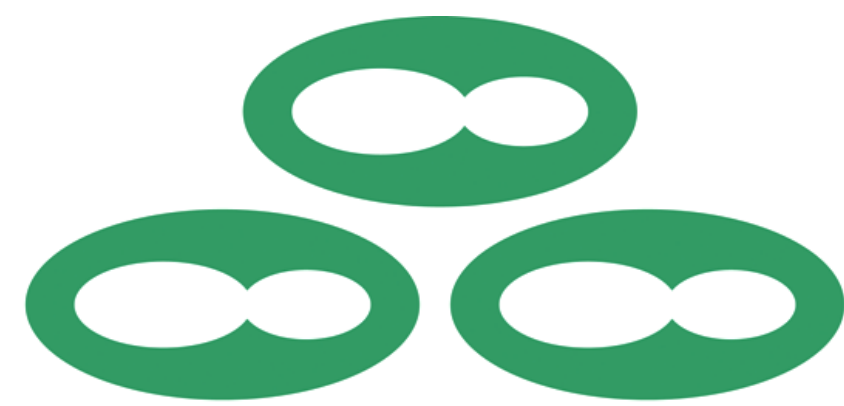


Genome-Wide DNA Methylation Analysis In Plants and Animals



ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

Xueguang Sun, TzuHung Chung, Eliza Bacon, Ron Leavitt, Nikolas Isely, Marc Van Eden & Xi-Yu Jia

Zymo Research Corporation, Irvine, CA

Abstract

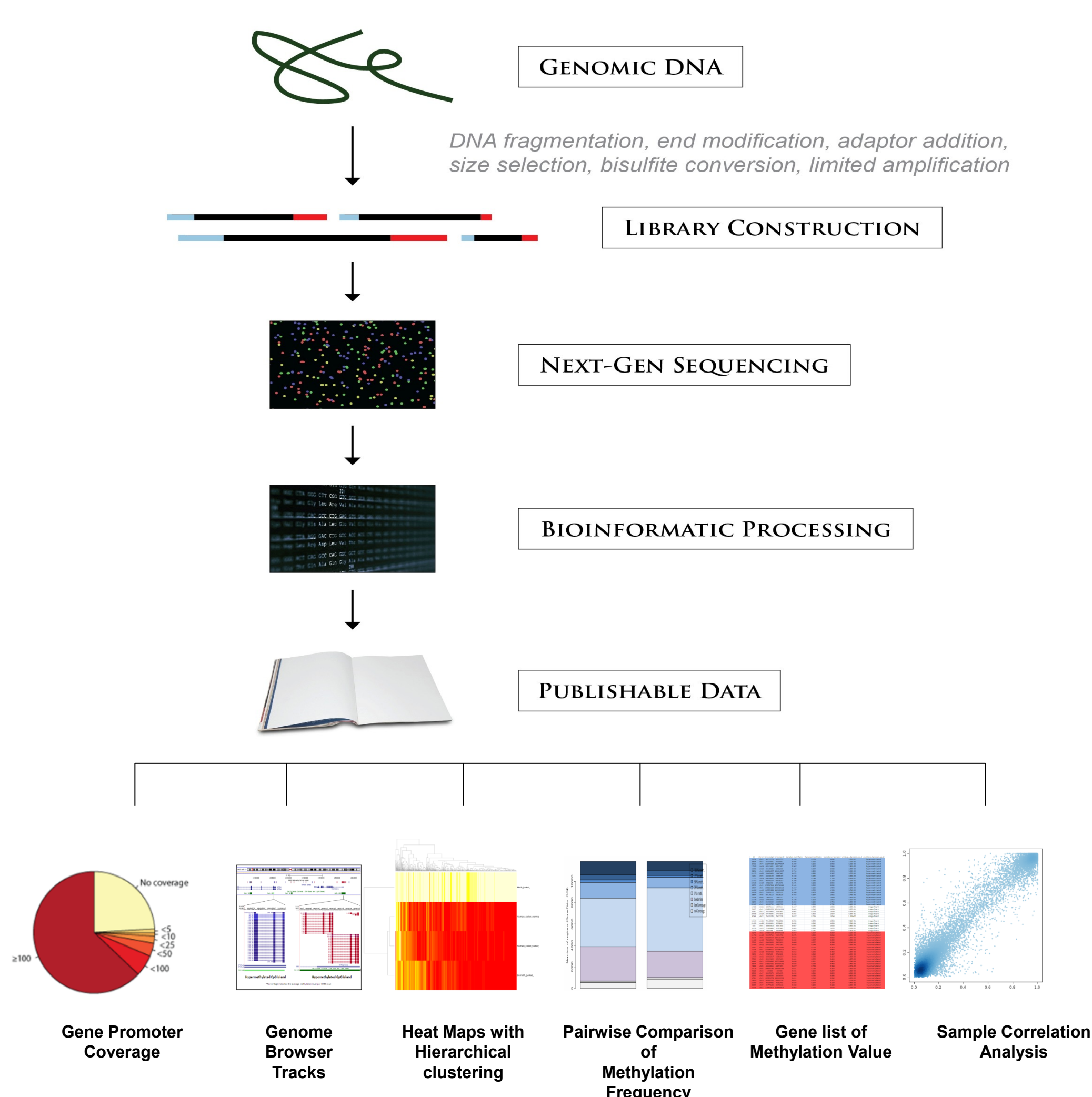
DNA methylation is a highly conserved epigenetic mark present in many eukaryotic organisms including plants, animals, and fungi. It plays an important role in the regulation of gene expression. A number of studies have shown its involvement in plant and animal growth and reproduction primary through the processes of genomic imprinting, X-chromosome inactivation, and the silencing of transposons and other repetitive DNA elements. As such, the understanding of DNA methylation has become a major focus of the research conducted during the “post-genomics” era. However, the precise determination of a DNA’s methylation pattern on a genomic scale has posed a challenge especially for those complex genomes. Combining well-established bisulfite conversion chemistry with NextGen sequencing, we have established a robust service platform for analyzing DNA methylation with single base resolution at the genomic scale. The service features a streamlined workflow coupled with a comprehensive bioinformatics pipeline to provide both a consolidated and cost-effective solution for epigenetic analysis of plant and animal genomes. This technology has been used successfully for the analysis of methylomes from many organisms including soybean, mouse, and chicken. Additional analyses of other species are ongoing. Those data should provide a means to understanding how environment as well as other factors may alter an organism’s fitness through heritable changes in epigenetic gene expression.

