Ovation® Ultralow Library Systems

Prepare next-generation sequencing libraries with as little as 1.0 ng DNA

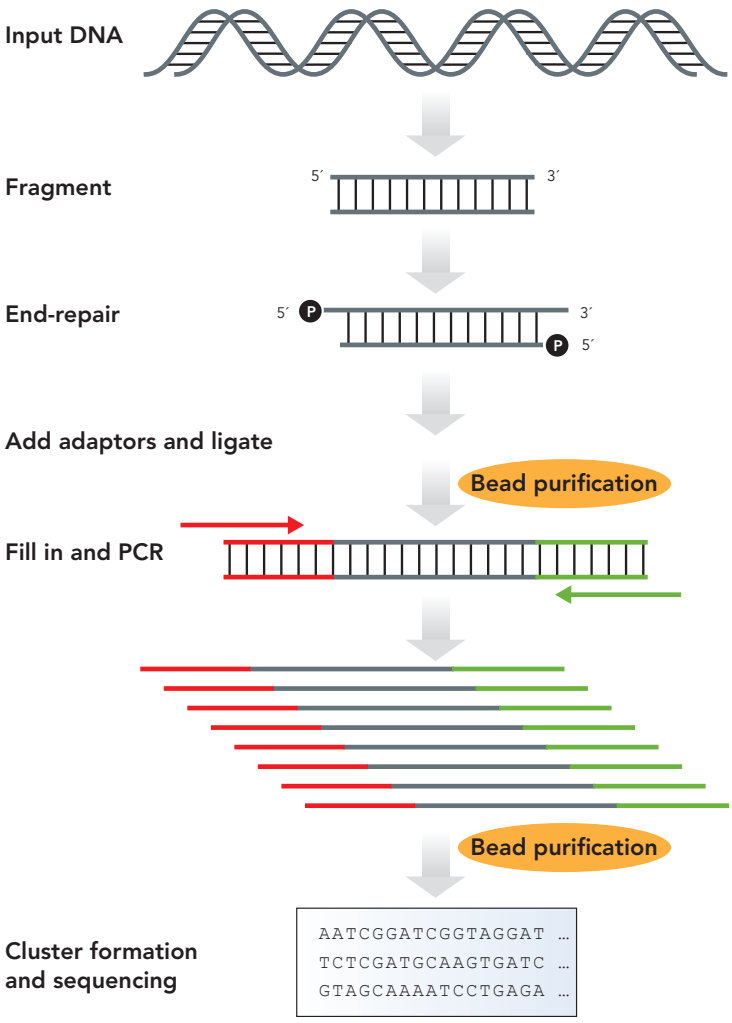
Highlights of the Ovation® Ultralow Library Systems

- **Low input requirements —** Library construction with as little as 1.0 ng DNA to enable sequencing of low abundance samples without pre-amplification.
- **Simple, fast and automatable solution —** Library construction in as little as four hours, with only two purification steps and no gel purification.
- **Cost-effective and scalable solution —** Optional barcoding for multiplex sequencing to improve sample throughput and reduce costs on Illumina NGS platforms .

Introduction

The Ovation® Ultralow Library Systems provide a simple, fast and scalable solution for producing libraries used in next-generation sequencing starting with as little as 1.0 ng of double-stranded DNA. The library construction workflow is suitable for a wide range of sequencing applications including RNA-Seq, Digital Gene Expression (DGE), genomic DNA/exome sequencing, amplicon sequencing, ChIP-Seq and more.

FIGURE 1 **Schematic of Ovation Ultralow Library System Workflow**



As shown in **Figure 1**, the streamlined workflow consists of four main steps:

(1) Fragmentation of either genomic DNA or double-stranded cDNA, (2) End repair to generate blunt ends, (3) Adaptor ligation (with or without indexing for multiplexing), and (4) PCR amplification to produce the final library. The entire workflow, including fragmentation, can be completed in as little as four hours, and yields DNA libraries ready for cluster formation and either single read or paired-end sequencing on the Illumina sequencing platforms.

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The Ovation Ultralow Library System (Part No. 0303) contains reagents for production of non-barcoded libraries, while the Ovation Ultralow IL Multiplex System 1–8 (Part No. 0304) and IL Multiplex System 9–16 (Part No. 0305) each provide eight unique barcoded adaptors for multiplex sequencing. In combination these latter two kits enable up to 16-plex sequencing. The Ovation Ultralow DR Multiplex System 1–8 (Part No. 0330) and DR Multiplex System 9–16 (Part No. 0331) offer the same level of multiplexing but use a dedicated read (DR) barcode design.

