Genomics

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Sequence Analysis

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New INDEL polymorphisms in ZFY and TSPY genes, in the indicine breed Gir, discovered through Next Generation Sequencing

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Brazil has the world's largest cattle herd and livestock share is about 9% of GDP. Minas Gerais State has the second largest cattle herd in the country and the highest primary livestock production value, accounting for 28% of the total milk production in the country. Among the breeds targeted by genetic breeding programs for milk production, Gir and Guzerat are the most important ones. Reproductive disorders have often been described in bovines selected for high milk production, and, in Brazil, these are frequently found in Gir cattle, but are also present in others breeds. A preliminary estimate suggests that the frequency of gonadal hypoplasia and others reproductive abnormalities, in both sexes, are in the order of 10%, which consequently leads to losses of semen and of any investment made in the animal. In humans and mice, the Y-chromosome has been frequently implicated in the etiology of male subfertility. The bovine genome was published in 2009, based on sequences generated from Hereford and Fleckvieh taurine breeds. Therefore, sequences generated from indicine breeds are rare in databases. The knowledge of Ychromosome genes and genetic variations may be used in research as well as for breeding strategies. This project is part of a larger one that aims to complete sequencing of both Gir and Guzerat genomes through next generation sequencing. Its objective is to ascertain quality, coverage and genetic conservation of sequences of genes mapping to the Ychromosome that have been generated in a first sequencing effort. Initially, sequences of a Gir bull have been produced. Two sequencing runs were done, yielding approximately 204,771,110 reads with a 1.55X coverage. An initial mapping of all reads was performed by using the software BioScope. The complete Bos taurus genome sequence (NCBI Project ID: 10708, BTA4.0) was used as a reference. Four Y-chromosome genes were selected for an initial comparative analysis. They were DDX3Y (Asp-Glu-Ala-Asp box polypeptide 3, Y-linked), TSPY (testis specific protein, Y-linked), ZFY (zinc finger protein, Y-linked) and SRY (sex-determining region Y protein). The sequences of these genes were obtained from public database (GenBank) and later compared to our unscaffolded sequences by using the BLAST software in the Linux operational system and MUMmer package v 3.22. After analysis, approximately 56% of identity for TSPY gene was found. The region of identity includes the exon 2 to exon 4 and two variations were found in the 726 bp and 741 bp positions for intron 3 this gene. A 15% of identity was found for SRY gene, but no variation was observed between our Gir database sequences and the Bos taurus reference. For ZFY and DDX3Y genes, only 4% of identity was represented among the sequences obtained for the Gir breed. Some variations were observed for these genes, but the low coverage obtained for the sequencing so far does not allow validation. These data allow a preliminary comparative analysis of two Gir Y-chromosome genes (TSPY and SRY). In comparison to Bos taurus genes, both TSPY exons 2-4 and SRY exon Gir showed 100% of sequence identity. Thus, in spite of the low coverage, Y-chromosome regions that have been specifically searched for exhibit high conservation levels among the subspecies.