Introduction

The EpiQuest™ **Genome-Wide DNA methylation Profiling Service** expands upon the conventional RRBS (Reduced-Representation Bisulfite Sequencing) method to greatly increase sequence analysis of CpG-rich DNA. Through an unique library preparation procedure and an optimized workflow, the EpiQuest™ team at Zymo Research has been able to expand coverage to **≥80%** of all gene promoters and **≥85%** of CpG islands (for human samples).

The EpiQuest™ **Whole-Genome DNA methylation Profiling Service** utilizes a streamlined library preparation and sophisticated alignment algorithms to deliver Whole Genome Bisulfite-Sequencing data that covers **>80%** of all the bases in the human genome at single-base resolution.

Workflow



Key features

- ✓ Next-Gen sequencing based platforms
- ✓ Low DNA input
- ✓ Applicable to a broad range of sample sources (human, mouse, rat, plant, etc.)
- ✓ Streamlined workflows with comprehensive bioinformatic analysis and high quality publishable data delivery.
- ✓ Customizable, rapid turnaround at an affordable cost.

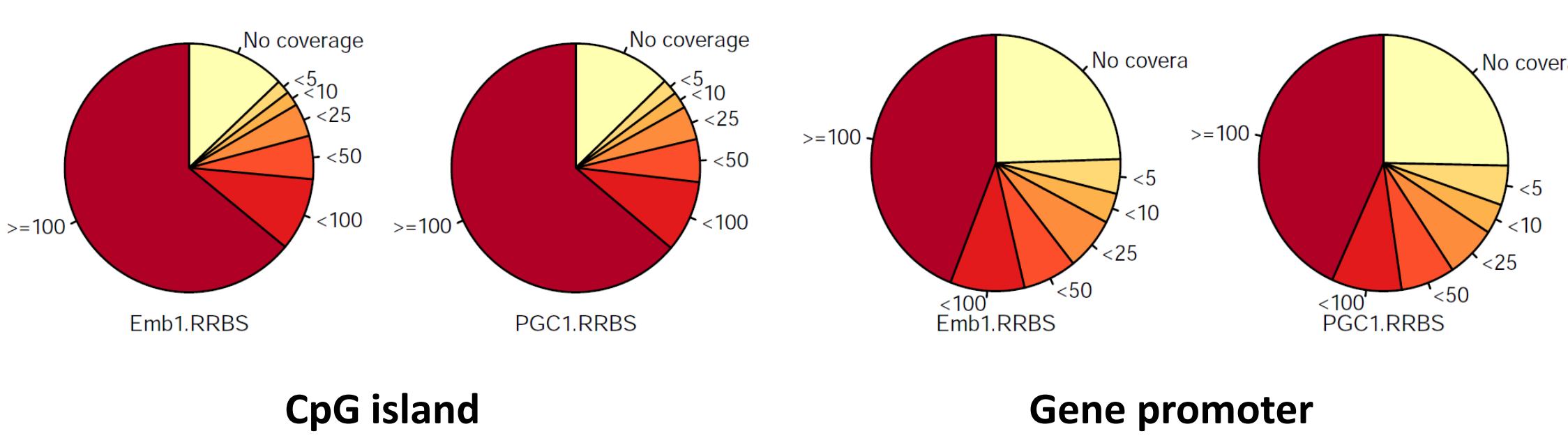
Service comparison

EPIQUEST™ NEXT-GEN SEQUENCING DNA METHYLATION ANALYSIS	BASIC SERVICE PACKAGE		FULL SERVICE PACKAGE	
	Genome Wide	Whole Genome	Genome Wide	Whole Genome
Service Performed	Description			
Genomic DNA Sample Validation*	Quality validation of samples supplied by client prior to processing			
Sample Processing & Library Preparation	Unique one-step procedure for optimized performance and limited sample loss: endonuclease digestion, end modification, adaptor addition, size selection, bisulfite treatment (conversion), limited amplification.			
Next-Gen Sequencing	Next-Gen sequencing using the latest technologies available from Illumina™.			
Sequencing Validation & Bioinformatic Analysis*	Alignment of sequencing data to the corresponding reference genome using proprietary, state of the art bioinformatic algorithms and software.			
Data Output & Delivery	Delivery of a consolidated printed report including: service checklist, performance metrics, statistics, select graphical data, and more. Project data will be provided to the client on a hard drive.			
Data Provided	Description			
Genome Browser Tracks	Three (3) UCSC browser tracks: CpG track, Next-Gen sequencing read track, and endonuclease-digested fragment track. Reference UCSC Genome Browser: (http://genomes.ucsc.edu)			
Sequence Coverage Information	Statistical coverage (pie chart) of genomic features analyzed (i.e., CpG islands, promoter regions, etc.)			
Hierarchical Clustering	Distribution (heat map) of methylated vs. non-methylated loci between two (2) or more samples			
Methylation Frequency	Pairwise sample comparisons (bar chart) of methylation frequencies in CpG islands, promoter regions, etc.			
Methylation Index	Global methylation (percentage) for CpG islands, promoters, etc.			
Differentially Methylated Regions	Index of hyper- and hypo-methylated regions for biomarker candidate determination.			
Correlation Data	Pearson correlation for pairwise data comparison between samples.			
...and much more!	Our unique bioinformatics pipeline is used to generate >35 publication-ready figures and tables for easy data interpretation and sample comparison.			
	Cost (per sample)		Inquire!	
ADDITIONAL CUSTOMIZED SERVICES ARE AVAILABLE				
Non-Human & Mouse Sampling	Samples from organisms other than human and mouse having annotated genomes can also be processed with the EpiQuest™ Genome-Wide DNA Methylation workflow.			
DNA Extraction/Purification from Difficult Sample Sources	DNA can be isolated from cells, blood, tissue, FFPE tissue, environmental sources, and more!			
Expanded Genomic Analyses	Analysis of gene bodies, repeated elements, exons, introns, non-coding RNAs, miRNAs, enhancers, and more!			
Analysis of Non-CpG Methylation	Expanded analysis of CHG and/or CHH methylation.			
Customized Bioinformatic Processing & Data Output	Our bioinformaticians can customize data delivery and graphics upon request.			
	Cost (per sample)		Inquire!	

