Identifying Tissue-Specific Regulatory Regions in Two Thoroughbred Stallions for the Functional Annotation of Animal Genomes Project

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The Functional Annotation of Animal Genomes (FAANG) consortium aims to functionally annotate livestock genomes and work in the horse has substantially contributed to that goal. Most recently, chromatin immunoprecipitation with sequencing (ChIPseq) was performed to identify histone modifications corresponding to enhancers (H3K4me1), promoters (H3K4me3), activators (H3K27ac), and repressors (H3K27me3) in eight tissues from two Thoroughbred stallions: adipose, parietal cortex, heart, lamina, liver, lung, skeletal muscle, and testis. Chromatin preparation and sequencing was performed in a comparable manner to previously published work in two mares. The average number of peaks identified by MACS2 for H3K4me1, H3K4me3, and H3K27ac were 120K, 34K, and 77K, respectively. Peaks were called for H3K27me3, a broad mark, using both MACS2 and SICERpy, with MACS2 identifying a greater average number of peaks (158K) than SICERpy (32K). Peaks unique to tissues were identified for each histone mark using BEDTools. Unique peaks varied between tissues with the fewest peaks (324) identified in skeletal muscle H3K27me3 by SICERpy and the most (99K) in liver H3K27me3 by MACS2. The greatest number of unique peaks for H3K4me1 and H3K27me3 was observed in liver while testis had the greatest number for H3K4me3 and H3K27ac. Comparison of this dataset to ChIPseq data previously reported for two Thoroughbred mares will allow for identification of sexual variation of regulatory regions. These data expand a growing resource available for identifying the function of regions within the equine genome, within and between sexes, and serve as a reference for the activation state of genes across healthy tissues.