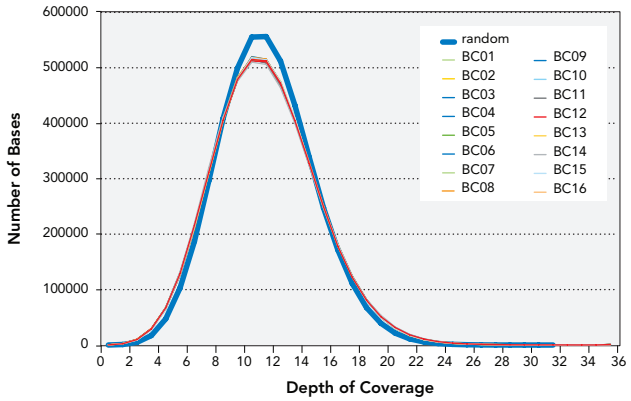
Highly Reproducible, Unbiased Results Using 1.0 ng Genomic DNA

To evaluate the performance of the Ovation Ultralow Library Systems with genomic DNA, sixteen independent sequencing libraries were constructed using 1.0 ng of *E. coli* DNA with the Ovation Ultralow IL Multiplex System 1–8 and 9–16 (Part Nos. 0304 and 0305) and sequenced on the Illumina Genome Analyzer IIx. The distribution of reads from each sample was plotted to determine the depth of coverage across the *E. coli* genome, as well as to assess the reproducibility of library construction and sequencing.

In **Figure 2**, the distribution of 1.4 million reads from each library mapped to the reference genome is shown along with a Gaussian distribution of the equivalent number of reads generated randomly from the genome sequence. No coverage bias was observed, and mapping of these 1.4 million reads provided an average 12X genomic coverage with very high reproducibility.

As shown in **Table 1**, minimal bias was observed with microbial genomes having extreme GC content, 33% (*S. aureus*) and 69% (*R. sphaeroides*), as well as *E. coli* genome (~51% GC). The GC content observed by sequencing matches closely with the known GC content of each genome. Taken together, these results demonstrate Ovation Ultralow Library Systems generate high complexity libraries with no significant coverage or GC bias in a reproducible fashion.

FIGURE 2 **Sequencing coverage with *E. coli* genomic DNA**



Sixteen independent sequencing libraries were constructed using 1.0 ng of *E. coli* genomic DNA with the Ovation Ultralow IL Multiplex Systems (Part Nos. 0304 and 0305) and sequenced on the Illumina Genome Analyzer IIx using 40 base-pair single end reads. The distribution of reads from each sample is plotted to determine the depth of coverage across the *E. coli* genome. 1.4 million reads were randomly sub-sampled from each data set to generate the above plots. The blue track represents the Gaussian distribution with randomly chosen 36 base-pair sequences mapped to the *E. coli* genome. The other overlapping tracks are from the experimental libraries.

TABLE 1 **Sequencing alignment metrics with a range of GC content**

Species	Total Reads	% Aligned Reads	Known GC Content	Observed GC Content
E. coli	1,072,561	99.2%	50.8%	51.6%
S. aureus	1,912,058	99.3%	32.8%	34.0%
R. sphaeroides	1,246,746	99.4%	68.5%	67.5%

Consistent RNA-Seq Performance

To examine the utility of the Ovation Ultralow Library Systems for RNA-Seq applications, the Ovation RNA-Seq System V2 (Part No. 7102) was used to produce double-stranded cDNA from 2.0 ng of Universal Human Reference total RNA (MAQC A) followed by the library construction workflow. As shown in **Table 2**, input of 1.0, 10 or 100 ng of cDNA to the Ovation Ultralow Library System produced very good sequencing alignment metrics with the expected level of uniquely mapped reads, low levels of rRNA reads and good detection of RefSeq transcripts. There was very little difference in the overall sequencing performance using libraries constructed with different amounts of input material, and the detection of expressed transcripts was highly concordant across all library inputs (see **Figure 3**).

