

1 **Transcriptome analysis of crossbred and purebred cattle using CAGE and mRNA-Seq for the**
2 **BovReg Project**

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4 Mapping of transcription start sites (TSS) is a key first step in understanding transcript regulation and
5 diversity and how these might influence phenotypic plasticity in cattle. Including data from multiple
6 tissues and non-reference breeds or crosses will increase our understanding of TSS usage, transcription
7 complexity and will enhance the annotation of the reference genome for cattle (ARS-UCD1.2). For the
8 BovReg project, we performed Cap Analysis Gene Expression (CAGE) sequencing and mRNA-
9 Sequencing of 105 tissue samples (from 24 different tissues) from dairy (Holstein, n=43), composite
10 beef (KC-composite, n=31) and beef/dairy cross (Charolais x Holstein, n=31) cattle. A *de novo*
11 assembled transcriptome of the tissue samples was produced using the mRNA-Seq data and isoform
12 expression estimates were calculated using Kallisto (TPM). Network cluster analysis was performed in
13 Graphia and large clusters of genes with tissue-specific expression patterns were identified. CAGE
14 libraries were mapped to ARS-UCD1.2_Btau5.0.1Y. The mapped reads were analysed using the
15 CAGEfightR package to generate uni-directional (TSS) and bi-directional (TSS-Enhancer) clusters.
16 Cross population analysis revealed that the greatest number of population-specific TSS were detected
17 in the KC-composite (3,102), relative to the Holstein (1,152) and the Charolais x Holstein (1,092). A
18 similar pattern was observed in the TSS-Enhancer clusters (419 KC-composite, 286 Charolais x
19 Holstein and 202 Holstein) indicating that the total number of TSS observed was greater in composite
20 relative to purebred cattle. The gene annotation datasets we have generated from crossbred and purebred
21 cattle, with BovReg partners, create a new high-resolution map that captures additional transcriptional
22 complexity in the bovine genome.

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