Comparative Genomics

The International Swine Methylome Consortium (ISMC): Supporting Epigenomics and Biomedical Research

Schachtschneider Kyle,[1,2] Rund Laurie A.,[1] Madsen Ole,[2] Johnson Rodney W.,[1] Archibald Alan L.,[3] Park Chankyu,[4] Groenen Martien A.,[2] Schook Lawrence B.,[1]

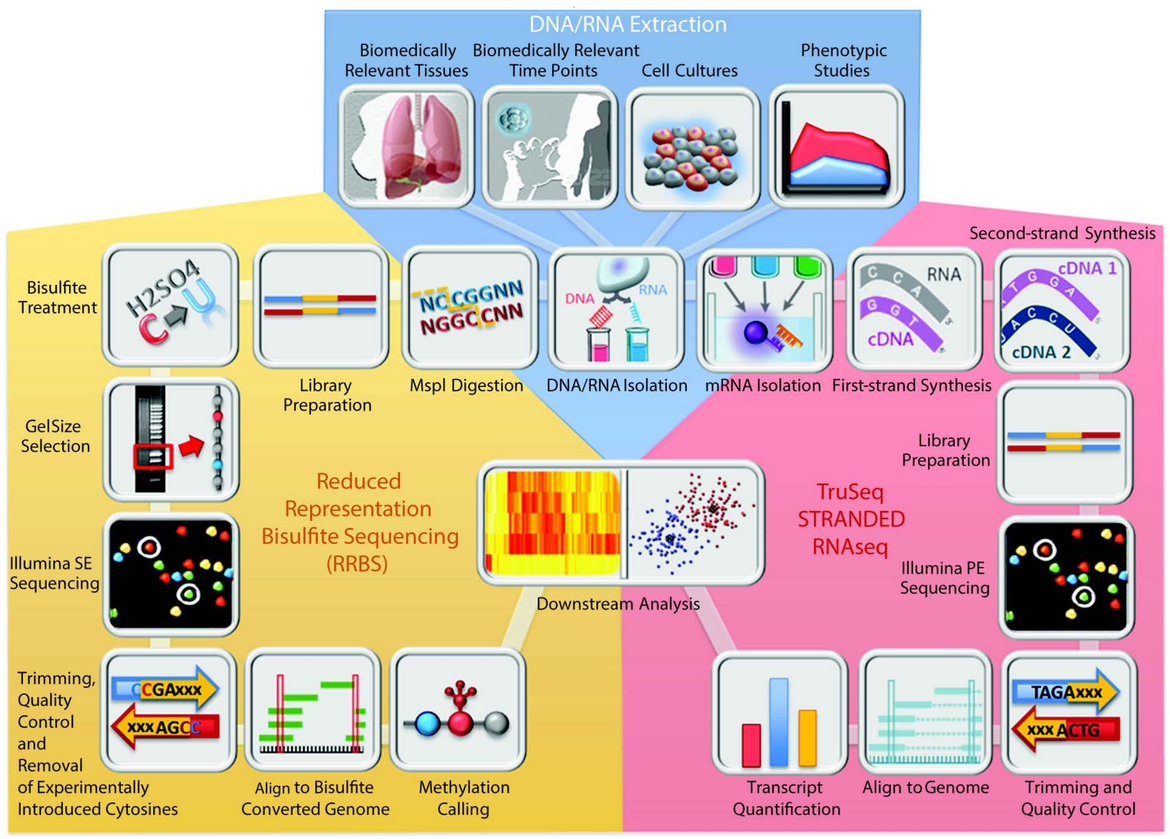
[1]Department of Animal Sciences, [5]Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Illinois, USA
[2]Animal Breeding and Genomics Center, Wageningen University, Wageningen, The Netherlands
[3]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, U.K.
[4]Department of Animal Biotechnology, Konkuk University, Gwangjin-gu, Seoul, South Korea

Introduction:

- Pigs (Sus scrofa) are an important biomedical model due to their anatomical, behavioural, genetic and physiological similarities with humans, as well as their availability, short generation interval and large litter size.
- Altered DNA methylation levels are associated with aberrant gene transcription and represent a link between genetics and environmental signals that has been reported to play an important role in human pathologies including cancer and neurological disorders.
- Increased promoter methylation is associated with transcriptional repression, while increased intragenic methylation is associated with increased transcription (The ENCODE Project Consortium, 2012).
- The International Swine Methylome Consortium (ISMC) was created to produce a porcine methylome map in order to enhance studies of DNA methylation patterns and their association with the development and detection of relevant human diseases.
- In a preliminary exploratory study, Reduced Representation Bisulfite Sequencing (RRBS) and RNA-seq were performed on tissue samples from the adult female Duroc utilized for the pig genome sequencing project.

Materials and Methods:

Figure 1: Protocol for Porcine Methylome Map Production



- DNA and RNA extracted from 8 tissue samples:
  - Fat, Heart, Kidney, Liver, Lung, Lymphnode, Muscle and Spleen
- RRBS and RNA-seq performed on Illumina HiSeq 2000
- RRBS Analysis:
  - 30–160 bp fragments selected
  - Alignment and methylation calling performed using BSseeker2 (Guo et al., 2013)
- RNA-seq Analysis:
  - Alignment performed using Tophat2 (Kim et al., 2013)
  - Transcript quantification performed using Cufflinks (Trapnell et al., 2013)

Results:

Table 1: RRBS Alignment Statistics

Sample	Total # Reads	Uniquely Aligned	Genomic Coverage	Average Coverage	Average Methylation	CpG Sites Minimum 1X Coverage	CpG Sites Minimum 10X Coverage
Fat	40,306,207	59.42%	1.42%	17.36	41.69%	2,153,622	650,251
Heart	32,860,936	59.53%	1.42%	15.38	41.24%	2,174,918	705,125
Kidney	46,220,679	59.88%	1.43%	21.06	41.99%	2,178,718	753,252
Liver	40,708,178	59.13%	1.32%	19.16	41.74%	2,052,654	663,199
Lung	34,772,539	60.73%	1.14%	18.58	41.22%	1,794,249	550,080
Lymphnode	53,116,075	59.57%	1.41%	22.86	41.98%	2,205,121	705,719
Muscle	50,707,203	60.79%	1.60%	21.39	39.14%	2,457,110	927,828
Spleen	62,273,308	58.17%	1.52%	25.05	48.29%	2,370,816	890,417
Theoretical Max			1.75%			2,812,047	

