DATA SHEET

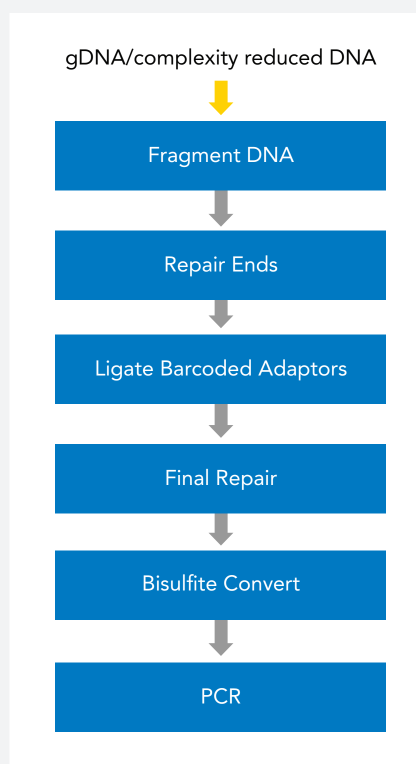
# Ovation® Ultralow Methyl-Seq Library Systems

## Libraries for DNA methylation analysis from just 10 ng DNA

### Highlights of the Ovation Ultralow Methyl-Seq Library Systems

- **Low input requirements:** Library construction and analysis of DNA methylation from as little as 10 ng human DNA to enable sequencing of low abundance samples
- **Fast and simple:** Library construction using a simple add-and-incubate workflow with easy purification steps for less hands-on time and faster results
- **Cost-effective and scalable solution:** Barcoding for multiplex sequencing to improve sample throughput and reduce costs on Illumina NGS platforms

FIGURE 1 The library construction workflow for the Ovation Ultralow Methyl-Seq Library Systems




### Introduction

The Ovation Ultralow Methyl-Seq Library Systems provide a simple, fast and scalable solution for producing libraries used in conjunction with bisulfite sequencing to analyze DNA methylation. The system requires as little as 10 ng of DNA, enabling methylation studies for a broad range of sample types. The protocol is compatible with whole genome bisulfite sequencing and can be completed in approximately 6 hours, including bisulfite conversion. As shown in **Figure 1**, the streamlined workflow consists of four main steps: fragmentation of genomic DNA or DNA that has been reduced in complexity, end repair to generate blunt ends, adaptor ligation, and a final fill-in reaction. Bisulfite conversion is performed following adaptor ligation to yield libraries ready for PCR amplification and cluster formation.

This system preserves directionality, dramatically simplifying the alignment process and saving data processing time and expense.

The Ovation Ultralow Methyl-Seq Library Systems are available in two kit configurations to enable multiplexing on Illumina sequencing platforms. The Ovation Ultralow Methyl-Seq DR Multiplex System 1–8 (Part No. 0335) and Ovation Ultralow Methyl-Seq DR Multiplex System 9–16 (Part No. 0336) each provide eight unique barcoded adaptors for multiplex sequencing. In combination these kits enable up to 16-plex sequencing using a dedicated read (DR) barcode design. The resulting library design is illustrated in **Figure 2**. The process is compatible with complexity reduction schemes such as methylated DNA capture[1,2] and RRBS.[3,4] Most processes that produce double-stranded DNA are compatible with this library system.

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## Whole Genome Bisulfite Sequencing

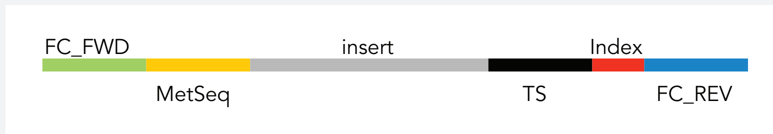
A key benefit of the Ovation Ultralow Methyl-Seq technology platform is elimination of adaptor dimer artifacts. This advantage enables successful construction of dimer-free libraries from very small amounts of DNA. **Figure 3** shows the Bioanalyzer profiles of libraries generated from 100 ng, 10 ng and 1 ng of human genomic DNA. These libraries were sequenced on an Illumina Genome Analyzer IIx and analyzed using the Bismark[5] analysis tool.

