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.

**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.
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 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.

### Table 1

<table>
<thead>
<tr>
<th>Input</th>
<th>PCR cycles</th>
<th>Yield (nM)</th>
<th>% Mapping</th>
<th>% MeCpG</th>
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<tr>
<td>100 ng</td>
<td>12</td>
<td>54.1</td>
<td>77.6</td>
<td>79.2</td>
</tr>
<tr>
<td>10 ng</td>
<td>15</td>
<td>67.7</td>
<td>77.4</td>
<td>79.7</td>
</tr>
<tr>
<td>1 ng</td>
<td>18</td>
<td>40.6</td>
<td>77.0</td>
<td>79.3</td>
</tr>
</tbody>
</table>

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.

**References**

6. www.bioinformatics.babraham.ac.uk/projects/sherman/
**ORDERING INFORMATION**

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**Technical Documents**

- Ovation Ultralow Methyl-Seq Library Systems User Guide

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**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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