Figure 2: Average Number of CpG Sites within Genomic Locations

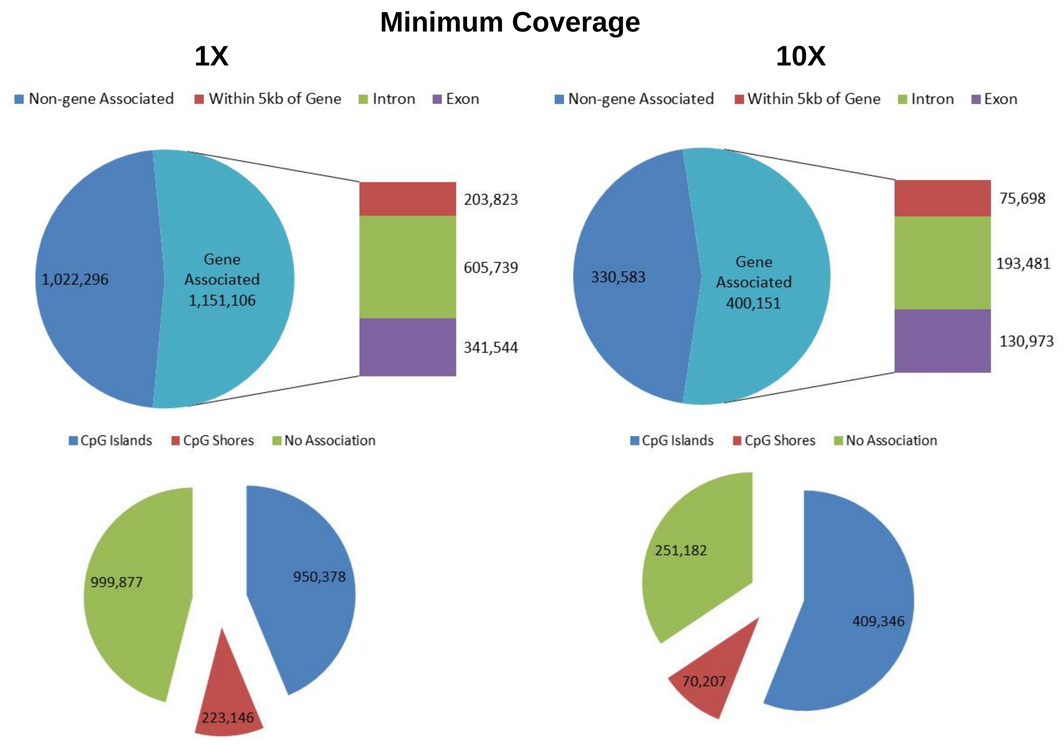


Figure 3: CpG Methylation Across Sites

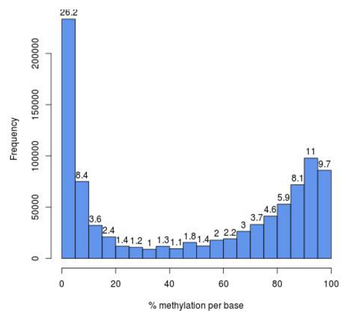
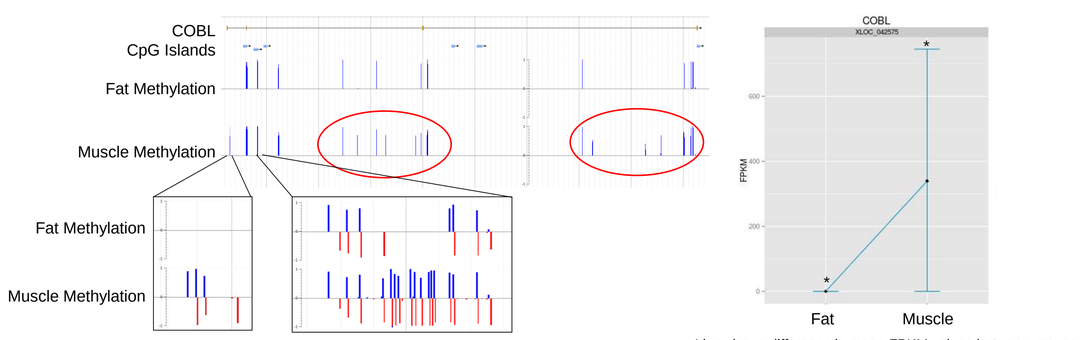


Table 2: RNA-seq Alignment Statistics and Differential Gene Expression Analysis

Total # Read Pairs	Aligned		Heart	Kidney	Liver	Lung	Lymphnode	Muscle	Spleen
46,429,351	86.00%	**Fat**	8834	8039	8029	7948	8500	9278	7766
45,916,393	91.70%	**Heart**		406	396	267	364	271	139
46,832,040	87.90%	**Kidney**			468	333	526	260	194
51,750,214	88.90%	**Liver**				197	400	139	196
41,264,298	86.10%	**Lung**					216	180	196
41,939,163	91.30%	**Lymphnode**						218	186
47,678,837	87.60%	**Muscle**							87
38,757,795	87.00%	**Spleen**							

Figure 4: Increased Intragenic Methylation Associated with Increased Expression



Line shows difference in mean FPKM values between groups.
* Denotes statistical significance (p < 0.05)

Conclusions and Future Plans:

- RRBS is a viable approach for targeted methylation profiling of CpG sites associated with genes and CpG Islands in pigs, and can provide insights into potential epigenetic regulation of gene transcription when coupled with RNA-seq.
- Develop a prioritized list of biomedically relevant tissues and time points for further analysis.
- Identify phenotypic studies of interest for epigenetic profiling.
- Perform RRBS and RNA-seq analysis on additional samples and develop a plan for data distribution.

Funding:

Jeju National University of Rural Development Administration of the Republic of Korea 538 JNU Korea 2012-06052 to LBS