Genome-wide 5mC profiling in chicken

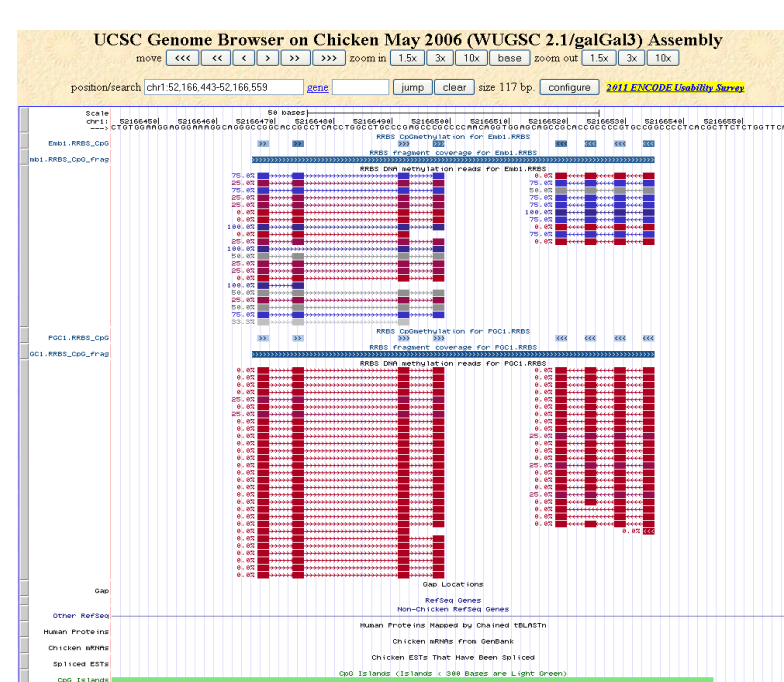


Genome Coverage :



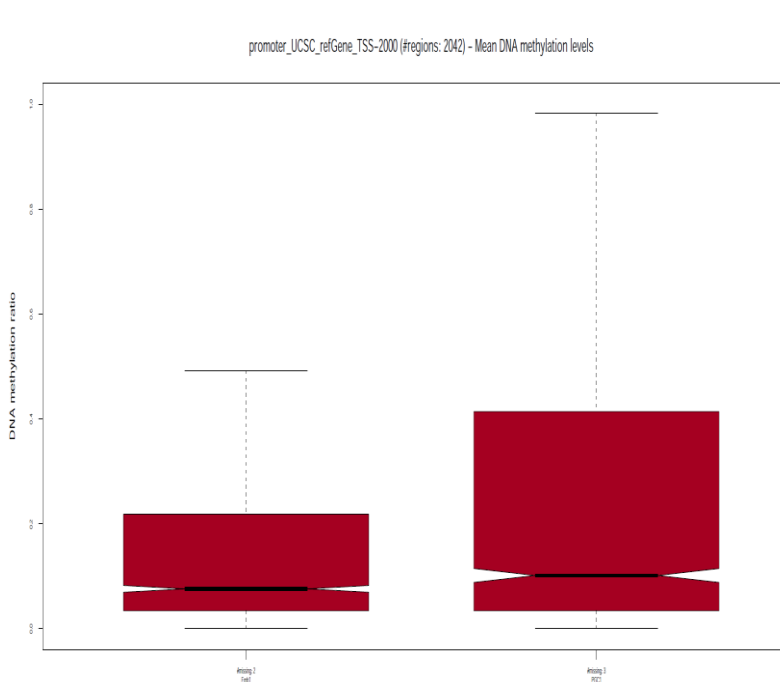
Pie charts shows the overall distribution of sequencing reads coverage

UCSC genome browser track



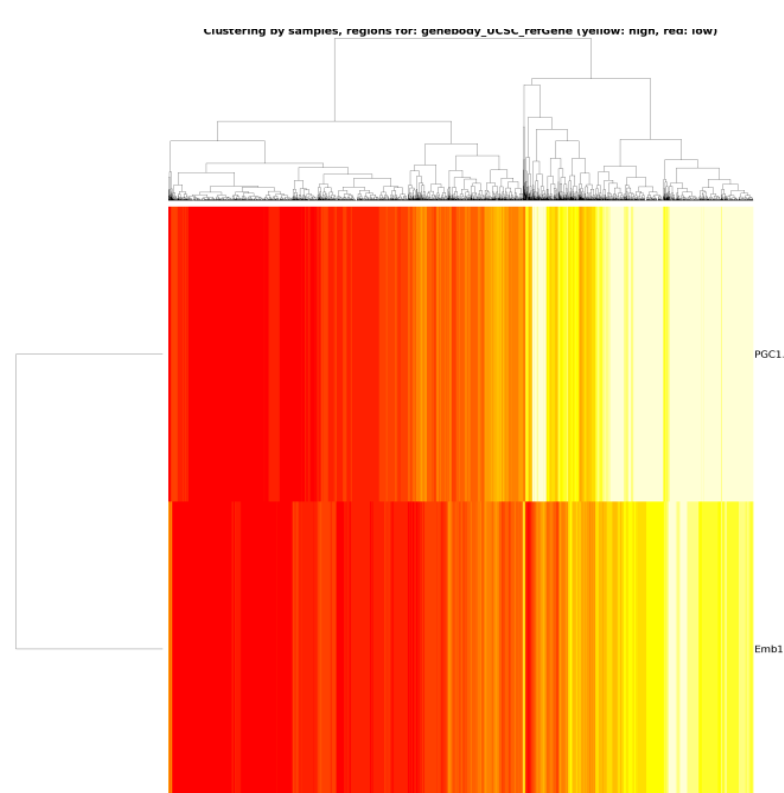
Browser tracks show the sequencing reads and methylation value

