total of 446 SNPs were identified but only 127 SNPs, with a minor allele frequency (MAF) lower than 0.05, were considered: 92 upstream, 8 in the promoter, 20 in gene and 7 in the downstream region. Association analysis between SNPs and SCS(EBV) was carried out using two different approaches. The first approach was the MAX test proposed for case-control studies by Fridlin et al. (2002), used to verify the association between the binary trait 'negative-positive tails' and SNPs. The second approach was the heteroscedastic effects model (HEM) (Shen et al. 2013). This model was used with the objective to capture genetic effects that are often quite small. All analyses were performed in R, using the package bigRR (Shen et al., 2013) and Rassoc (Zang et al., 2010), bootstrapping (with 50,000 replicates) to approximate the distribution of the MAX test under the null hypothesis of equal genotype distribution in the 2 tails as described in Fontanesi et al. (2012) and using a binomial distribution. With the MAX test five SNP were found significant using a threshold of p -value <0.1 (0.013-0.075): rs137746604(A/G), rs210417381(C/T) and rs136687125 (C/T), located in the promoter region, rs42175271 (C/G) and rs378094124, located upstream of the promoter. With the HEM method three SNPs identified in MAX test showed a highest heteroscedastic

effect: rs137746604 (0.030), rs136687125(0.012) and rs42175271 (0.017). In addition, rs42175273(A/T) and rs384439423(C/T), located upstream of the promoter, had effect > 0.0123. Analysis with different approaches (e.g. Bayesian, GRAMMAR) are underway. Although these results must be confirmed by the analysis of a large number of individuals, this investigation is our first contribution to the identification of markers for genetic resistance to mastitis in Italian Holstein.