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MULTI-TISSUE GENOME WIDE EXPRESSION OF MRNA AND MICRORNA IN CATTLE SELECTED FOR HIGH AND LOW RESIDUAL FEED INTAKE

Y. Chen[1], C. Gondro[2], W. Al-Husseini[2], S. De las Heras Saldana[2], R. Herd[3] and P. Arthur[1]

[1] Elizabeth Macarthur Agricultural Institute, NSW Department of Primary Industries, Menangle, NSW, Australia
[2] Environmental and Rural Science, University of New England, Armidale, NSW, Australia
[3] Beef Industry Centre, NSW Department of Primary Industries, Armidale, NSW, Australia

SUMMARY

Residual feed intake is a measure of feed efficiency in beef cattle which is an economically important trait in beef production. Gene expression is the key determinant of cellular phenotype and genome-wide expression analysis will provide insight into the molecular events underlying biology of feed efficiency. We carried out genome-wide gene expression in liver, muscle and blood with total 199 samples and micorRNA expression with 96 samples. These datasets provide a resource of expression QTL mapping for understanding the functional consequences of genetic variation, and how it affects feed efficiency in beef cattle.

INTRODUCTION

Feed use and feed efficiency are important for the survival and selective advantage of an animal. In animal production enterprises, feed intake and feed efficiency are important factors that affect overall profitability. In a typical beef production system, about 65–85% of the feed is used to maintaining the cow breeding herd (Montaño-Bermudez and Nielsen 1990). Feed efficiency in beef cattle can be measured as Net feed intake (NFI) or residual feed intake (RFI). This is the difference between an animal's actual feed intake recorded over a test period and the expected feed intake based on the animal's size and growth rate (Koch et al. 1963).

There is strong evidence that genetic variation in RFI exists. The estimated heritability of RFI in cattle populations is moderate ranged from 0.07 to 0.62 (Berry and Crowley 2013). In Australia, heritability estimates for NFI at feedlot is 0.41 and 0.34 for post-weaning (Jeyaruban et al. 2009).

However, the accurate measurement of RFI for individual animals is an expensive process, and this has been a major limitation to the adoption of feed efficiency as an economically important trait in animal breeding. Much of the research projects were focus on to to develop genetic markers that can be used for genomic selection using high density single nucleotide polymorphism (SNP) chips and more recently, next generation sequencing technologies that enable breeders to select animals based on genomic sequences (Meuwissen et al. 2001; Barendse et al. 2007; Meuwissen and Goddard 2010; Bolormaa et al. 2011; Khansefid et al. 2014).

In the past decades, we have gained considerable knowledge of understanding of animal's development with the advance of genome sequence of human and many other species (The-ENCODE-Project-Consortium 2012). The genome sequence contains all the information necessary to develop from the initial zygote to an adult with full set of organs to respond to the environmental influence. Although all cells from an individual have the same genome sequence, there are more than 400 distinct cell types which and their cellular developments, morphology, and function are governed by precise patterns of gene expression which are regulated by the functional elements in the genome.

A number of studies of gene expression in beef cattle have been published (Chen et al. 2011; Tizioto et al. 2015; Weber et al. 2016) with a limited number of samples and a number of differentially expressed genes between high and low NFI cattle were revealed by comparing two extreme phenotype groups. However, a large gene expression dataset with sufficient number of

animals and crossing multiple tissues is essential to study the patterns of transcriptome variation across individuals and tissues. We carried out genome-wide gene expression in liver, muscle, and blood tissues with total 199 samples, In addition, we also carried out genome-wide expression of micorRNA for 96 samples. These datasets provide a resource of expression QTL mapping for understanding the functional consequences of genetic variation, and how it affects feed efficiency in beef cattle.

MATERIALS AND METHODS

All animals used for the genome-wide gene expression by RNA-sequencing were from Angus divergent selection line for NFI at the Agricultural Research Centre, Trangie, NSW (Arthur *et al.* 2001). The first set of animals consisted of 48 young bulls born in 2005 which were approximately three generations of divergent selection for NFI. Liver biopsies were taken from 24 animals with the lowest RFI and 24 animals with the highest NFI at the end of the test at feedlot Tullimba. The second set of animals contained 48 young bulls born at 2008. Muscle biopsies were taken at post weaning at the Agricultural Research Centre, Trangie. The third dataset was 30 steers and 30 heifers born in 2012, the blood tissue and liver biopsies were taken at the end of NFI test.

All animals used in these experiments were recorded for average daily gain during the 70day; net feed intake, average daily feed intake; P8 fat thickness (ultrasound) at the end of NFI test; RIB fat thickness (ultrasound); eye muscle area (ultrasound).

All experiments were approved by the University of New England Animal Ethics Committee (AEC 06/123, AEC14-002 and AEC14-036) and New South Wales Department of Primary Industries (NSW DPI) Animal Research Authority ((ORA09/015, ORA 13/16/004). Male calves were castrated at 4 months of age. After weaning animals were grown on native pastures until they reached feedlot entry weight of approximately 400 kg BW. NFI was tested in the Beef Research Feedlot Tullimba, NSW with an automated recording system. During the 70-day test, the animals had ad libitum access to a barley-based feedlot ration containing 12 MJ metabolizable energy per kilogram dry matter and 15–17% crude protein.

Ninety-seven RNA-sequencing samples were obtained using HiSeq 2000 (Illumina Inc) and the RNA-sequencing libraries were created from the polyadenylated fraction of RNA from each animal by using modified protocol of Illumina sample preparation. The remaining 104 RNA-sequence samples were obtained by using HiSeq 2500 (Illumina Inc) at Beijing Genome Institute and the sequencing library were created by using non-strand specific protocol with poly-A selection of mRNA (the Illumina Tru Seq™) protocol.