Methylation Index



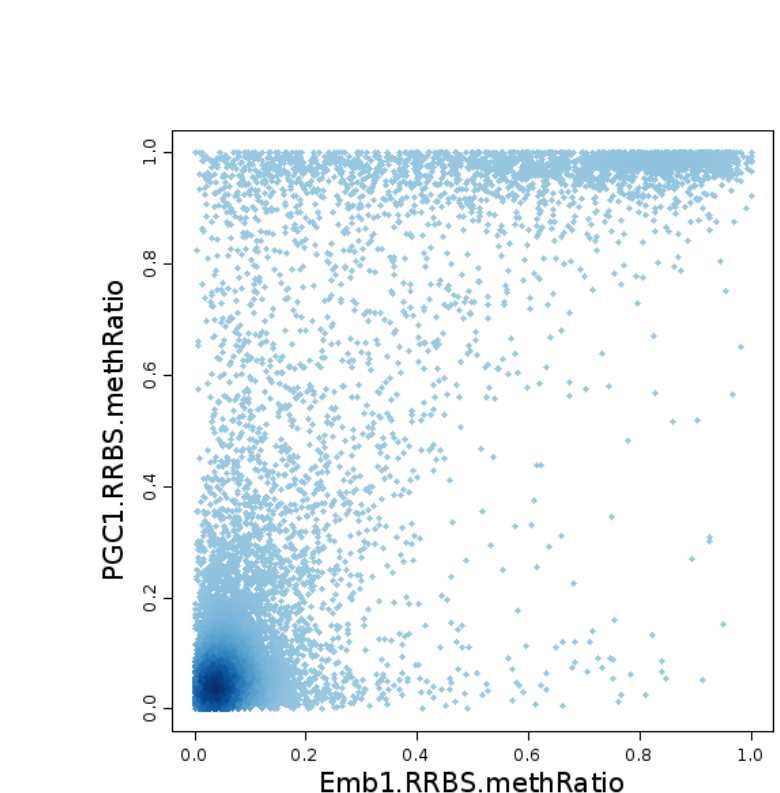
Mean DNA Methylation Levels for Gene Promoter

Hierarchical clustering:



Heatmap shows the hierarchical clustering by region and sample

Correlation analysis



Pearson correlation for pairwise data comparison between samples

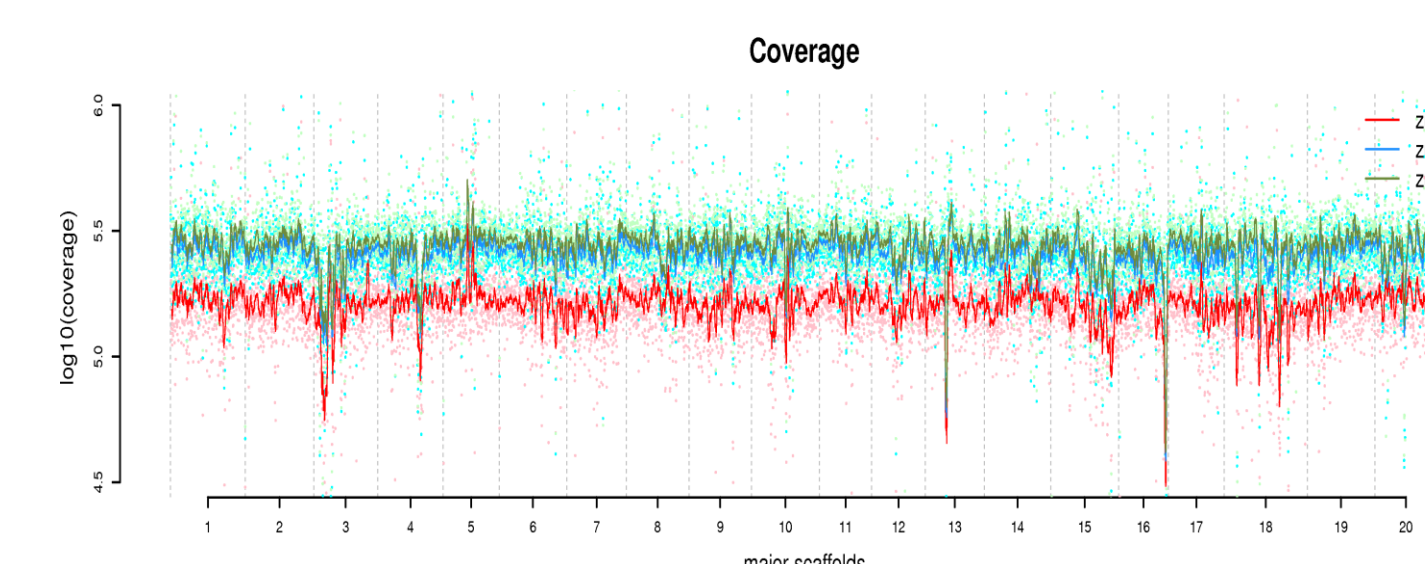
hyper/hypo methylation gene list

Pair-wise comparison was performed between the different samples, DMR were counted according to hyper/hypo methylation.

