Methyl-Seq Solutions

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Truly accurate interrogation of DNA methylation and hydroxymethylation

TrueMethyl® oxBS conversion technology is the breakthrough approach of oxidative bisulfite conversion to obtain accurate 5-methylcytosine (5mC) identification for NGS. In addition, this method enables interrogation of 5-hydroxymethylcytosine (5hmC), a modified base that is not assayed by traditional bisulfite conversion approaches. Differentiating these modifications is key since each can have different effects on gene expression[1]. When used in conjunction with the Ovation Ultralow Methyl-Seq and Ovation RRBS Methyl-Seq systems, the result is an accurate, low-cost, solution for whole genome bisulfite sequencing (WGBS) or reduced representation bisulfite sequencing (RRBS).

Why use NuGEN Systems?

1. **Ovation Ultralow Methyl-Seq:** Methylation detection from as little as 10 ng input enable samples such as cfDNA[2].
2. **Ovation RRBS Methyl-Seq:** Inclusion of diversity adaptors eliminates the need for PhiX spike-in, reducing sequencing costs without sacrificing data.
3. **TrueMethyl® oxBS**[3] conversion for accurate detection of both 5mC and 5hmC.

Features

- **DimerFree** library preparation
- Integrated oxidative bisulfite conversion
- Uniform whole genome coverage
- Reproducible RRBS enrichment
- PhiX-free sequencing with diversity adaptors
- Detection of 5mC and 5hmC with the same workflow
- Directional libraries
- **All in one:** From DNA to sequencable library in a single kit.

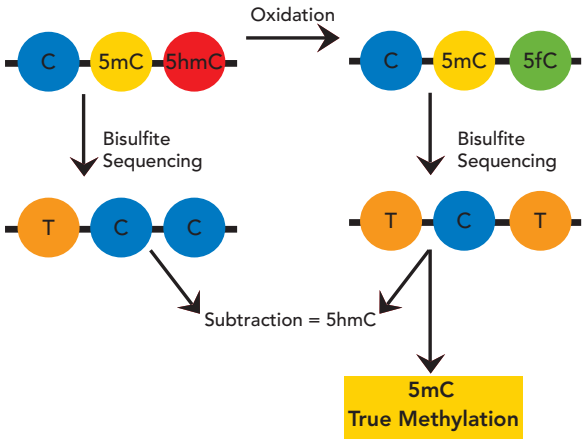

Figure 1: Achieve complete measurements of methylation with oxBS. The schematic (Left) shows classic bisulfite conversion, which creates a library that detects both 5mC and 5hmC. Processing with the oxidation of 5hmC (Right) generates a bisulfite-convertible base that leads to detection of only 5mC. Differences between the libraries can then be used to deduce the sites of 5hmC modifications.

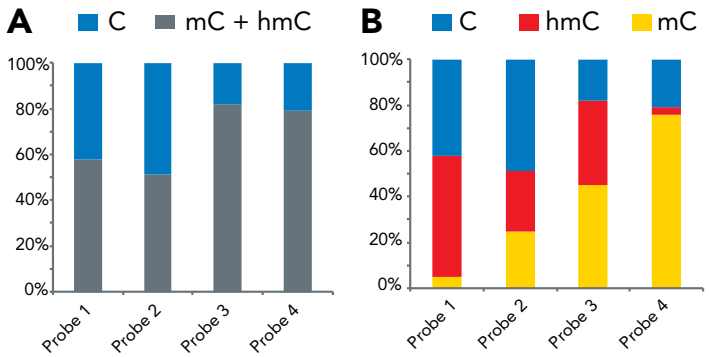

Figure 2: TrueMethyl® oxidative bisulfite conversion enables accurate measurements of methylation. A) Standard bisulfite conversion cannot distinguish between 5mC and 5hmC, resulting in a single readout. B) TrueMethyl oxidative bisulfite conversion provides an accurate methylation profile of each.

NuGEN®

Ovation Ultralow Methyl-Seq Library Systems

- Unique **DimerFree** ligation chemistry eliminates adaptor dimer artifacts even at low inputs, enabling access to a broader range of samples
- Simple add and incubate protocol with easy purification steps
- Directional libraries reduce the computational time and expense required for alignment
- Investigate multiple epigenetic modifications using the same workflow

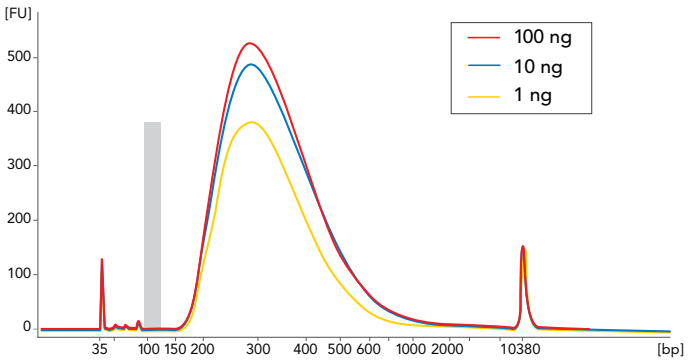

Figure 3: Obtain high quality results regardless of input. WGBS libraries were generated from 100 ng, 10 ng and 1 ng of human genomic DNA. Bioanalyzer analysis indicates no adaptor artifacts (grey box) regardless of input and without the need for adaptor dilution during library construction.

Ovation RRBS Methyl-Seq System

- Unique **DimerFree** ligation chemistry eliminates adaptor dimer artifacts
- Streamlined, single day protocol
- Built in sequence diversity eliminates the need for PhiX
- Integrated molecular tag (N6) enables removal of non-unique reads from the dataset
- Methylation data concordant with whole genome bisulfite sequencing data

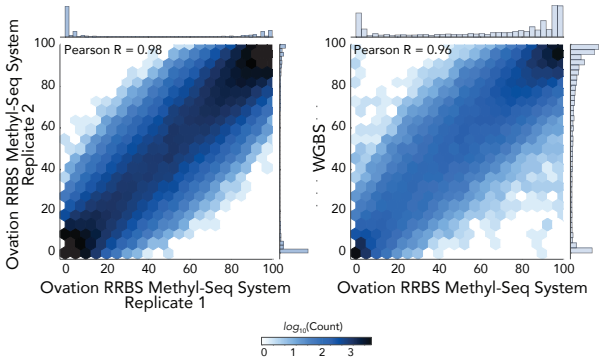

Figure 4: Obtain results that are highly reproducible and concordant. Concordance in methylation levels for CpG's covered at 20x or greater depth using 25 ng of IMR90 gDNA between Ovation RRBS Methyl-Seq System technical replicates (Left) and between Ovation RRBS Methyl-Seq System versus WGBS (Right).

Ordering Information	No. of Reactions	No. of Barcodes	Part No.
Ovation Ultralow Methy-Seq DR Multiplex System 1-8, with TrueMethyl® oxBS	32	8	0535
Ovation Ultralow Methy-Seq DR Multiplex System 9-16, with TrueMethyl® oxBS	32	8	0536
Ovation RRBS Methyl-Seq System 1-16, with TrueMethyl® oxBS	32	16	0553
TrueMethyl® oxBS module	32	--	0414

References

1. Pastor et al., Genome-wide mapping of 5-hydroxymethylcytosine in embryonic stem cells. Nature. 2011.
2. Legendre et al., Whole-genome bisulfite seuqencing of cell-free DNA indentifies signature associated with metastatic breast cancer. Clin Epigenetics. 2015.
3. Booth M. J., et al. Quantitative sequencing of 5-methylcytosine and 5-hydroxymethylcytosine at single-base resolution. Science. (2012).

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