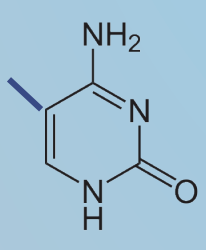
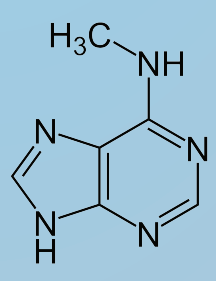
CD Genomics
The Genomics Services Company

5mC/5hmC Identification
with Second- and Third-Generation Sequencing

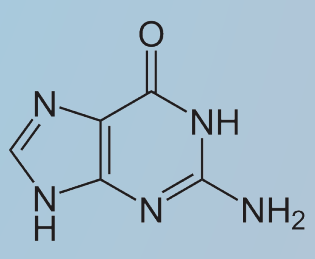
DNA methylation, one of the first identified forms of epigenetic modification for genes, is a major epigenetic factor in research studies. The dynamic DNA methylation-demethylation process plays a critical role in controlling gene expression and cell development. DNA methylation can induce gene inactivation, while demethylation can reactivate gene activity and expression. The major forms of methylation are 5-methylcytosine, N6-methyladenine, and 7-methylguanine.



5-Methylcytosine



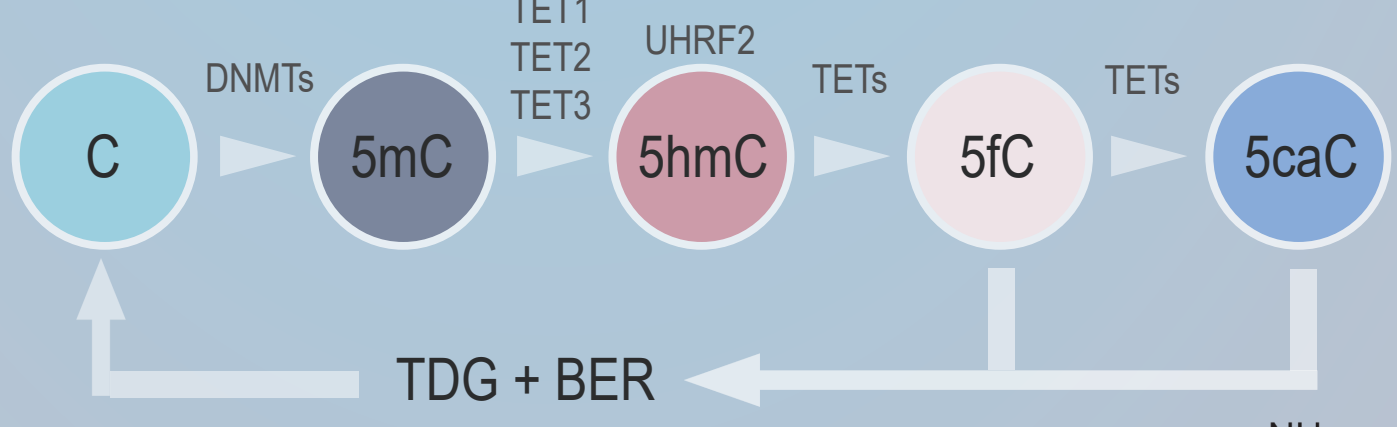
N6-Methyladenine



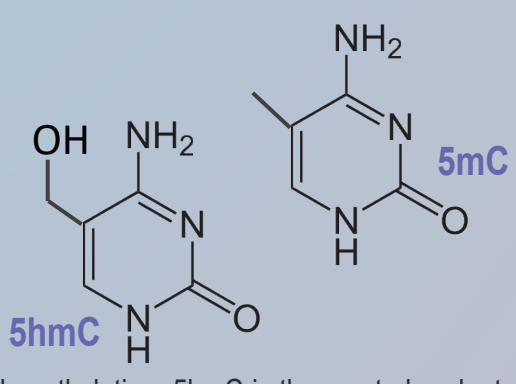
7-Methylguanine

5mC and 5hmC

Cytosine DNA methylation (5mC) groups introduced by DNA Methyl-Transferases can be iteratively oxidized to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC) by the action of Tet Methylcytosine Dioxygenases. DNA methylation and hydroxymethylation at the 5-position of cytosine are essential epigenetic alterations that play vital roles in gene expression regulation and cell differentiation.



DNA methylation typically represses gene transcription. The oxidation derivatives of 5mC could impact biological functions by altering DNA properties or recognizing potential reader proteins, and this dynamic process is critical for cellular reprogramming and development. Aberrant methylation is a common feature of many human disorders, including cancer, autoimmune diseases, heart diseases, skin diseases, etc. Thus, this dynamic process has been considered an important factor in studying tumorigenesis mechanisms and discovering therapeutic targets.



