AB.32

Identification of polymorphisms in a QTL region on chicken chromosome 2 associated with muscle deposition

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Abstract:

Next-generation sequencing technologies are great tools for the identification of SNPs (single nucleotide polymorphism) and INDELs (deletions/insertions) of complete genomes with high accuracy. This technique can help the study of quantitative trait loci (QTL) previously mapped by the identification of alleles associated with the traits of economic interes t for the poultry industry, such as breast muscle, part of greater commercial value. This study aims to sequence the genome of two chicken lines: a broiler TT and layer CC, both developed by Embrapa Swine and Poultry for the detection of SNPs/INDELs by bioinformatic tools in a QTL region on chicken chromosome 2 (105,800-112,600 bp) that was previously associated with breast muscle deposition (Baron et al., 2010). Sample preparation, cluster generation (cBot) and sequencing (HiSeq1000, Illumina) were performed according to the manufacture's protocols: Nextera DNA Sample Preparation kit, KAPA Library Quantification kit (KAPA Biosystems), TruqSeq PE Cluster V3 kit (Illumina) and TruqSeq SBS V3 kit - 200 cycles (Illumina), respectively. After the sequencing, the quality of the reads was verified by FastQC tool, and the quality trimming was performed by Seqyclean software (v.1.3.12) by the use of quality of 24 and minimal fragment size of 65 bp. The next step was to align the re ads against the chicken reference genome (Gallus gallus-4.0) with Bowtie 2 (v.2.1.0). The identification of SNPs/INDELS was performed by SAMtools (v.0.1.19) with mpileup option and mapping and base qualities of >20. The filtration of the SNPs/INDELs was based on variant quality score ≥30 and coverage ≥5. The percentage of reads kept after cleaning for the TT and CC line was 77.9% (~101 million of reads) and 77.4% (~96 million of reads), respectively. The average coverage was between 9.3 to 16.0 X. Initially we identified 38,692 SNPs and 3,753 INDELs from CC layer and 46,354 SNPs and 4,308 INDELs from TT broiler. After the filtration ~92% of SNPs and 74% of INDELs were kept and we obtained 71,122 unique SNPs and 6,367 unique INDELs for all 6 chickens. The minor allele frequency (MAF) of SNPs/INDELs filtered was calculated and we identified 59,830 SNPs and 6,110 INDELs fixed (MAF=0), and 69,356 SNPs and 3,737 INDELs segregating (MAF≠0) in broiler line, and 70 ,036 SNPs and 6,445 INDELs fixed and 36,082 SNPs and 1,784 INDELs segregating in layer line. The largest number of variants segregating in broiler line shows high variability for this line, and it can be explained partially by the genetic background of this line which was originated from different lines. The layer line was originated from only one line and the breast muscle development is slower and more homogeneous compared to the broiler line. The functional role from the INDELS and SNPs found will be evaluated in further studies.