Genomic Approach for DNA Methylation and Hydroxymethylation Analysis

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Abstract

DNA methylation and hydroxymethylation are some of the most important epigenetic modifications that can occur in the human genome. For instance, DNA methylation plays a vital role in the regulation of gene expression in normal cell development and aging, and also in the formation and progression of cancer and other diseases. Large scale identification of putative epigenetic biomarker candidates is now achievable with the ability to profile DNA methylation and hydroxymethylation at the genomic level. Once validated, specific biomarkers could be applied to clinical and molecular diagnostic fields. Due to the increased availability of Next-Gen sequencing technology, a number of new technologies have been developed for interrogating DNA methylation and hydroxymethylation at the genomic scale. Zymo Research has recently perfected sample prep and bioinformatics analysis as part of its new DNA Methylation and Hydroxymethylation Profiling Services. These epigenetic services combine next-generation sequencing with Zymo's well-established epigenetic technologies and innovative bioinformatics algorithms for the most streamlined, comprehensive genome scale data generation to date. With these new services, hundreds of epigenomic biomarker candidates can be discovered simultaneously. Furthermore, Zymo Research offers services for validation of biomarker candidates via targeted sequencing or qPCR.

Introduction

The **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 single-base DNA methlation coverage to **≥80% of all gene promoters and ≥85% of CpG islands** (for human samples).

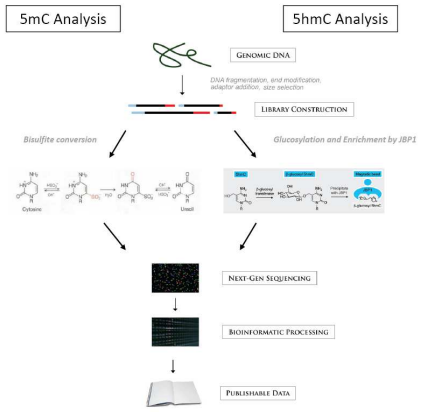
The **Whole-Genome DNA methylation Profiling Service** utilizes a streamlined library preparation and sophisticated alignment algorithms to deliver Whole-Genome Bisulfite Sequencing data that covers **>80%** of all the bases in the human genome provides DNA methylation analsyis at single-base resolution.

Also, the **Genome-wide DNA hydroxymethylation Profiling Service** provides a sensitive and accurate tool to analyze DNA hydroxymethylation at genomic level by coupling JBP-based enrichment with Next-Gen sequencing.

Features

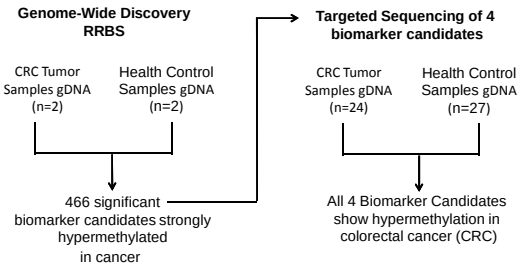
- ✓ Next-Gen sequencing based platforms for genomic profiling of 5-mC and 5-hmC
- ✓ Low DNA input
- ✓ Applicable to a broad range of sample sources (human, mouse, rat, plant, etc.)
- ✓ Streamlined workflows with comprehensive bioinformatic analysis and high quality publishable data delivery.
- ✓ Customizable, rapid turnaround at an affordable cost.
- ✓ Platforms for validation of biomarker candidates

Workflow

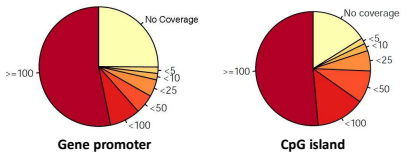


Genomic 5mC profiling

➢ **Workflow**



➢ **Genome Coverage RRBS**



Pie charts shows the overall distribution of sequencing reads coverage at gene promoters and CpG Islands for this experiment

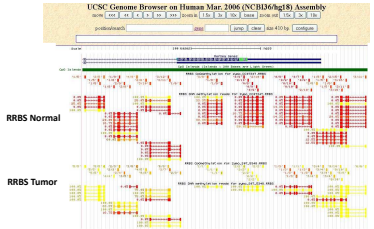
Genomic 5-mC profiling

➢ **Number of Differentially Methylation Regions**

CRC Tumor vs. Normal Tissue		
	Gene Promoter	CpG Island
Strong hypermethylation	155	311
Hypermethylation	1541	1734
Insignificant	9554	10802
Hypomethylation	2065	2283
Strong hypomethylation	260	797

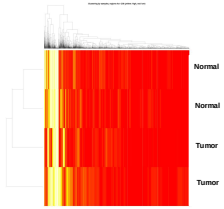
Pair-wise comparison was performed between the different samples, different DNA methyation regions were counted according to hyper/hypo methylation

➢ **UCSC genome browser track**



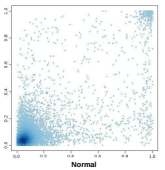
UCSC genome browser shows the sequencing reads and DNA methylation value for the samples.
Yellow = Hypermethylated; Red = Hypomethylated

➢ **Hierarchical clustering**



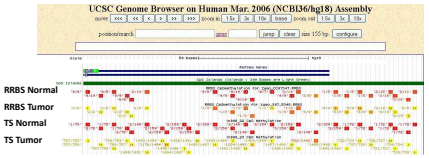
Heatmap shows the hierarchical clustering by region and sample

➢ **Correlation analysis**



Pearson correlation for pairwise data comparison between samples

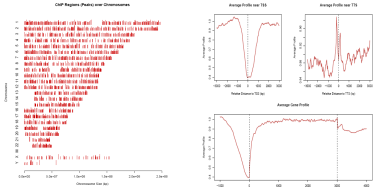
➢ **Validation**



Hypermethylated loci from RRBS screen were confirmed by targeted sequencing via 48.48 Access Array (© Fluidigm)

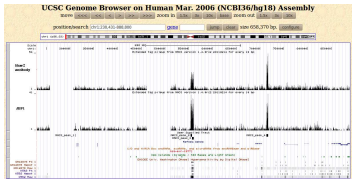
Genomic 5-hmC profiling

➢ **5hmC profiling by hmeDIP -Seq**



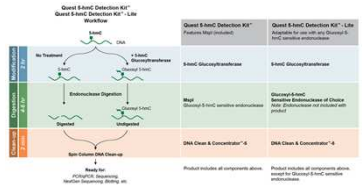
hmeDIP peaks distribution over the genome and the average hmeDIP enrichment signal s around TSS, TTS and the whole gene

➢ **UCSC genome browser track**

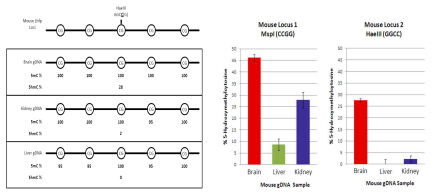


UCSC genome browser show the hmeDIP peaks by antibody and JBP

➢ **Locus-specific validation with Zymo's Quest 5-hmC Detection Kit**



➢ **DNA Methylation Profile at Lhfp loci - 5-mC and 5-hmC**



Five CpG residues were inspected by bisulfite conversion to determine DNA methylation signature. Use of Quest 5-hmC Detection Kit™ with HaeIII was used to detect 5-hmC at a single CpG sites.