Methyl-Seq: library preparation and bisulfite sequencing workflows.

PRODUCT SHEET

Solutions for methylation studies

Our product line comprises library preparation and bisulfite sequencing conversion tools for a broad range of applications, including NGS and methylation arrays. The proprietary TrueMethyl® oxBS chemistry provides insight into epigenetic modifications and enables both 5mC and 5hmC to be interrogated in a single protocol. Methyl-Seq products feature DimerFree® ligation technology to enable simple, streamlined workflows for a broad range of samples, including intact genomic DNA, FFPE DNA, cell-free DNA and ChIP DNA.

The Ultralow Methyl-Seq™ library preparation kit provides a comprehensive approach for the detection of DNA methylation across the entire genome from many different sample types. The Ovation® RRBS Methyl-Seq kit enables a focused and cost-efficient method to assess the methylation state of regions with high CpG density using reduced representation bisulfite sequencing (RRBS).

Why use Tecan's solutions for your epigenetic research?

1. **Ultralow Methyl-Seq:** Methylation detection from as little as 10 ng input enables studies of samples such as cfDNA[1]
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[2]** conversion for accurate detection of both 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC)

TrueMethyl oxBS

Our breakthrough TrueMethyl oxBS technology accurately detects 5mC and 5hmC, a modified base that is not assayed by traditional bisulfite conversion approaches. Differentiating these modifications is key, since they can have different effects on gene expression.[3] When used in conjunction with the Ultralow Methyl-Seq and Ovation RRBS Methyl-Seq systems, the result is an accurate, low cost solution for whole genome bisulfite sequencing (WGBS) or RRBS.

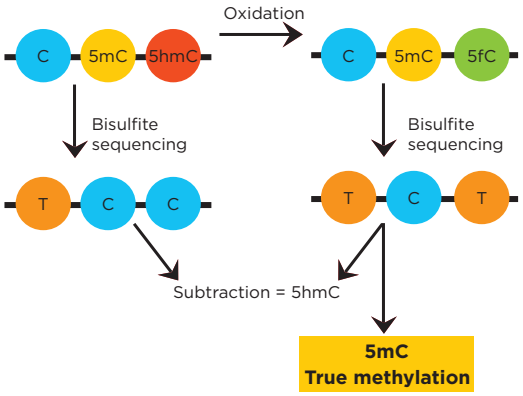

Figure 1: **Achieve complete measurements of methylation with oxBS.** The schematic (left) shows classic bisulfite conversion, which creates a library that detects both 5mC and 5hmC. Oxidation of 5hmC (right) generates a bisulfite-convertible base that leads to the detection of 5mC only. The differences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

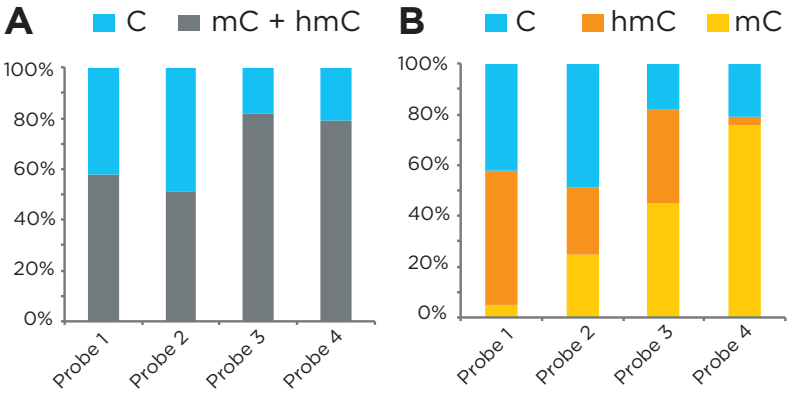

Figure 2: **TrueMethyl oxidative bisulfite conversion enables accurate measurement of methylation.** A) Standard bisulfite conversion cannot distinguish between 5mC and 5hmC, resulting in a single readout. B) TrueMethyl oxidative bisulfite conversion provides an accurate methylation profile of each modification.

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
- Integrated oxidative bisulfite conversion allows the investigation of multiple epigenetic modifications using the same workflow

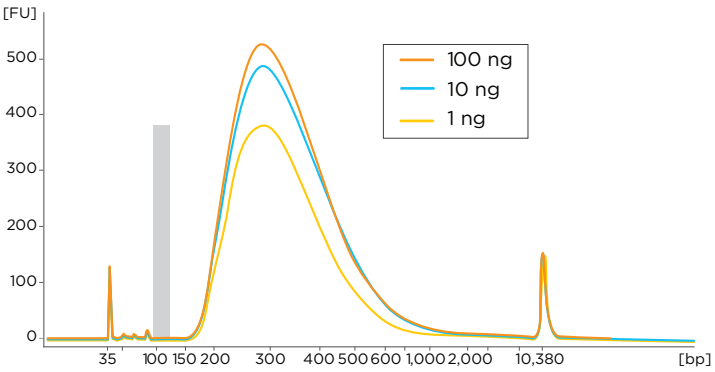

Figure 3: **Obtain high quality results regardless of input.** WGBS libraries were generated from 100, 10 and 1 ng of human genomic DNA. Bioanalyzer analysis indicates no adaptor artifacts (grey box) regardless of input and without the need for adaptor dilution during library construction.

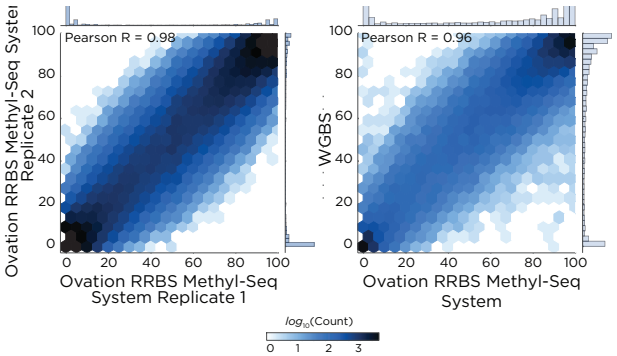

Figure 4: **Obtain highly reproducible and concordant results.** Concordance in methylation levels for CpGs covered at 20x or greater depth using 25 ng of IMR90 gDNA between Ovation RRBS Methyl-Seq System technical replicates (left) and versus WGBS (right).

Ovation RRBS Methyl-Seq System

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

References

1. Legendre C., *et al.,* Whole-genome bisulfite sequencing of cell-free DNA identifies signature associated with metastatic breast cancer. *Clin Epigenetics.* 2015. **7**(1):100. doi: 10.1186/s13148-015-0135-8
2. Booth M.J., *et al.,* Quantitative sequencing of 5-methylcytosine and 5-hydroxymethylcytosine at single-base resolution. *Science.* 2012. **336**(6083):934-7. doi: 10.1126/science.1220671
3. Pastor W.A., *et al.,* Genome-wide mapping of 5-hydroxymethylcytosine in embryonic stem cells. *Nature*. 2011. **19**;473(7347):394-7. doi: 10.1038/nature10102

Ordering information

Description	Part No.	No. of reactions	No. of barcodes	Automation fill availability
Ultralow Methyl-Seq with TrueMethyl oxBS	0541, 9513	32, 96	Up to 96	yes
Ovation RRBS Methyl-Seq System with TrueMethyl oxBS	0553, 9522	32, 96	Up to 96	yes
TrueMethyl oxBS module	0414	32	–	no

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