Similar results were obtained using the Ovation Ultralow Library Systems in

TABLE 2 **Ovation RNA-Seq System V2 results with a range of library inputs**

Library Input	%Unique Reads	%Multi Reads	%Mito rRNA Reads	% Cyto rRNA Reads	% Inter-genic	%Exon	%Intron	%RefSeq
100 ng	61.1%	28.9%	27.7%	5.4%	19.5%	37.3%	41.8%	73.9%
10 ng	57.9%	28.1%	26.7%	5.7%	19.5%	38.9%	40.3%	72.9%
1 ng	59.4%	27.2%	26.0%	4.7%	19.5%	36.9%	42.5%	70.5%

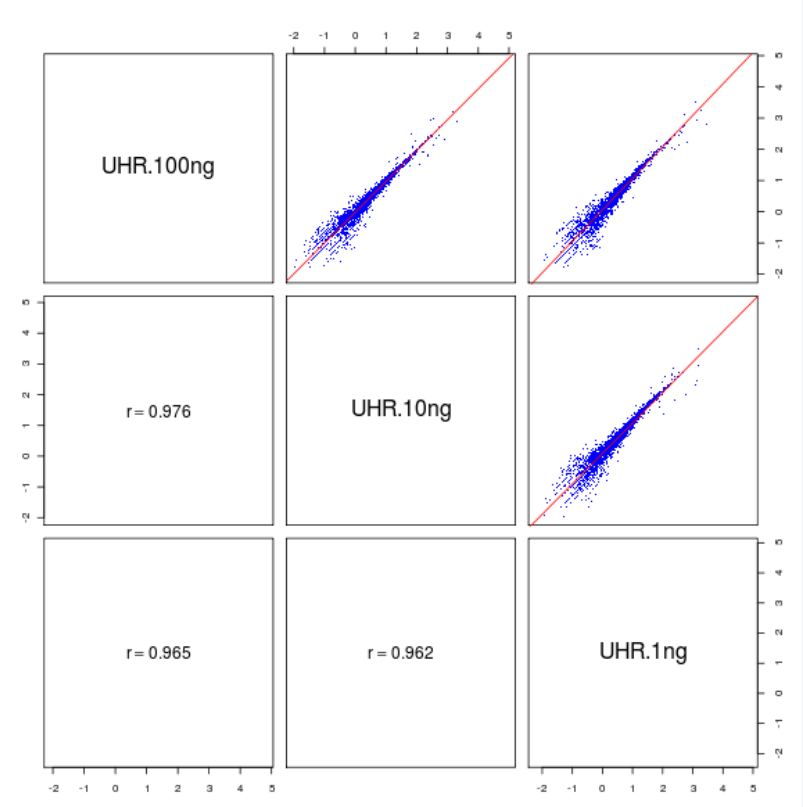
conjunction with the Ovation Prokaryotic RNA-Seq Systems (Part No. 9030) with 100 ng cDNA produced from *E. coli* total RNA (not shown). The integration of these RNA-Seq solutions with Ovation Ultralow Library Systems further illustrates the versatility of this library construction system for NGS applications starting with either RNA or DNA.

Conclusion

The Ovation Ultralow Library Systems offer a number of advantages for a broad range of NGS applications:

- **Low input requirements —** Library construction from as little as 1.0 ng DNA. This low input capability enables sequencing of your most precious genomic DNA samples without pre-amplification for the study of cancer genomes, stem cell biology, circulating tumor cells or immune cells.
- **Simple, fast and automatable solution —** Library construction in as little as four hours, with only two purification steps and no gel purification
- **A complete library solution for a range of NGS applications —** RNA-Seq, whole genome or targeted DNA sequencing, ChIP-Seq or Amplicon sequencing
- **Cost-effective and scalable solution —** Optional barcoding for multiplex sequencing to improve sample throughput and reduce costs on Illumina NGS platforms

FIGURE 3 **Correlation between varying inputs of double-stranded cDNA**



Scatterplots of the $\log_2$ RPKM values for Human Universal Reference samples (MAQC A) prepared using 2.0 ng of total RNA and amplified with the Ovation RNA-Seq System V2. Libraries were constructed using the Ovation Ultralow IL Multiplex System 1–8 (Part No. 0304) with either 1.0, 10 or 100 ng input of double-stranded cDNA. RPKM stands for Reads Per Kilobase of exon model per Million mapped reads. The RPKM measure of read density reflects the molar concentration of a transcript in the starting sample by normalizing for RNA length and for the total read number in the measurement. Sequencing results were obtained using the Illumina Genome Analyzer IIx platform.

ORDERING INFORMATION

Part No.	Product Name	No. Reactions
0303	Ovation® Ultralow Library System	8
0304	Ovation® Ultralow IL Multiplex System 1–8	32
0305	Ovation® Ultralow IL Multiplex System 9–16	32
0330	Ovation® Ultralow DR Multiplex System 1–8	32
0331	Ovation® Ultralow DR Multiplex System 9–16	32
Related Products		
7102	Ovation® RNA-Seq System V2	
7150	Ovation® RNA-Seq FFPE System	
9030	Ovation® Prokaryotic RNA-Seq System	
Technical Documents		
Ovation® Ultralow Library Systems User Guide		

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