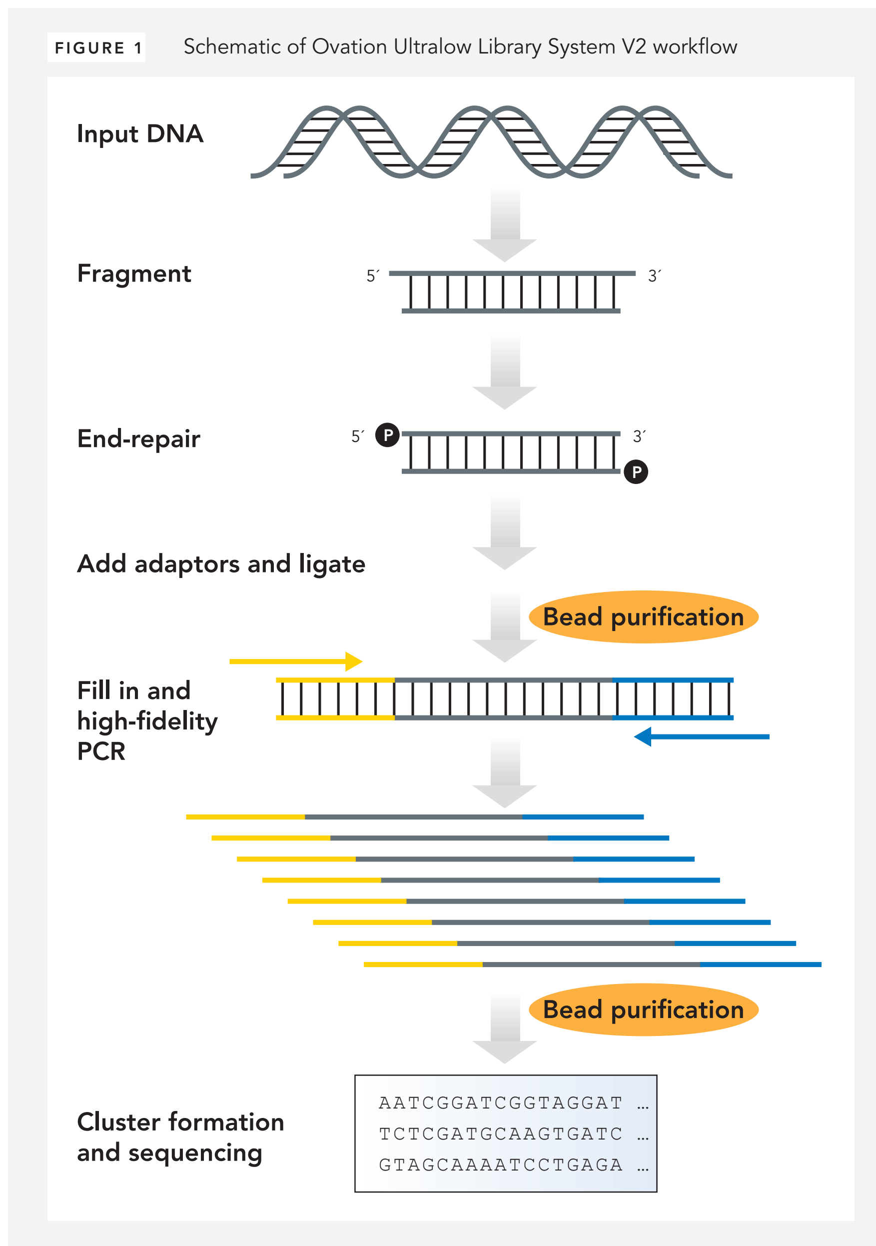
Ovation® Ultralow Library Systems V2

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

Highlights of the Ovation Ultralow Library Systems V2

- **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
- **Cost-effective and scalable solution:** Optional barcoding for multiplex sequencing to improve sample throughput and reduce costs on illumina NGS platforms

FIGURE 1 Schematic of Ovation Ultralow Library System V2 workflow



Introduction

The Ovation Ultralow Library Systems V2 provide a simple, fast and scalable solution for producing libraries that can be used in a broad range of next-generation sequencing applications starting with 1 to 100 ng of DNA. The method used for adaptor ligation provides for low bias libraries suitable for RNA-Seq, Digital Gene Expression (DGE), genomic DNA sequencing, target capture, ChIP-Seq and more. In this second version of the product, a high-fidelity DNA polymerase has been integrated for the library amplification step.

This high fidelity enzyme provides superior accuracy for library enrichment resulting in more uniform coverage of both AT and GC rich sequences and accurate variant detection.

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,

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(3) Adaptor ligation (with or without indexing for multiplexing), and (4) High-fidelity 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.

The Ovation Ultralow Library System V2 1–16 (Part Nos. 0344 and 0344NB) contain reagents for generating 32 libraries with 16 unique barcoded adaptors for multiplex sequencing. Designed for automated workflows, the Ovation Ultralow Library System V2 1–96 (Part No. 0347) enables multiplexing of up to 96 libraries per lane.

