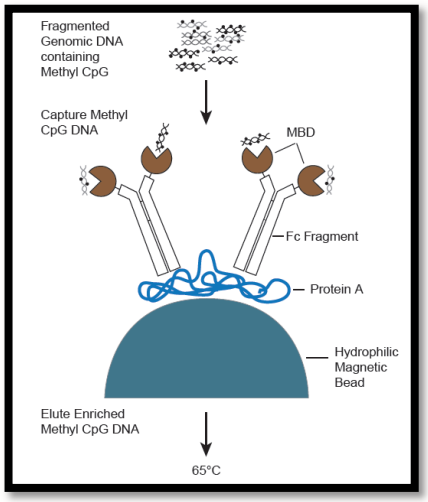
Methylome analysis

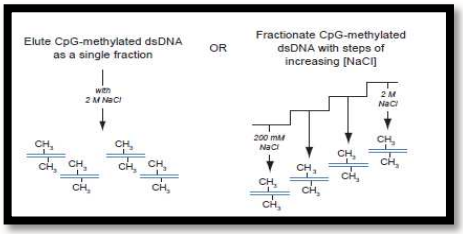
Aims

DNA methylation is an important biochemical process involved in the development and cellular differentiation of superior organisms. In adult somatic tissues, DNA methylation typically occurs in a CpG dinucleotide context, grouped in islands (CpG islands) at the 5' of regulatory regions, in CpG shores (<2 kb flanking the islands) and in CpG shelves (<2 kb from the shores). The methyl cytosine distribution along the genome has an epigenetic role controlling genes expression and alterations of DNA methylation pattern (hypermethylation and hypomethylation) have been recognized as an important component of many disease processes such as cancer, genetic diseases, cellular senescence and aging.

Procedure

Methyl enrichment procedure can be set-up and performed in the customer laboratory or in Genomnia when specifically requested. It is mandatory that Genomnia receives genomic DNA enriched for methylated double strand regions (dsDNA) compatible with the construction of Ion Torrent fragment libraries. GENOMNIA staff will be always available for technical help and scientific advices. The figure below summarizes the procedure.





The MBD pull-down procedure uses the properties of the methyl-CpG-binding domain (MBD) present in different protein components of the chromatin to capture DNA regions with CpG dinucleotide methylated.

In detail, as shown in the image above, in the system selected by Genomnia methylated DNA is isolated by binding to the methyl-CpG binding domain of human MBD2 protein fused to the Fc tail of human IgG1 (MBD2-Fc). The latter is coupled to paramagnetic hydrophilic protein A beads. Two Fc domains can be bound to one site on protein A and as the Fc fragment is a dimer, four MBD2 domains are exposed to the solvent per molecule of protein A, increasing the relative equilibrium constant 100-fold.

The genomic DNA is initially reduced to fragments of size 50-500 bp, with a peak around 200 bp. Subsequently, the CpG methylated regions are stably and selectively captured from the biochemical mechanism described above. The enriched double strand DNA is then eluted in a small volume of nuclease-free water by incubation at 65°C and used for the production of a fragment library. The sequencing is performed on the Ion Torrent S5 platform, with 200 bp reads and a sequencing depth of around 20 million per sample.

Sample amount

Amounts listed in the table depend on each protocols' enrichment yield. Minimal amounts are reported just as an indication of the lower limits of the technique and as a basis for discussion to meet the requirements of very particular experimental conditions

Starting material	Quantità (ng)
Genomic DNA	500 - 5000
Methylated DNA, MBD method	10 - 100

Bioinformatic Analysis

Bionformatic analysis of Methilseq experiments is available at two different levels.

In the first level bioinformatic analysis [MBD-BF01], we first align the sequence read to the reference genome and we elaborate the mapping statistics. We generate quality metrics and enrichment diagnostics in textual and graphical format and we identify with a proprietary procedure based on the Bioconductor MEDIPS package the methylated genome regions. We also elaborate differentially methylated regions according to the project-associated comparisons. Gene-level annotation of the differentially methylated regions is performed, followed by functional analysis of the differentially methylated genes.

In the second level of bioinformatic analysis [MBD-BF02], we perform all the analyses described in MBD-BF01. In addition, we estimate the methylation of the transposable sequences at elevated redundancy level (LINE, SINE) and we perform differential analysis of methylation in repeat families according to the project-associated comparisons.

Ordering information

Item	Catalog N.
QC: Quality and size control of MBD/CHIP enriched DNA	DNA05
QC: Quality control of DNA preparations	DNA06
MBD-enrichment from genomic DNA	MBD
Barcoded DNA library preparation	LDb
Forward sequencing 200 bp tags with barcode	SEQI200B
Methylation Bioinformatic Analysis	MBD-BF01
Methylation Bioinformatic Analysis (advanced)	MBD-BF02

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