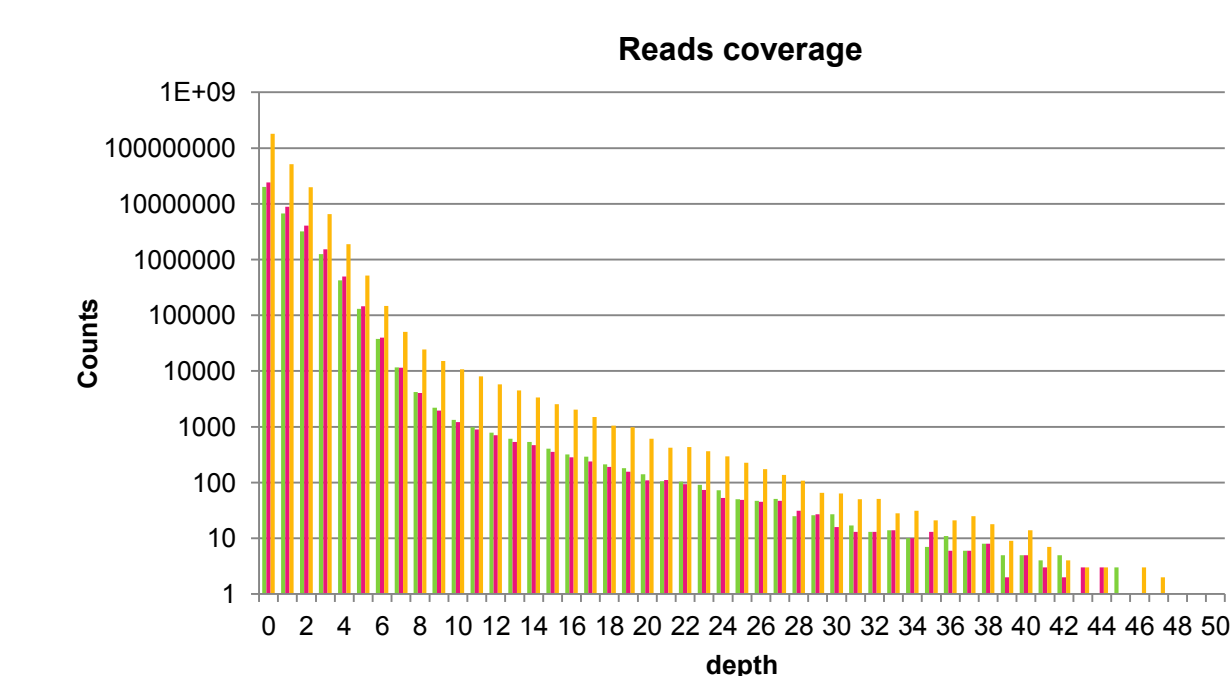
Whole Genome 5mC profiling in soybean



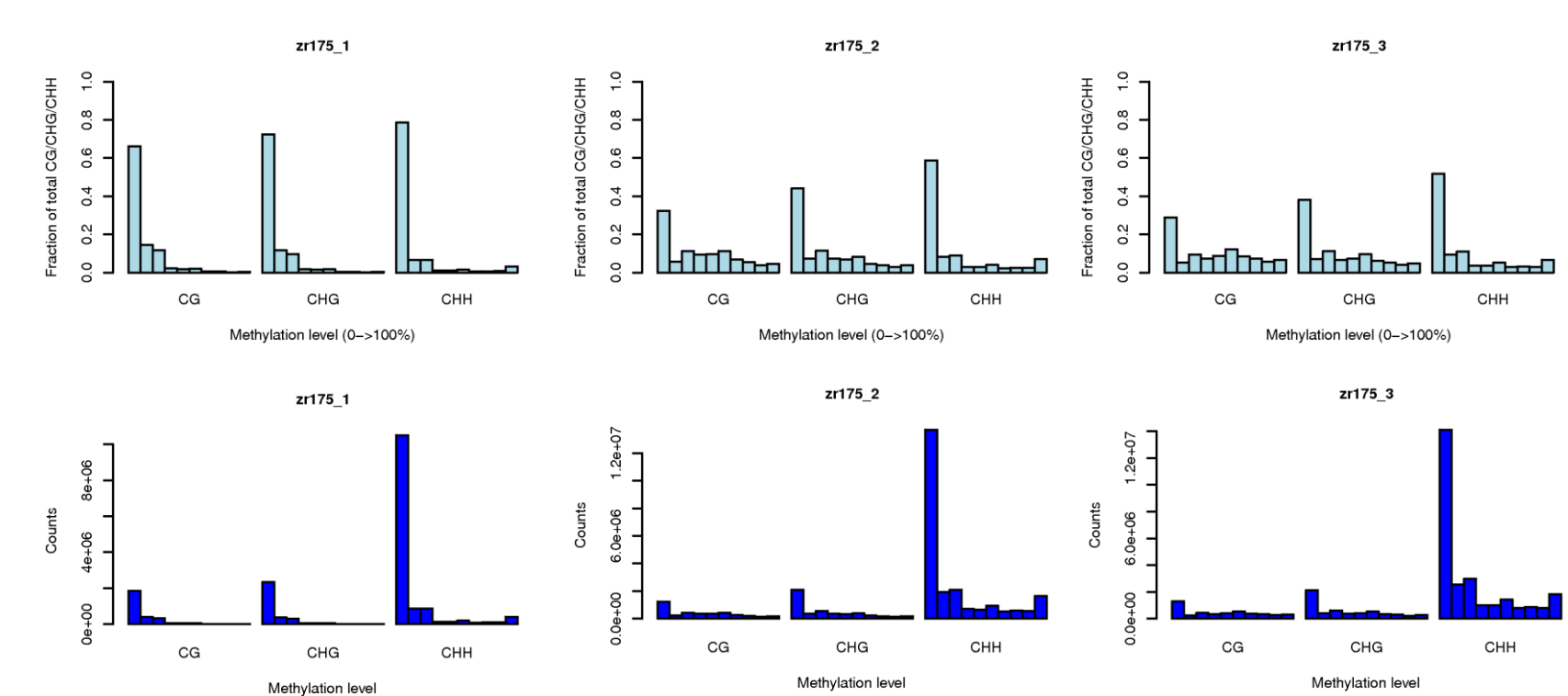
Genome coverage



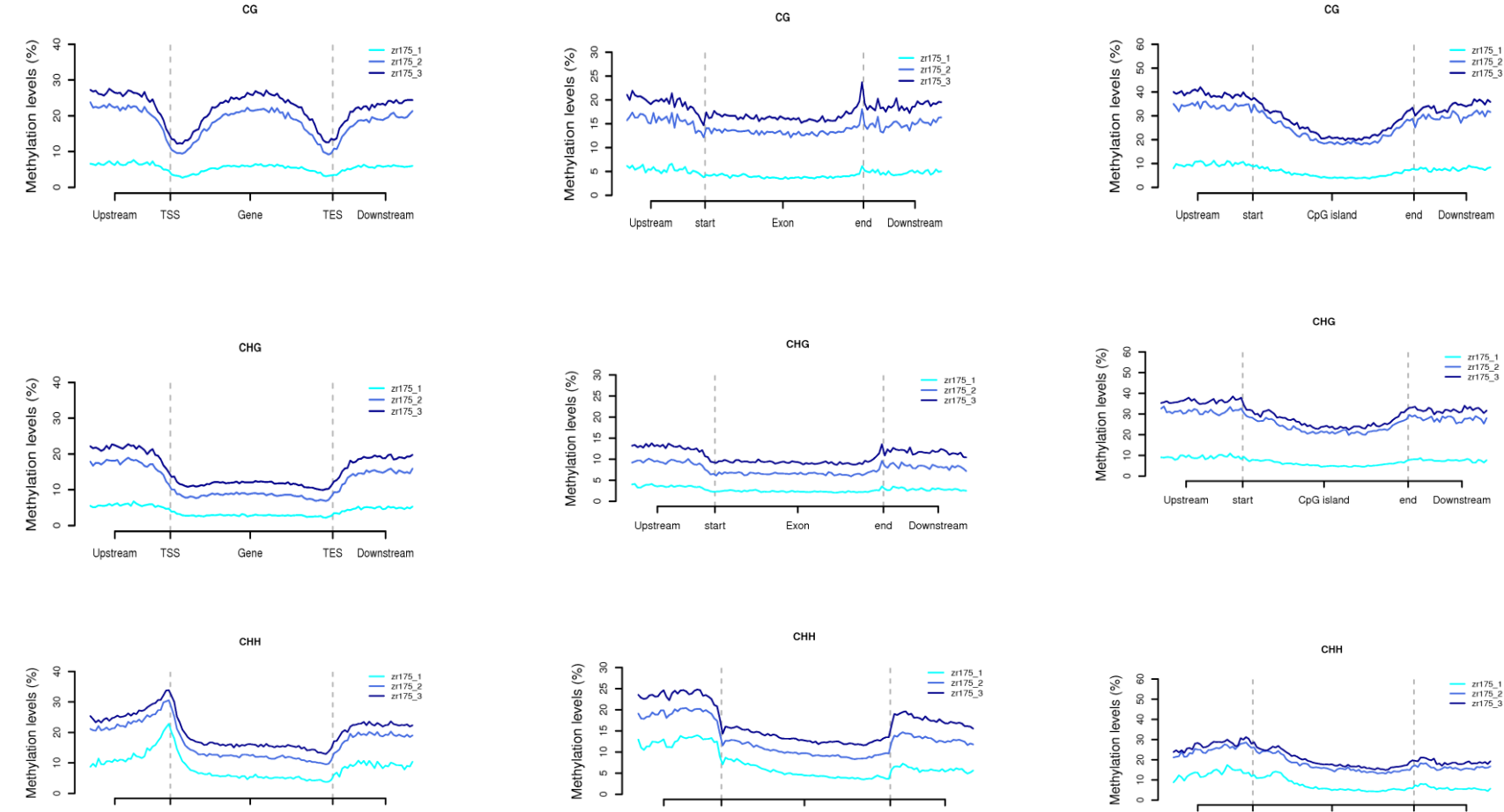
