

EpiQuest- A Genomic Approach for DNA Methylation and Hydroxymethylation Analysis

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ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

Abstract

DNA methylation and hydroxymethylation are some of the most important epigenetic modifications that can occur to the human genome. For instances, DNA methylation plays a vital role in the regulation of gene expression in normal cell development and aging, but also in the formation and progression of cancer and other diseases. Profiling DNA methylation and hydroxymethylation at the genome level promises large-scale identification of epigenetic biomarkers that could be applied to clinical and molecular diagnostic fields. Due to the 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 bioinformatic analysis as part of its new EpiQuest™ Methylation and Hydroxymethylation Profiling Services. These services combine next generation sequencing with Zymo's well-established epigenetic technologies and innovative bioinformatic algorithms for the most streamlined, comprehensive genome scale data generation to date. With EpiQuest... hundreds of epigenomic biomarker candidates can be discovered at once.

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 ≥75% of all gene promoters and ≥80% of CpG islands (for human samples).

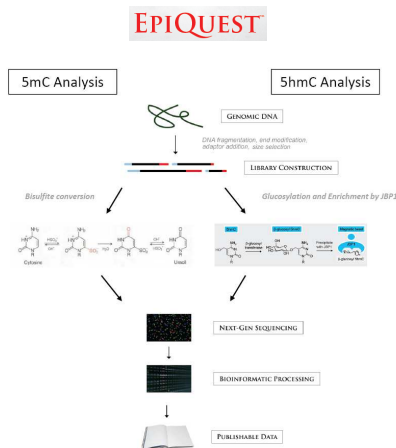
The EpiQuest™ Whole-Genome DNA methylation Profiling Service utilizes a streamlined library preparation and sophisticated alignment algorithms to deliver Whole Genome Bisulfite-Sequencing data that covers >80% of all the bases in the human genome at single-base resolution.

Also, the EpiQuest™ 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.

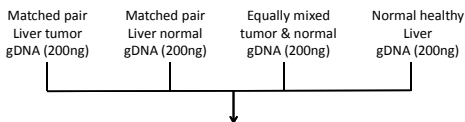
Features

- ✓ Next-Gen sequencing based platforms for genomic profiling of 5mC and 5hmC
- ✓ 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.

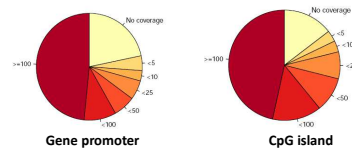
Workflow



Genomic 5mC profiling

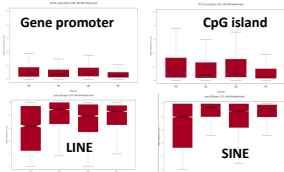


Genome Coverage :



Pie charts shows the overall distribution of sequencing reads coverage at gene promoters and CpG islands

Methylation Index



The global DNA methylation level calculated in terms of the regions (gene promoter, CpG island, LINE and SINE)

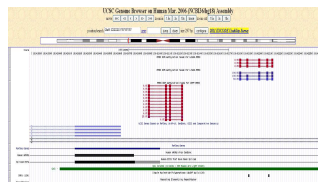
Genomic 5mC profiling

Number of Different Methylation Regions

	Gene Promoter	CpG Island	mRNA	LTR	LINE	SINE
Tumor						
Strong hypermethylation	277	253	0	0	0	16
Hypomethylation	2376	2979	0	0	0	16
Insufficient	13633	14890	143	2838	1896	8801
Hypomethylation	723	1317	9	66	5	29
Strong hypomethylation	235	706	4	739	138	1013
Normal						
Strong hypermethylation	520	1173	14	36	17	53
Hypomethylation	4734	5643	3	16	0	3
Insufficient	10080	12318	323	2544	1621	7005
Hypomethylation	273	670	2	92	5	18
Strong hypomethylation	235	603	7	626	161	179
Tumor-Normal						
Strong hypermethylation	144	302	5	1	0	3
Hypomethylation	1212	1908	7	6	0	0
Insufficient	13606	16714	154	3487	2215	8864
Hypomethylation	933	1443	8	21	1	1
Strong hypomethylation	129	373	3	152	24	175

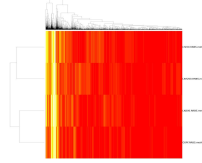
Pair-wise comparison was performed between the different samples, different methylation regions were counted according to hyper/hypo methylation

UCSC genome browser track



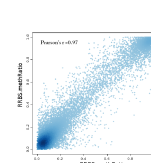
UCSC genome browser shows the sequencing reads and methylation value for the samples

Hierarchical clustering:



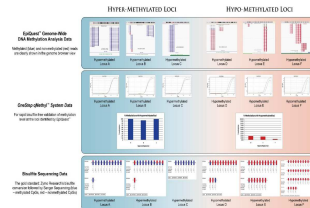
Heatmap shows the hierarchical clustering by region and sample

correlation analysis



Pearson correlation for pairwise data comparison between samples

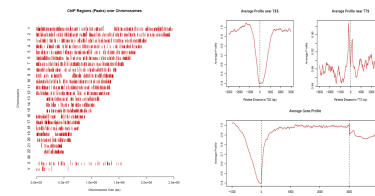
Validation



Three hyper/hypo methylated loci from EpiQuest screen were validated by our one-step qMethyl method and direct bisulfite sequencing

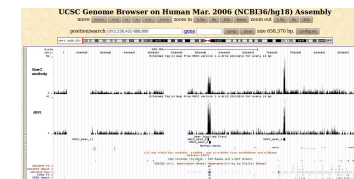
Genomic 5hmC profiling

5hmC profiling by hmeDIP-Seq



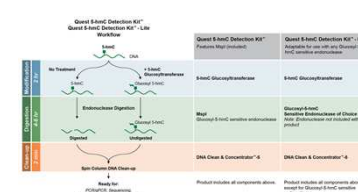
hmeDIP peaks distribution over the genome and the average hmeDIP enrichment signal s around TSS, TTS and the whole gene

UCSC genome browser track

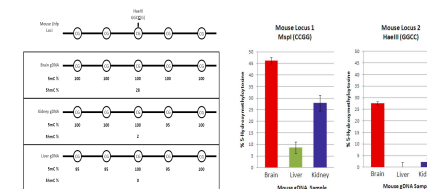


UCSC genome browser show the hmeDIP peaks by antibody and JBP

locus-specific validation with Zymo's Quest 5hmC Detection Kit



DNA Methylation Profile at Lhfp loci – 5mC and 5hmC



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