Hydroxymethylation, on the contrary, activates gene expression or prompts DNA demethylation. 5hmC is the most abundant component in vivo among the three 5mC oxidative derivatives and can be identified in almost all mammalian tissues and cells. 5hmC is now regarded as an epigenetic alteration and is one of the medium states of DNA demethylation. 5mC and 5hmC genome-wide mapping uncover the alternated areas, which are crucial for elucidating cellular mechanisms.

Sequencing Technologies for 5mC/5hmC Identification

With the development of next-generation sequencing technology (NGS) and third-generation sequencing (TGS), modifications including but not limited to 5mC and 5hmC can be analyzed genome-wide. These technologies are so-called "DNA methylation sequencing" and allow access to genetic information beyond traditional genomics research. DNA methylation sequencing methods can be divided into four main categories according to their principles.

- Bisulfite sequencing
- Restriction endonuclease-based sequencing
- Targeted enrichment methylation sequencing
- Third-generation sequencing

Representative methods include Bisulfite sequencing (BS-Seq), oxidative bisulfite sequencing (oxBS-Seq), Tet-assisted bisulfite sequencing (TAB-Seq), Oxford Nanopore and PacBio single-molecule real-time (SMRT) sequencing, which allows methylation analysis at a single-base level. Depending on the sample processing, these methods can achieve detection of 5mC and/or 5hmC.

BS-Seq

BS-Seq treats DNA with bisulfite to deaminate unmethylated cytosine into uracil before sequencing. Then bisulfite-treated and untreated sequenced samples are compared to uncover methylated sites. However, BS-Seq was not able to distinguish between 5mC and 5hmC.

hMeDIP-Seq
MeDIP-Seq

MeDIP-Seq and hMeDIP-Seq are commonly used to study 5mC or 5hmC modification. Specific antibodies can be used to study cytosine modifications. If using 5mC-specific antibodies, methylated DNA is isolated from genomic DNA via immunoprecipitation, followed by incubation, DNA purification, and sequencing.

oxBS-Seq

In the oxBS-Seq process, 5mC was retained while 5hmC was oxidized and then deaminated to uracil for sequencing. By comparing the sequencing of oxidized and untreated samples, 5mC and 5hmC can be distinguished from each other at the single-base level.

SMRT

Single-molecule real-time sequencing can be adapted to detect 5mC and 5hmC. In SMRT sequencing, DNA methylations were presented with corresponding kinetic information. Thus, DNA methylations, including 5mC and 5hmC, can be sensitively and quantitatively detected by comparing kinetic signatures.

Nanopore

With α-hemolysin (αHL) or mycobacterium smegmatis porin A (MspA) protein nanopore, current trace changes according to the blockage of nanopore with nucleotides. Thus, DNA methylation (including 5mC and 5hmC) is detected through the different numbers of nucleotides.

5mC/5hmC Sequencing Methods Comparison

	BS-Seq	oxBS-Seq	TGS
Deamination	C→U	C/5hmC→U	N/A
Detection	5mC + 5hmC	5mC	5mC, 5hmC
Resolution	Single base-pair	Single base-pair	Single base-pair
Features	◆Highly integrated single-base resolution DNA methylation patterning; ◆Providing insights into gene cell-fate commitment and reprogramming, as well as gene regulation; ◆Identifying novel epigenetic markers and targets for disease; ◆Unable to distinguish between 5mC and 5hmC	◆CpG and non-CpG methylation at single-base resolution throughout the genome; ◆Covering 5mC in dense and less thick repeat areas; ◆Clearly distinguishing between 5mC and 5hmC; ◆Needs to be integrated with BS-Seq to differentiate and evaluate C, 5mC, and 5hmC.	◆Both Nanopore sequencing and single-molecule real-time sequencing can be adapted to detect 5mC and 5hmC; ◆Quantify 5mC or 5hmC by detecting the value of electrics; ◆In SMRT sequencing, different kinetics information represents different DNA methylations; In Nanopore sequencing, different nucleotides lead to distinct current trace changes when a nucleotide goes through and blocks the nanopore; ◆Single base sensing.

CD Genomics aims at providing the research community with high-quality Next Generation Sequencing, PacBio SMRT sequencing, and bioinformatics services. Our long-standing experience and advanced platforms allow us to offer a comprehensive service package from project consultation, experiment design, sequencing, to bioinformatics analysis of epigenomics. We strive to provide the most suitable scheme for your project.

Contact CD Genomics for more inspiration and service content.

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