Small RNA libraries were constructed for each animal using 1µg total RNA with NEXTflex™ Small RNA-Seq Kit v2 (Bioo Scientific, TX, USA) following the protocols supplied by the manufacturer. The libraries were sequenced at Ramaciotti Center, University of NSW with Illumina HiSeq 2000 Sequencing System.

The quality of the sequence was assessed with FastQC v0.11.3 (Andrews 2010) and the low quality bases and adaptor sequences were removed by Trimmomatic v0.33 (Bolger et al. 2014). We used topHat v2.1.1 (Trapnell et al. 2009) to align all paired reads against the Bos taurus reference genome (Ensembl UMD3.1). Read counts for each sample was obtained with HTSeq v 0.6.0 (Anders et al. 2014). In order to visualize the clustering of the tissues samples, batch effects, and possible outliers we performed plots from the output of Principal Component Analysis (PCA) on the raw counts.

We used R package ComBat (Johnson et al. 2007) to adjust for batch effect in liver samples of the bull and steer datasets. The differential expressed (DE) genes between tissue were obtained by Edge R (false discovery rate <0.05 and the logarithm fold change (logFC) ≥ 1.5).

RESULTS AND DISCUSSION

In total, we obtained more than 1772 million high quality paired sequence reads for RNA-sequencing and 240 million microRNA sequence reads. Among all the RNA-sequencing samples, more than 90% of the paired sequence reads from liver and muscle tissue were aligned to the bovine genome (bostau6, UMD_3.1) albeit, the mapping rate for sequence reads in blood is lower (~ 81%) (Table 1). For the microRNA sequencing, more than 80% of the sequence reads mapped to the bovine genome and 69% sequence reads mapped to known bovine mature miRNA (bostau6, UMD_3.1).

Table 1. Summary of the RNA-sequencing results

Dataset	Liver /bull	Liver /steer	Liver /heifer	Muscle /bull	Blood /steer	Blood /heifer	Liver [2] miRNA	Muscle[2] miRNA
No sample	47	25	27	48	25	27	48	48
Paired reads (10^6)	6.0	11.3	11.3	6.8	11.2	11.2	2.81	2.2
Range (10^6)	1.6-12.5	11.0-11.5	11.0-11.5	0.6-9.4	9-11.5	11-11.5	0.32-9.8	0.4-7.5
Mapped pair reads %[1]	81	90.8	90.8	88.7	80.6	80.6	88 (0.69)[3]	84 (0.69)[3]

[1] the concordant pair alignment rate; [2]:miRNA-sequencing using 1x75 single read; [3]:the sequence reads mapped to known miRNA in bovine genome sequenced (UMD3.1).

We explored gene expression similarity between tissues and across samples by principal component analysis (PCA) on the raw counts. Liver, muscle and blood tissues show a characteristic transcriptional signature (Figure 1 A). Furthermore, the expression profiles in liver of young bulls are quite distinct to steers and heifers, while there is little difference of expression pattern in liver between steer and heifer (figure 1B). Indeed, we found the largest number of differential expressed genes between tissues (>12000). In liver expression, there are more than 9000 differentially expressed genes between young bull and steer/ heifer, only 2 differentially expressed genes between steers and heifers (data not shown due page limits). This suggests that male hormones played important roles in liver expression. The liver is one of the most essential organs involved in the regulation of energy homeostasis and associated with lipid formation and breakdown, glucose production and catabolism, and cholesterol synthesis and secretion. It was well documented in mice and human that males, testosterone works via androgen receptors to increase insulin receptor expression and glycogen synthesis, decrease glucose uptake and lipogenesis, and promote cholesterol storage in the liver (Shen & Shi 2015).

Understanding the gene regulation and the identification of the functional elements in genome are important to increase the accuracy of genome selection by increase the level of linkage disequilibrium in the marker panel by including functional SNPs. These gene expression datasets provide a potential resource for mapping functional elements by eQTL mapping.

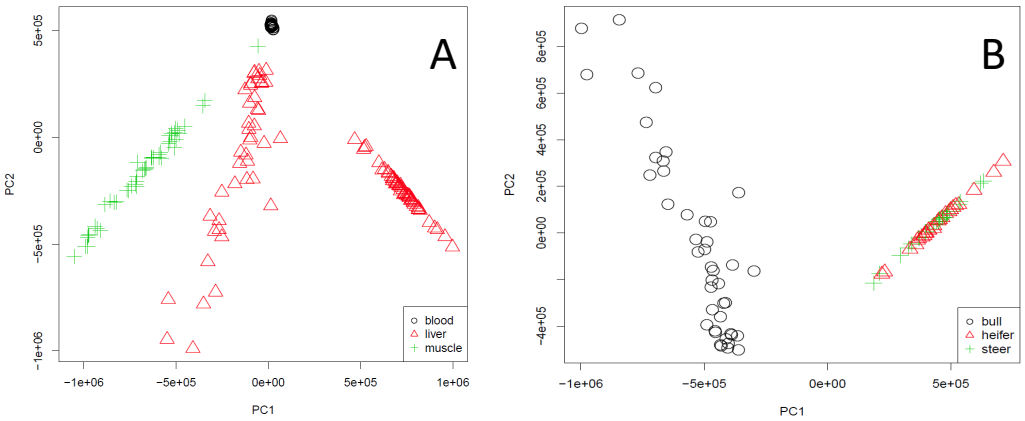

Figure 1. Gene expression similarities between tissues and sex by principal components analysis. A:gene expression between tissues across all samples. B:Liver gene expression between young bulls, steers and heifers

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