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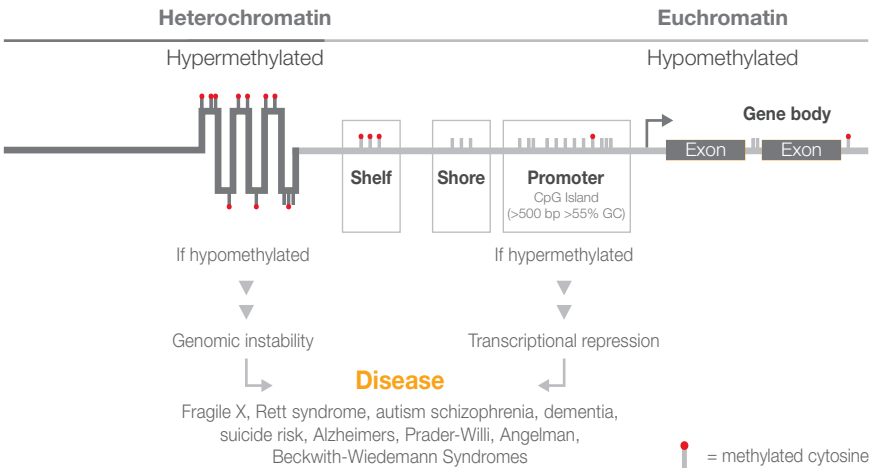
Field Guide to Methylation Methods

Methylation and Cellular Processes

Cytosine methylation (5-mC) has a significant effect on gene expression and chromatin remodeling. Cytosine methylation and hydroxymethylation (5-hmC) regulate spatial and temporal gene expression, and are critical for embryonic development and cellular differentiation processes.

- **Modulation of gene expression.** Cytosine methylation in promoter regions often reduces gene expression. 88% of active promoters are associated with unmethylated CpGs in mammals. Hydroxymethylation often correlates with increased gene expression
- **Change in chromatin structure.** Heterochromatin formation is initiated by hypermethylation. Methyl binding domain (MBD) proteins specific for 5-mC recruit histone-modifying and chromatin-remodeling proteins. Abnormal hypomethylation of heterochromatin (normally hypermethylated) is linked to chromosomal instability and loss of imprinting. Controlled changes in chromatin structure are responsible for selective X chromosome inactivation and suppression of transposable elements.
- **Cellular differentiation.** Methylation change usually drives one-way differentiation; that is, differentiated cells do not typically revert to stem cells.
- **Onset of diseases.** Perturbation of any of the above processes may result in disease.

Perturbation of Methylation



Methylation Enzymes

Mammalian

DNMT1 (DNA methyltransferase) isoforms methylate hemimethylated CpGs, maintaining methylation patterns during DNA replication.

DNMT3a and DNMT3b, *de novo* methyltransferases that set up DNA methylation patterns early in development; can methylate unmethylated and methylated DNA.

DNMT3L unknown; facilitates *de novo* methyltransferase activity.

TET (Ten eleven translocation) enzymes involved in methyl group oxidation with the production of 5-hydroxymethylation as an intermediate.

Plant

DRM2 (RNA directed DNA methylation) homologous to DNMT3.

MET1 (methyltransferase 1) homologous to DNMT1.

CMT3 unique to plants. Function for other methyl transferases unknown.

Bacteria

Dam (DNA adenine methyltransferase), methylates 'A' in GATC, key role in mismatch repair, DNA replication timing, and gene regulation. Independent of restriction modification systems.

Dcm (DNA cytosine methylase) produces 5-mC in CCAG and CCTGG sites.

EcoKI methylates adenine in AAC(N6)GTGC and GCAC(N6)GTT.

Glossary

5-Methylcytosine (5mC) Formed by addition of -CH3 to N5 position of cytosine. 70–80% of CpG dinucleotides are methylated (high proportion in repetitive elements). In plants, cytosine methylation is observed at CpG, CpHpG, and CpHpH (H = nucleotide other than guanine).

5-Formylcytosine Oxidation product of 5-hmC. Detected in mouse embryonic stem cells.

Base modification Addition of methyl- or other groups to nucleic acid bases. In addition to 5-methylcytosine, other common modifications are 7-methylguanosine (5'-cap for RNA), and 6-methyladenosine (common RNA modification.)

CpG island Defined as regions > 500 bp, > 55% GC and expected/observed CpG ratio of > 0.65. 40% of gene promoters contain islands.

CpG shelves ~4Kb from islands.

CpG shores ~2Kb from islands, > 75% of tissue-specific differentially methylated regions found in shores. Methylation in shores shows higher correlation with gene expression than CpG islands.

Differentially methylated regions (DMR) Cell-, tissue-, and condition- specific differences in methylation.

Enhancer A short region of DNA that can activate transcription and is often regulated by methylation.

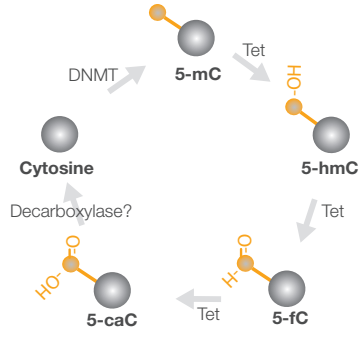
Expected/observed CpG ratio The human genome contains 25% of the expected number of C-G pairs due to spontaneous deamination of meC to T over evolutionary time scales.

