

we analyzed the correlation between the levels of DNA methylation and gene expression among neocortex, liver, muscle, spleen, and a macrophage cell line. We identified 21 neocortex-, 42 liver-, 20 muscle-, 28 spleen-, and 652 macrophage-specific differentially expressed genes (DEG) harboring tissue specific DMCs in upstream 10 kb region from TSS and gene bodies, including exons and introns. These results confirm and support that changes in DNA methylation in the swine genome are associated with alteration in gene expression and phenotypic differences.

**Key Words:** pig, DNA methylation, gene expression, RRBS, RNA-seq, epigenetics

#### **P2011 Novel analysis of global DNA methylation in the limbic system of the bovine brain.**

B. A. Cantrell<sup>1</sup>, S. D. McKay<sup>1</sup>, R. L. Weaver<sup>2</sup>, R. N. Funston<sup>3</sup>, H. Lachance<sup>1</sup> (<sup>1</sup>University of Vermont, Burlington, VT, <sup>2</sup>Kansas State University, Manhattan, KS, <sup>3</sup>University of Nebraska, West Central Research and Extension Center, North Platte, NE)

There has been limited research focusing on the genetic-environmental interactions in bovine brains. Global DNA methylation has been measured in brains of several species, but has yet to be examined in bovine. The objective of this study was to characterize global DNA methylation in 9 regions of the limbic system in the bovine brain: amygdala, bed nucleus of the stria terminalis, cingulate gyrus, dorsal raphe, hippocampus, hypothalamus, nucleus accumbens, periaqueductal gray, and prefrontal cortex. DNA was extracted from brain and blood samples of 6 Red Angus × Simmental steers (less than 20 mo of age), using the DNA Extraction Kit from Agilent Technologies (Santa Clara, CA) and a phenol chloroform extraction. Percent of global DNA methylation was determined using the MethylFlash Methylated DNA Quantification Kit (Colorimetric) from Epigentek (Farmingdale, NY). Varying amounts of global DNA methylation were observed among the 9 functionally distinct regions of the bovine limbic system. Amygdala, bed nucleus of the stria terminalis, cingulate gyrus, dorsal raphe, periaqueductal gray, prefrontal cortex, and nucleus accumbens are all significantly different ( $P < 0.05$ ) from 1 or more brain tissue type, using a paired t test in SPSS (IBM, Armonk, NY). Conversely, global DNA methylation of blood was not significantly different ( $P < 0.05$ ) from any brain tissue type. This study shows significant differences in global DNA methylation among different tissue types in the limbic system of the bovine brain. Understanding the differences in global

DNA methylation within different tissues in the brain will facilitate future research involving the effects of differential methylation with regard to economically important traits.

**Key Words:** bovine, brain, methylation

#### **P2012 Investigation of genomic imprinting in chicken embryonic brain and liver through RNA sequencing.**

Z. Zhuo<sup>\*1</sup>, S. J. Lamont<sup>2</sup>, B. Abasht<sup>1</sup> (<sup>1</sup>Department of Animal and Food Sciences, University of Delaware, Newark, DE, <sup>2</sup>Department of Animal Science, Iowa State University, Ames, IA)

Genomic imprinting refers to the epigenetic phenomenon that some autosomal genes are exclusively expressed from either the maternal or paternal allele, whereas, based on Mendelian inheritance, expression of alleles is expected to be in equal amount and independent of their parental origin. DNA methylation in *cis*-acting manner is the major mechanism for genomic imprinting. Imprinted genes have been identified in several animal species and are frequently associated with embryonic growth and survival functions. Yet, whether genomic imprinting exists in chickens is still debatable, as previous studies reported conflicting evidence regarding the topic. Albeit no genomic imprinting has been found in the chicken embryo as a whole, we investigated whether certain embryonic tissues exhibit genomic imprinting. In this study, we interrogated the existence or absence of genomic imprinting in chicken the embryonic brain and liver by examining mRNA expression of parental alleles in an F1 generation. Eggs from 2 highly inbred chicken lines (Fayoumi and Leghorn) and their reciprocal crosses were collected and incubated for 12 d; then, brain and liver were harvested from embryos for cDNA library preparation. To establish the genotypes of the inbred lines and F1 hybrids, and to minimize reference bias of RNA-Seq sequence alignment, genomic DNA from inbred Fayoumi and Leghorn chickens were pooled separately and each pool was sequenced at 20X coverage. The SNP loci identified from DNA-Seq data were masked to create a customized reference genome (based on Ensembl Galgal4) for RNA-Seq reads mapping. Of 65 million RNA-Seq reads per sample generated using the Illumina HiSeq 2000 sequencer, 88% were mapped to the customized reference genome. The genome-wide ratio of mapped reads containing reference allele was reduced by 1.5% when compared with results from the original reference genome. Our analyses indicated that in the F1 crosses, about 9.2% of the heterozygous loci show allele-specific expression (binominal test,  $p$  value  $\leq$