The data in **Table 1** illustrate that after the indicated number of PCR cycles, yields were far in excess of the 2 nM recommended for loading the Illumina cBot for cluster generation.

**Figure 4** shows how the number of unique reads increases as a function of total reads analyzed for these three libraries. This figure includes a theoretical curve based on Poisson sampling statistics for a perfectly complex library. The curves for the 100 ng and 10 ng libraries closely track the theoretical curve, while the 1 ng library curve falls away from the theoretical curve, indicating a higher rate of PCR duplicates present in the 1 ng input library. For this reason, NuGEN typically does not recommend DNA input of less than 10 ng for samples from complex genomes with the Ovation Ultralow Methyl-Seq Library Systems. With smaller genomes such as *Arabidopsis*, the minimum input requirement is considerably lower.

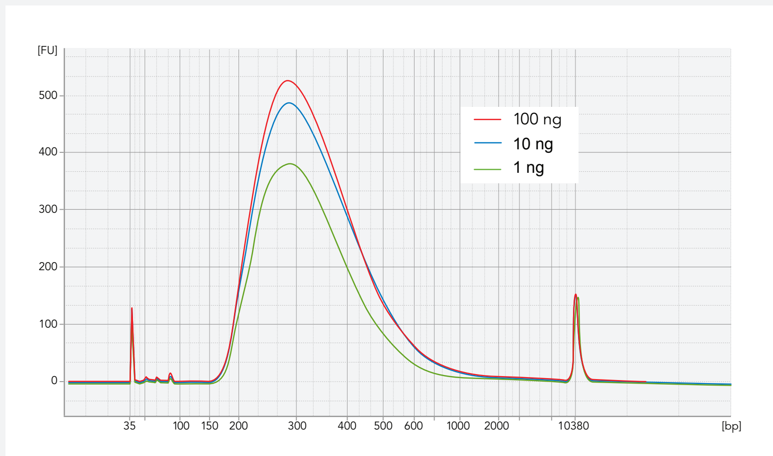
Coverage was further analyzed as shown in **Figure 5**. Read data from the 100 ng and 10 ng libraries were analyzed using Bismark and compared with similarly analyzed-theoretical read data. Of those reads, 225,000 reads mapping to a 40 Mb region of Chromosome 5 (between 100 Mb and 140 Mb) were selected and the number of reads per 3 Kb bin was graphed. This figure illustrates how closely the real data matches theoretical coverage.

**FIGURE 2** Detailed Schematic of Library Product




Library features include Illumina flowcell priming sites (FC_FWD, FC_REV), forward custom priming site (MetSeq), bisulfite converted insert, standard Illumina TruSeq reverse read and index (barcode) read priming sites (TS).

**FIGURE 3** High Sensitivity DNA Chip Bioanalyzer Profiles of Ovation Ultralow Methyl-Seq Library Systems Sequencing Libraries




Libraries were generated from 100 ng, 10 ng and 1 ng of human genomic DNA. 1.0 µL was analyzed on a High Sensitivity DNA Bioanalyzer chip.

**TABLE 1** Ovation Ultralow Methyl-Seq Library Systems Sequencing Libraries Made from Human DNA

| Input | PCR cycles | Yield (nM) | % Mapping | % MeCpG |
|---|---|---|---|---|
| 100 ng | 12 | 54.1 | 77.6 | 79.2 |
| 10 ng | 15 | 67.7 | 77.4 | 79.7 |
| 1 ng | 18 | 40.6 | 77.0 | 79.3 |

Mapping and %MeCpG are two metrics produced by Bismark. The % Mapping metric refers only to reads that map uniquely to the bisulfite converted genome. Reads that map to multiple locations are not included. Sequences that fail to map are less than 10% of all reads. The percentage of methylated CpG (% MeCpG) remains relatively constant across the three libraries, indicating a lack of PCR bias, even as the number of PCR cycles increases from 12 cycles to 18 cycles.

