

## RRBS Service Guidelines

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

### RRBS Service Workflow

#### 1. QC of the genomic DNA

- Measurement of DNA concentration using the Qubit (Thermo Fisher)
- Assessment of the DNA quality using the Fragment Analyzer (Advanced Analytical)

#### 2. Preparation of RRBS libraries

- MspI digestion
- Library preparation (ends preparation, adaptor ligation)
- Size selection
- Sample pooling
- Bisulfite conversion
- Library amplification and clean-up
- QC of the RRBS library pool (DNA concentration, analysis of the pool profile)

(Detailed instructions on the protocol used can be found on the manual of Diagenode’s “Premium RRBS kit” <https://www.diagenode.com/documents/premium-rrbs-kit-manual-24rxns>)

#### 3. Deep sequencing

- Samples are sequenced on an Illumina® platform, paired-end reads, read length 50 bp (PE50)
- 40 million raw reads on average are obtained per sample when pooling 10 samples

#### 4. Bioinformatics analysis

Quality check, alignment of bisulfite sequencing data to reference genome and determination of methylation status of CpG nucleotides (methylation calling) is included.

**Provided files:**

- Report with sequencing and CpG methylation statistics
- FASTQ files with the original raw reads
- FastQC and trimming reports for the quality of the sequenced reads
- Alignment files in BAM format
- Alignment and methylation reports
- Methylation files of covered Cytosine in bedGraph

#### 5. Additional bioinformatics analysis on request

- **Differential methylation analysis:** Identification and annotation (human, mouse, rat and drosophila) of differentially methylated CpG and region (DMR) between sample groups.

**Provided files:**

- Report with summary of differential methylation analysis and plots
  - Files containing the differentially methylated CpGs and breakdown of those positions in regional analysis (CpG islands, shelves, shores and open sea) and annotations (CpGs in exons, introns, promoters and intergenic regions) for human, mouse, rat and drosophila
  - File containing differential methylated region (DMRs)
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- **Annotation with genomic regions:** Regional analysis and annotation of methylated CpGs in CpG islands, shelves, shores, open sea and in introns, exons, promoters, intergenic regions for human, mouse, rat and drosophila.

- **Gene ontology terms analysis:** Enrichment analysis on gene sets. Gene Ontology terms that are overrepresented in methylated CpGs may indicate the underlying biological processes involved.
- **Pathway analysis:** Identify biochemical pathways in which genes associated with methylated CpG 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.