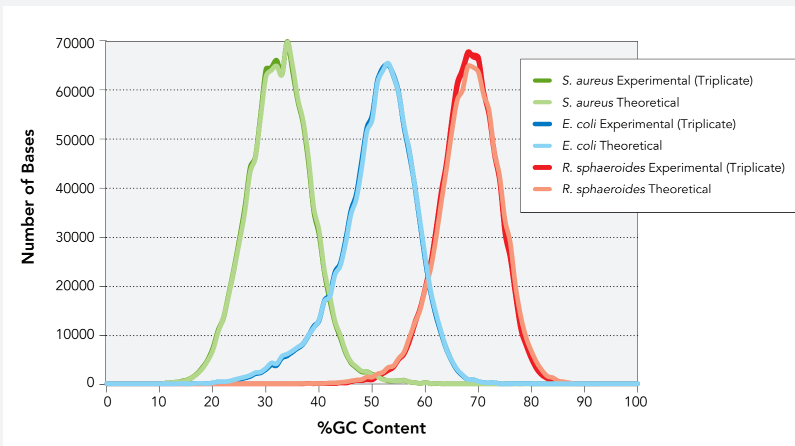
Highly Reproducible, Unbiased Results Using 1.0 ng Genomic DNA

To evaluate the performance of the Ovation Ultralow Library Systems V2 with genomic DNA, barcoded sequencing libraries were constructed using 1.0 ng of DNA from *E. coli*, *S. aureus*, or *R. sphaeroides* with the Ovation Ultralow Library Systems V2 1–16 and sequenced on the Illumina MiSeq platform. The distribution of reads (# of bases) from each sample was determined as a function of the %GC content for each genome. As shown in **Figure 2**, the reads display even coverage across a broad range of GC content with very good concordance to theoretical coverage plots.

Consistent RNA-Seq Performance

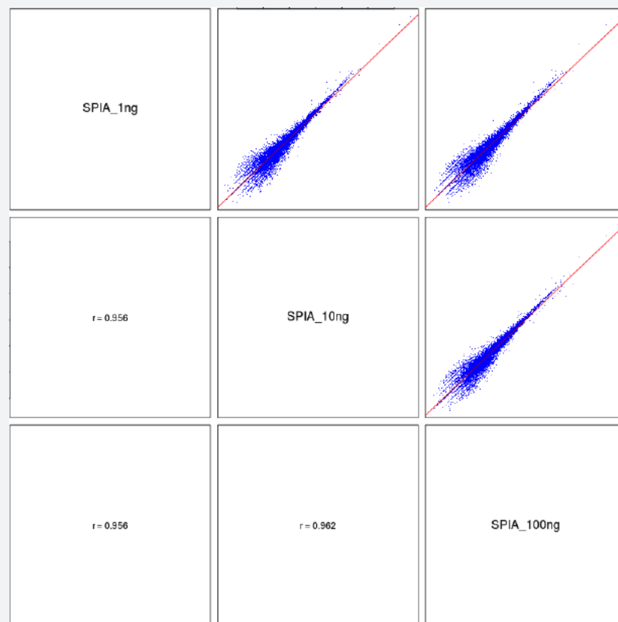
To examine the utility of the Ovation Ultralow Library Systems V2 for RNA-Seq applications, the Ovation RNA-Seq System V2 (Part No. 7102) was used to produce double-stranded cDNA from 2.0 ng of Human Brain Reference total RNA (MAQC B) followed by the library construction workflow. As shown in **Figure 3**, input of 1.0, 10 or 100 ng of double-stranded cDNA to the Ovation Ultralow Library Systems V2 showed little difference in the overall sequencing performance using libraries constructed with different amounts of input material, and the detection of expressed tran-

FIGURE 2 Sequencing Performance with a Range of GC content



Triplicate barcoded sequencing libraries were constructed using 1.0 ng of genomic DNA from *E. coli* (51% GC), *R. sphaeroides* (69% GC), or *S. aureus* (33% GC) with Ovation Ultralow System V2 1–16 (Part No. 0344-32) and sequenced on the Illumina MiSeq using 40 base-pair single reads. The GC content observed by sequencing matches closely with the known GC content of each genome (theoretical curves). These results demonstrate Ovation Ultralow Library System V2 generates high complexity libraries with no significant genome coverage bias, or bias due to the GC content of the input DNA.

FIGURE 3 Correlation between varying inputs of double-stranded cDNA



Scatterplots of the log RPKM values for Human Brain reference samples (MAQC B) prepared using 2.0 ng of total RNA and amplified with the Ovation RNA-Seq System V2. Libraries were constructed using the Ovation Ultralow Library Systems V2 1–16 (Part No. 0344) 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 transcript length and for the total read number in the measurement. Sequencing results were obtained using the Illumina MiSeq platform.

scripts was highly concordant across all library inputs examined.

The integration of RNA-Seq solutions and subsequent input of cDNA to the Ovation Ultralow Library Systems V2 workflow further illustrates the versatility of this library construction system for NGS applications starting with either RNA or DNA.

Conclusion

The Ovation Ultralow Library Systems V2 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, with low input capability that enables sequencing 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 —** barcoding for multiplex sequencing to improve sample throughput and reduce costs on illumina NGS platforms.

ORDERING INFORMATION

Part No.	Product Name	No. Reactions
0344	Ovation Ultralow Library Systems V2 1–16	32
0347	Ovation Ultralow Library Systems V2 1–96	96
0344NB	Ovation Ultralow Library Systems V2 1–16 (no beads)	32

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