

Human Methylome Service Guidelines

The **Human Methylome** is a Next-Generation Sequencing (NGS) service assay targeting DNA methylation in human samples from solid (Flash Frozen, FFPE) or liquid biopsy like plasma circulating cell-free DNA (cfDNA). Ideal for biomarker discovery and monitoring in cancer, neurodegenerative, cardiovascular, and metabolic diseases.

The assay analyzes more than 3.98M CpG sites including 84% of human CpG island sites with high coverage (150X).

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

Human Methylome 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 Human Methylome libraries

- DNA shearing on Bioruptor® Pico (if necessary) with successive profile analysis
- Library preparation including Enzymatic conversion (NEB)
- QC of the EM-seq libraries (DNA concentration, analysis of the profile)
- Targeted hybrid-capture using Human Methylome Panel (Twist Bioscience)
- QC of captured Human Methylome libraries (DNA concentration, analysis of the profile)

3. Deep sequencing

- Samples are sequenced on an Illumina platform
- Paired-end reads, 150bp read length (PE150)
- 60M raw reads/sample on average

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.

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.