diagenode
Innovating Epigenetic Solutions

WGBS/EM-seq Service Guidelines

Whole-genome bisulfite sequencing (WGBS) and **Enzymatic Methylation (EM-seq)** are the methods of choice for obtaining a comprehensive DNA methylation profiling, evaluating the methylation patterns of nearly every CpG site of the entire genome. By comparing the proportion of unconverted and converted cytosines at the same location, the methylation levels are determined with accuracy. Genome-wide methylation sequencing (WGBS and EM-seq) provides deep insights into gene regulation, allowing identification of novel epigenetic markers and targets for disease.

In-depth DNA methylation analysis

- Very powerful solution for genome-wide biomarker discovery compatible with low input and highly fragmented cfDNA and FFPE samples
- Single-nucleotide resolution
- Evaluation of the methylation status of nearly every CpG sites of the entire genome
- Detection of global methylation patterns including outside of CpG islands and in low-CpG-density regions
- Identification of regions or even loci with differential methylation between groups using bioinformatics tools
- Identification of methylation pattern/signature predictive and discriminating different groups with Data Mining approach
- Providing a better understanding of development and disease

DNA samples should be purified in a high-quality manner and sent to Diagenode (see “Sample submission guidelines for WGBS/EM-seq” document for detailed instructions).

WGBS/EM-seq Service Workflow

1. **QC of the DNA**
   - Measurement of DNA concentration using picogreen based technology
   - Assessment of the DNA quality using the Fragment Analyzer (Advanced Analytical)

2. **Preparation of WGBS/EM-seq libraries**
   - DNA shearing on Bioruptor® Pico (if necessary) with successive profile analysis
   - Library preparation including Bisulfite conversion (Zymo) / Enzymatic conversion (NEB)
   - QC of the WGBS/EM-seq libraries (DNA concentration, analysis of the profile)

3. **Deep sequencing**
   - Samples are sequenced on an Illumina platform
   - Paired-end reads, 150bp read length (PE150)
   - 400M raw reads/sample on average (can be adapted to specific requirements)

4. **Bioinformatics analysis**

Methylation calling: alignment of sequencing data to reference genome and determination of methylation status of CpG nucleotides.

5. **Additional analysis on request:**
   - **Differential methylation analysis**: Comparison of methylation status of CpG nucleotides between sample groups.
   - **Annotation with genomic regions:** Annotation of differentially methylated CpGs or of DMRs with genomic regions such as introns, exons, enhancers (when available), promoters, intergenic regions.
   - **Gene ontology terms analysis:** Enrichment analysis on gene sets. Gene Ontology terms that are overrepresented in differentially bound regions may indicate the underlying biological processes involved.
   - **Pathway analysis:** Identify biochemical pathways in which genes associated with differentially methylated regions (or individual differentially methylated CpGs) may be overrepresented.

6. **Additional information**

   For sample preparation and sample shipment it is mandatory to follow Diagenode's guidelines. If customer samples do not meet Diagenode's quality requirements, any additional QC of new samples will be charged to the customer. Any delay in sample shipment to Diagenode's facilities might result in delaying customer's project.

   Generated files will be available for download during 1 month and stored for an additional period of 3 months on Diagenode's servers. Additional long-term storage of data is available upon request. This offer includes a one hour call to walk you through the results if needed.

   Original samples are stored at Diagenode during 4 months after project completion, but will be discarded once this time is exceeded. Return shipment of samples is available upon request.

   Any additional service which is beyond the current project scope will be charged to the customer.