Coverage per cytosine



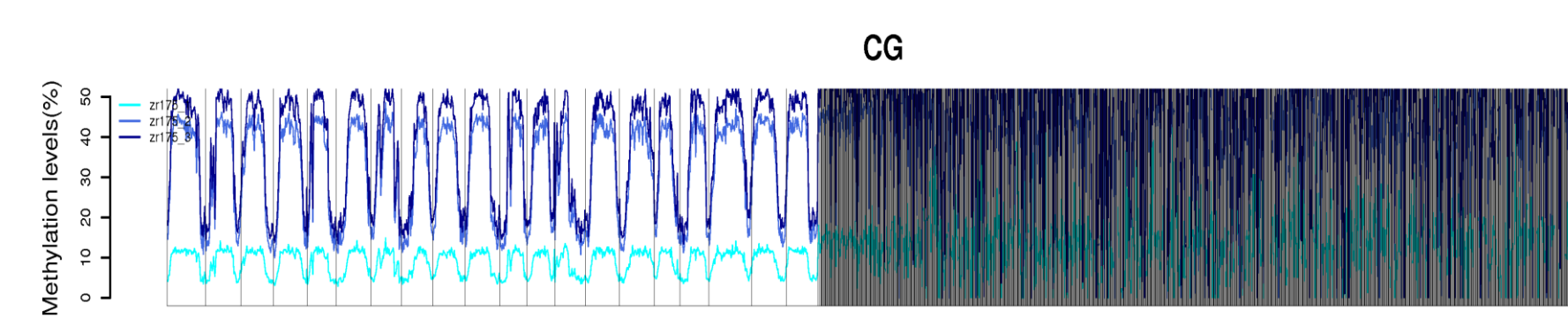
Distribution of CG/CHG/CHH sites for three soybean samples



Metaplot for gene, exon and CpG island



Genome methylation level



References

1. Meissner A, Mikkelsen TS, Gu H, Wernig M etc. Nature. 2008 Aug 7;454(7205):766-70.
2. Smith ZD, Gu H, Bock C, Gnirke A, Meissner A. Methods. 2009 Jul;48(3):226-32