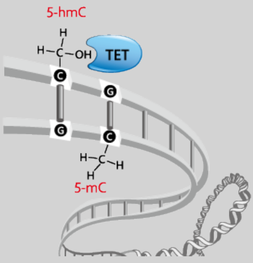
CD Genomics
The Genomics Services Company

SEQUENCING-BASED METHODS FOR PROFILING DNA METHYLATION

Introduction to DNA Methylation

DNA methylation, one of the most studied epigenetic modifications, refers to the addition of a methyl group to the fifth carbon of cytosine (C) catalyzed by DNA methyltransferases (Dnmts), forming 5-methylcytosine (5mC). DNA methylation predominantly occurs in CpGs but is also found in non-CpG contexts. DNA methylation is heritable and has been associated with multiple cellular processes, including transcriptional repression, transposon inactivation, X chromosome inactivation, embryonic development, genomic imprinting, and the alteration of chromatin structure.



Next-Generation Sequencing

Next-generation sequencing (NGS) has been widely used for genome-wide DNA methylation analysis, such as whole genome bisulfite sequencing (**WGBS**), reduced representation bisulfite sequencing (**RRBS**), and Methylated DNA immunoprecipitation sequencing (**MeDIP-Seq**).

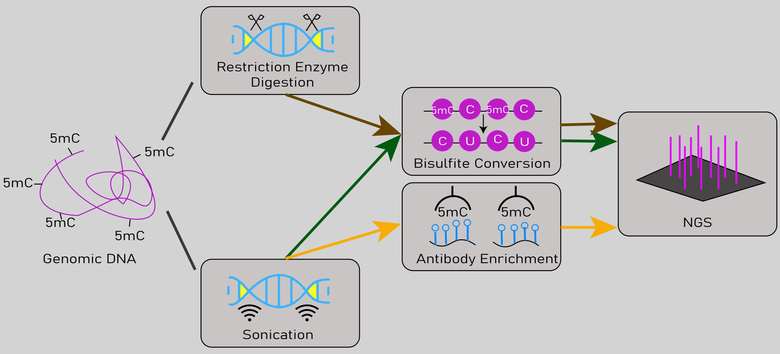


Figure 1. The workflow of WGBS, RRBS, and MeDIP-seq.

Table 1. A comparison among WGBS, RRBS, and MeDIP-seq.

Methods	Strength	Weakness	Resolution	Quantitative Nature	Cost
WGBS	Evaluate methylation state of almost every CpG sites	-High cost -Substantial DNA degradation after bisulfite treatment -Cannot discriminate between 5mC and 5hmC	Single base	Digital	High
RRBS	-High CGI coverage -High sensitivity -Cost-effective comparing to WGBS	-May exhibit a poor coverage at intergenic and distal regulatory elements -Substantial DNA degradation after bisulfite treatment -Limited to regions in proximity to enzymes' recognition sites -Cannot discriminate between 5mC and 5hmC	Single base	Digital	Moderate
MeDIP-seq	-Cost-effective -No mutation introduced -Specific to 5mC/5hmC depending on the antibody specificity -More sensitive in regions with low CpG density than MBDCap-Seq	-Biased toward hypermethylated regions -Do not identify individual 5mC sites -Inability to predict absolute methylation level	~100 bp	Abundance	Moderate

Third-Generation Sequencing

Emerging third-generation sequencing technologies, including PacBio single-molecule real-time sequencing (SMRT) and Oxford nanopore sequencing, have been recently applied in epigenetics research.

They have many **advantages** over NGS:

- Minimal chemical modification during library preparation;
- The requirement for DNA amplification is eliminated;
- Reduced requirement for input DNA;
- The ability to generate longer reads;
- The ability to directly detect different types of epigenetic modifications.

PACBIO®

PacBio SMRT allows the direct detection of DNA modifications by monitoring the activity of DNA polymerase during the incorporation of different fluorescently labeled nucleotides into complementary DNA strands. The direct detection of various modifications involves the measurement of the kinetics variation in the time between base incorporations.

Oxford NANOPORE Technologies

In **nanopore sequencing**, single-stranded DNA is pulled by a phage DNA polymerase through a bacterial pore in single-nucleotide steps, and the ion current through the pore is recorded. It can distinguish C from 5mC and 5hmC based on differences in the current traces.