Genomic imprinting An epigenetic process causing genes to be expressed only from one of the parental chromosomes.

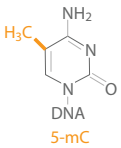
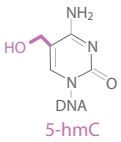
Hypermethylation Most cytosines are methylated

Hypomethylation Most cytosines do not have 5-mC. Euchromatin and active gene promoters are hypomethylated.

Cytosine Modifications



Primary Methods For Detecting Cytosine Modifications

Method	DNA Preparation	Considerations
WGBS-Seq Whole-Genome Bisulfite Sequencing with TruSeq® DNA Methylation	Convert nonmethylated Cs to U with sodium bisulfite, which are read as T. 5-mC and 5-hmC are protected from conversion and read as C. Sequence with random-primer extension, 2 × 75–100 bp reads.	Compatible with most species, covers all genes and > 38 M CpGs in humans Requires > 90 Gb sequencing Cost: US $5000/sample*
Methylation Array Infinium® MethylationEPIC	Bisulfite convert DNA to find C-to-T changes at defined genomic positions by the Infinium assay	Samples 95% of CpG islands, > 850K CpGs, 99% RefSeq promoters, coverage across the gene Cost: US $330/sample*
RRBS-Seq Reduced Representation Bisulfite Sequencing	Digest DNA with *Msp*I and isolate 100–150 bp fragments, representing > 85% CpG islands. Bisulfite treat fragments and sequence using 1 × 75 bp reads.	Samples 50% of RefSeq promoters with a focus on CpG-rich regions for ~ 85% of CpG islands Requires 3-5 Gb/sample Cost: US $300–350/sample*
 **MeDIP** Methylated DNA Immunoprecipitation	Sonicate DNA to 100–300 bp, end-repair, ligate to adapters, and denature. Capture 5-mC containing fragments with anti-5-mC magnetic beads, and sequence using 1 × 75–100 bp reads. May be combined with bisulfite conversion.	5-mC detected at ~150 bp resolution. Bias towards hypermethylated regions. Requires ~60 M reads, ~5 Gb/sample Cost: US $300–360/sample*
 **Tab-Seq** Tet-Assisted Bisulfite Sequencing	β-glucosyltransferase adds glucose to 5-hmC but not 5-mC. Tet enzymes oxidize 5-mC to 5-caC, and converts to U on bisulfite treatment but glucosylated 5-hmC cannot be oxidized. hmC will be read as C and mC will be read as T.	Detects genome-wide 5-hmC at single-base resolution Requires > 90 Gb sample Cost: US $5,000–6,000/sample*
Tab/OxBS Array hmC analysis on a methylation array	TAB or OxBS converted DNA is hybridized to the Infinium Methylation array for targeted analysis of > 850K genomic locations.	Detects 5-hmC at single-base resolution at 95% of CpG islands and 99% of RefSeq promoters Cost: US $430/sample*

*Cost is provided for guidance only, may not reflect your actual cost.

Techniques in Brief

COBRA Combined Bisulfite followed by Restriction Analysis. RE recognition affected by bisulfite treatment.

C-Subtraction *Mse*I (methylation independent) digestion, ligation to linkers, then digestion with methylation sensitive *Bst*UI or HpaII to reduce unmethylated DNA.

HELP *Hpa*II enrichment by ligation PCR. Differential restriction of methylated and unmethylated CpG sites.

MCA Methylated CpG island amplification. DNA cut with *Sma*I (blunt ends, cannot cut meC) followed with *Xma*I (sticky ends, cuts meC and C). Sticky end adapters select for meC fragments.

MeDIP Methylated DNA immunoprecipitation with anti-5-mC methylcytosine antibody

Methyl Light Methylation specific qPCR (see MSP).

Methylation Trapping of methyltransferases onto DNA with 5-Aza- 2'-deoxycytidine (decitabine) followed by immunoprecipitation.

MIRA Methylated CpG island recovery assay. Capture with MBD2b and MBD3L1 protein heterodimer.

MSDK Methylation-specific digital karyotyping. Cleavage with methylation sensitive *Asc*I. Sequence tags sequenced and mapped like SAGE.

MSP Methylation-specific PCR on bisulfite converted DNA with 2 sets of primers to amplify either -C or -T base.

OxBS K-perruthenate oxidizes only 5-hmC to 5fC that is converted to U by bisulfite

RLGS Restriction Landmark Genome Scanning with 2D gel of methylation-sensitive *Not*I and *Asc*I restricted DNA

RRHP Reduced Representation Hydroxymethylation Profiling.

RRBS Reduced Representation Bisulfite Sequencing.

Tab-Seq Tet-assisted bisulfite sequencing of 5-hmC.

WGBS-Seq Sodium bisulfite conversion of C to T. mC and hmC are not converted and are read as C by whole-genome, next-generation sequencing.

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