## Directional Libraries

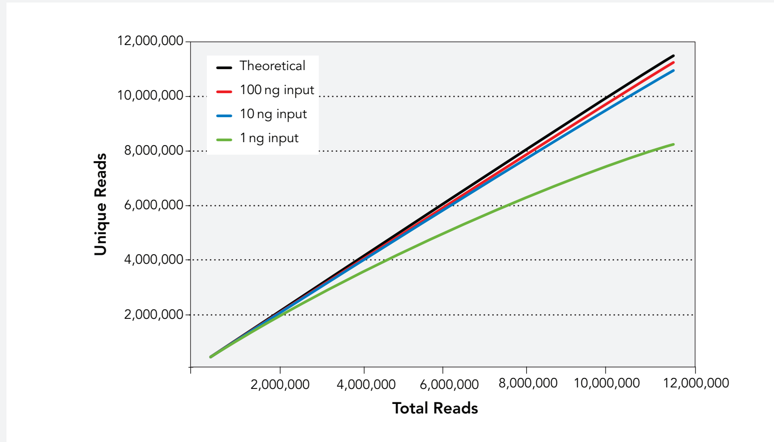
Libraries produced using the Ovation Ultralow Methyl-Seq Library Systems maintain the directional orientation of the original genomic DNA. The forward read is always the C-to-T converted (original genomic) strand, while the reverse read is the G-to-A reverse complement. **Figure 6** depicts the effect of this directionality on the nucleotide distributions of forward and reverse reads. When using Bismark for analysis, this conserved directionality reduces the computational burden by a factor of two over non-directional libraries.

## Conclusion

The Ovation Ultralow Methyl-Seq Library Systems offer a number of advantages for researchers engaged in DNA methylation studies:

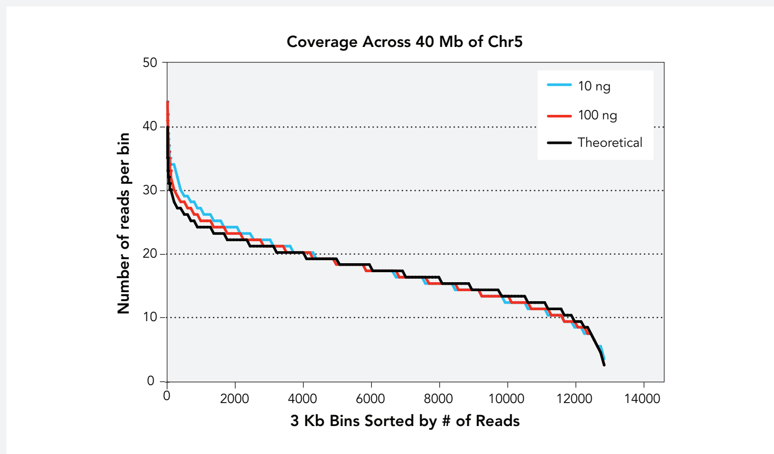
- Low input requirements — High efficiency coupled with adaptor artifact elimination enables high quality results from very low inputs.
- Fast, simple, gel-free workflow — Generates barcoded bisulfite sequencing libraries from as little as 10 ng human DNA in about 6 hours.
- Cost-effective and scalable — Barcoding for multiplex sequencing to improve sample throughput and reduce costs on Illumina NGS platforms.

**FIGURE 4** Sequence Complexity of Ovation Ultralow Methyl-Seq Library Systems Libraries




The number of unique reads increases with the total number of reads analyzed for each library. The theoretical library curve is based on Poisson sampling statistics for a perfectly complex library.

**FIGURE 5** Coverage Analysis of Ovation Ultralow Methyl-Seq Library Systems Sequencing Libraries




Coverage shown for 100 ng, 10 ng and theoretical libraries. Theoretical data consists of 30 million, 40 bp reads against the human genome, with no errors and a 100% percent conversion rate generated using the Sherman Bisulfite-treated Read FastQ Simulator.[6]

## References

1. Cross SH, Charlton JA, Nan X, and Bird, AP. (1994) Purification of CpG islands using a methylated DNA binding column. *Nat Genet*, 6, 236–244.

2. Bock C, Tomazou EM, Brinkman AB et al. (2010) Quantitative comparison of genome-wide DNA methylation mapping technologies. *Nat Biotechnol*, 28, 1106–14.

