Targeted DNA methylation service guidelines

Targeted DNA methylation allows for quantitative analysis of cytosine methylation at known and precise locations throughout the genome.

Depending on the size of the region of interest, several options are available. All techniques mentioned here below rely on bisulfite or enzymatic conversion and sequencing, in order to quantitatively detect the methylation status of the target regions with single nucleotide resolution.

Based on project specifications and requirements we will provide guidance on the most suitable technology for your project.

1. Targeted DNA methylation Service Workflow using Twist custom probes technlogy

1.1. QC of the DNA

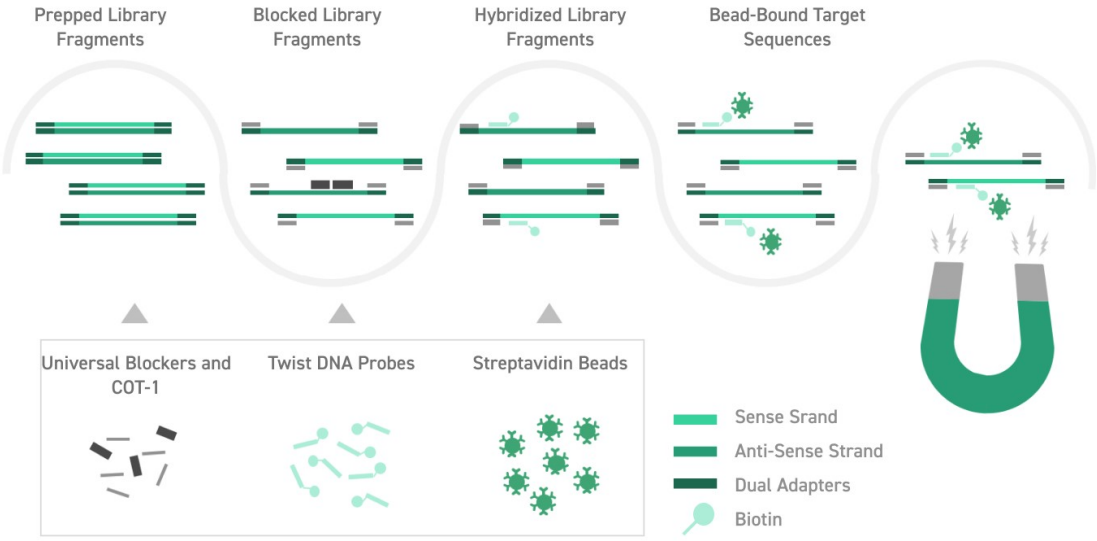
- Measurement of DNA concentration using picogreen based technology
- Assessment of the DNA quality using the Fragment Analyzer (Advanced Analytical)

1.2. Preparation of libraries

- DNA shearing on Bioruptor® Pico (if necessary) with successive profile analysis
- Library preparation including Enzymatic conversion (NEB) or Bisulfite conversion followed by library preparation
- QC of the libraries (DNA concentration, analysis of the profile)
- Targeted hybrid-capture using Custom Panel (Twist Bioscience)
- QC of captured libraries (DNA concentration, analysis of the profile)

How does it work?

Following DNA shearing, DNA samples are either processed using Bisulfite conversion of unmethylated cytosine and library preparation or undergoes EM-seq preparation to enzymatically convert the unmethylated cytosine and prepare the libraries.



Most adapted for projects with
1000 probes 50kb to 1M probes 50Mb (CpGs)
At least 96 samples

2. Targeted DNA methylation Service Workflow using Qiagen Single Primer Extension technology

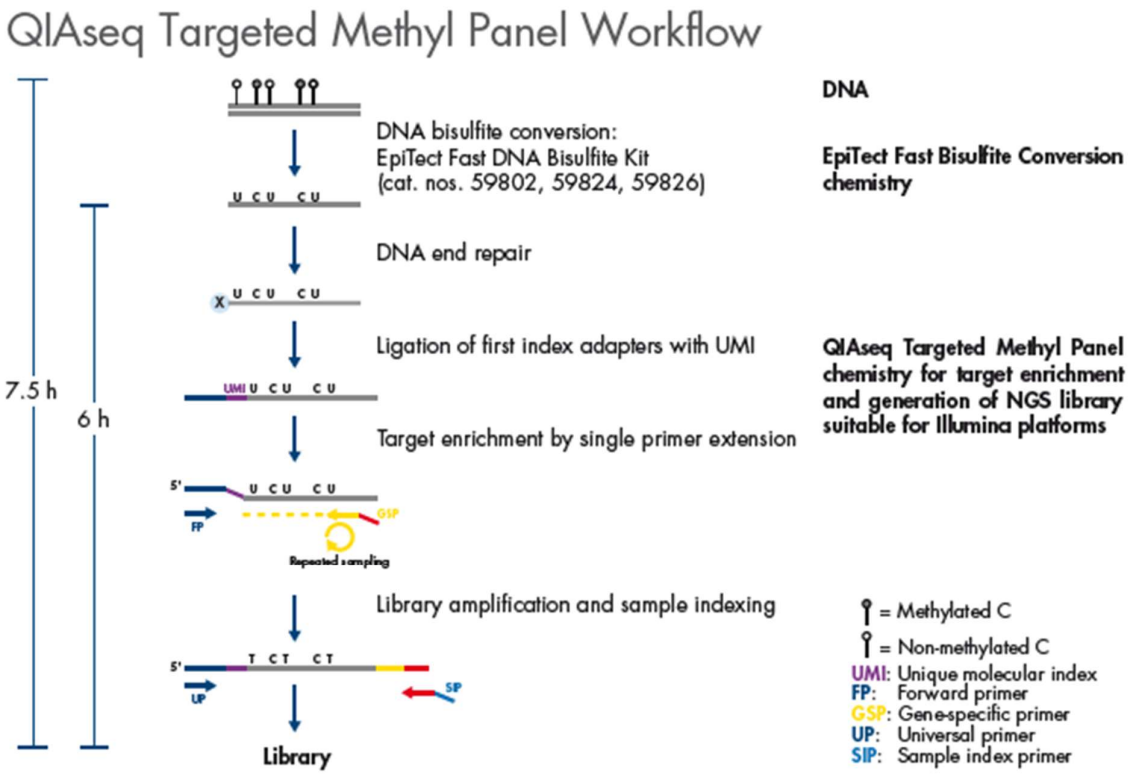
2.1. QC of the DNA

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

2.2. Preparation of libraries

- DNA shearing on Bioruptor® Pico (if necessary) with successive profile analysis
- Bisulfite conversion followed by library preparation
- Library preparation on regions of interest using custom primers
- QC of the targeted libraries (DNA concentration, analysis of the profile)

How does it work?
Following DNA shearing, DNA samples are processed using Bisulfite conversion of unmethylated cytosine and library preparation during which only the regions of interest are specifically enriched.



QIAseq Targeted Methyl Panel workflow.

Most adapted for projects with
Mininum 50 primers
At least 96 samples

3. Bioinformatics analysis

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

4. 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.

5. 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.