3. Meissner A, Gnirke A, Bell GW, et al. (2005) Reduced representation bisulfite sequencing for comparative high resolution DNA methylation analysis. *Nucleic Acids Res*, 33, 5868–5877.

4. Boyle P, Clement K, Gu H et al. (2012) Gel-free multiplexed reduced representation bisulfite sequencing for large-scale DNA methylation profiling. *Genome Biology*, 13, R92.

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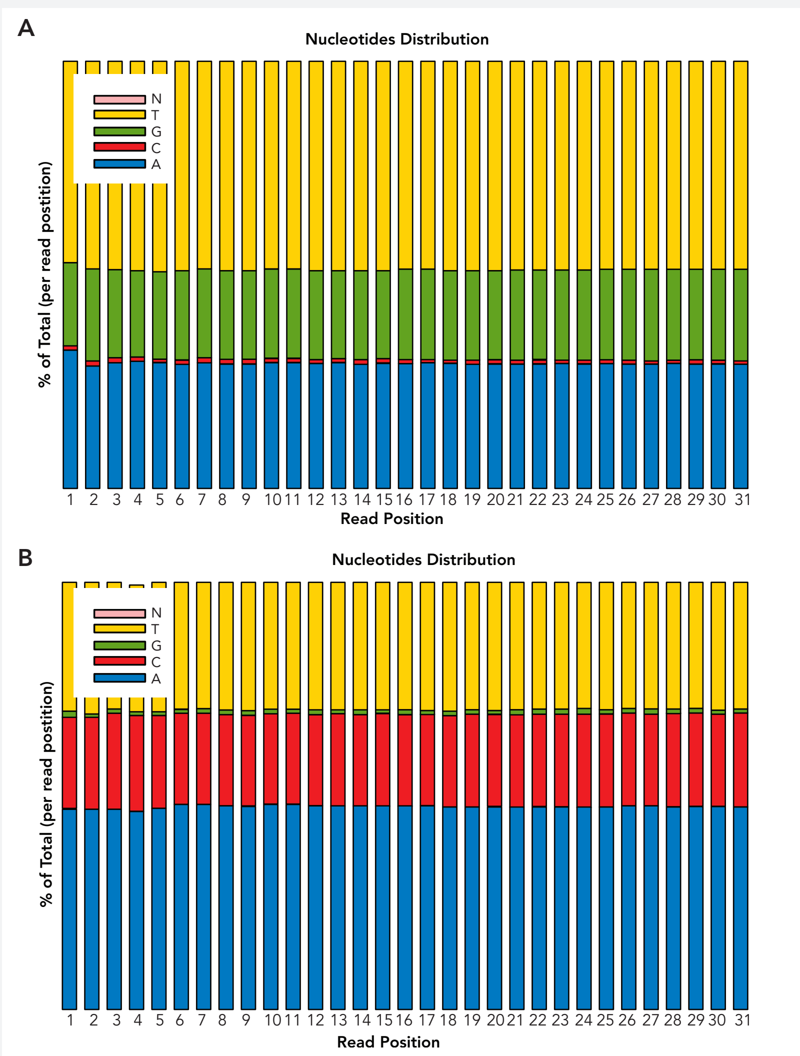
6. www.bioinformatics.babraham.ac.uk/projects/sherman/

## ORDERING INFORMATION

| Part No. | Product Name |
|---|---|
| 0335 | Ovation Ultralow Methyl-Seq Multiplex System 1–8 |
| 0336 | Ovation Ultralow Methyl-Seq Multiplex System 9–16 |
| **Related Products** | |
| 0330 | Ovation Ultralow DR Multiplex System 1–8 |
| 0331 | Ovation Ultralow DR Multiplex System 9–16 |
| 0329 | Ovation Ultralow DR Multiplex System 1–96 |
| 7102 | Ovation RNA-Seq System V2 |
| **Technical Documents** | |
| Ovation Ultralow Methyl-Seq Library Systems User Guide | |

**FIGURE 6** Nucleotide Distributions of Forward (A) and Reverse (B) Reads Generated from an Ovation Ultralow Methyl-Seq Library Systems Human Library During a MiSeq 2x31 nt Paired-end Run



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