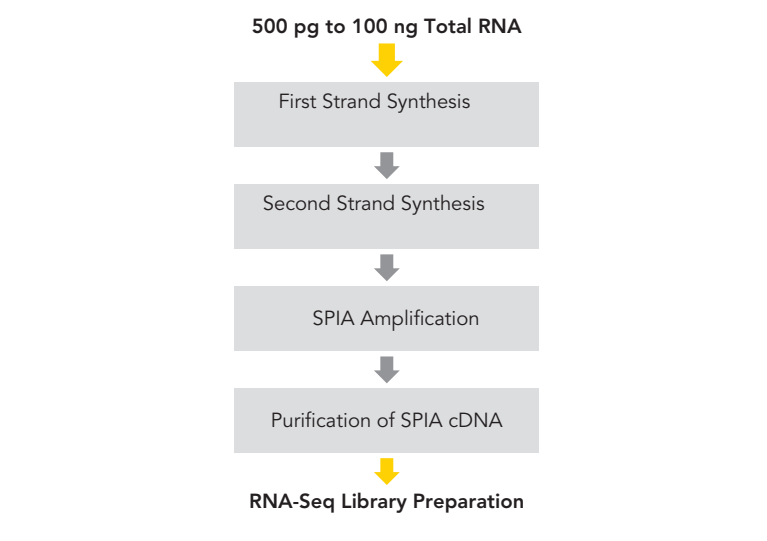
Ovation® RNA-Seq System V2

RNA-Seq sample preparation for complete transcriptome representation from picograms of total RNA

Highlights of the Ovation RNA-Seq System V2

- **Low RNA input requirements:** Requires just 500 pg – 100 ng total RNA to provide RNA-Seq data from mRNA and non-polyadenylated transcripts
- **More complete transcriptome representation:** Provides enhanced depth of transcriptome coverage with no 5′/3′ positional biases
- **Fast, easy protocol based on proven technology:** Leverages NuGEN's powerful Ribo-SPIA® technology to produce double-stranded cDNA, ready for RNA-Seq library construction in 4.5 hours without poly(A) selection or rRNA depletion

FIGURE 1 The Ovation RNA-Seq System V2 Workflow



The cDNA produced by the Ovation RNA-Seq System V2 may be input to NuGEN's Ovation Library Systems, or used with other library construction kits suitable for double-stranded cDNA samples.

Introduction

NuGEN continues to innovate with the introduction of the Ovation RNA-Seq System V2. This system uses NuGEN's proprietary Ribo-SPIA technology to provide high-quality cDNA samples from as little as 500 pg to 100 ng of total RNA in just 4.5 hours.

The Ovation RNA-Seq System V2 provides enhanced transcript coverage and uniform distribution of sequencing reads using a streamlined workflow — from sample to RNA-Seq. It enables researchers to conduct RNA-Seq research using small quantities of total RNA, with uniform read coverage across the full-length of poly(A)+ RNA and non-polyadenylated transcripts.

NuGEN
imagine more from less®

Amplification is initiated at the 3′ end as well as randomly throughout the whole transcriptome in each sample. As a result, NuGEN's Ovation RNA-Seq System V2 provides you with a more complete picture of the transcriptome without the need or added cost for rRNA reduction or poly(A) selection.

The Ovation RNA-Seq System V2 provides optimized reagent mixes and a protocol to process 8 or 32 total RNA samples, and is also available as an automation solution for processing 96 samples.

Simple, Fast Workflow Integrates Seamlessly with Library Construction Protocols

As illustrated in **Figure 1**, the Ovation RNA-Seq System V2 protocol yields double-stranded cDNA ready for the construction of RNA-Seq libraries. The protocol can be completed in approximately 4.5 hours, and yields sufficient cDNA for several sequencing runs.

The resulting cDNA is approximately 50 bases to 1.5 Kb long before adaptor ligation as measured on the Agilent Bioanalyzer (**Figure 2**), with >60% of the amplified product falling below 500 bases. The amplified cDNA product is ideal for use with NuGEN's Ovation Ultralow Library Systems and Ovation Rapid Library Systems and can be directly integrated into the protocols provided by NGS suppliers. **Figure 3** shows reproducibility of libraries produced using the Ovation Rapid Library Systems with differing amounts of input SPIA® cDNA product. The combined workflow takes just 6.5 hours from total RNA to sequence-ready library.

Reproducible Sequencing Results

To evaluate the performance of RNA-Seq using cDNA generated with the Ovation RNA-Seq System V2, libraries were constructed using total RNA from Human Brain Reference and Universal Human Reference (UHR) MAQC samples. These samples were sequenced on the Illumina Genome Analyzer IIx using 40-base-pair single-read sequencing.

As shown in **Table 1**, 2 ng of total RNA generated an average of 11.1 million total reads per flow cell lane. Approximately 44% of those reads were unique, mapping to the reference sequence only once. On average, 87% of the reads were mappable to the reference genome. Additionally, reads from these datasets mapped to approximately 17,000 genes in the NCBI Reference Sequence (RefSeq).

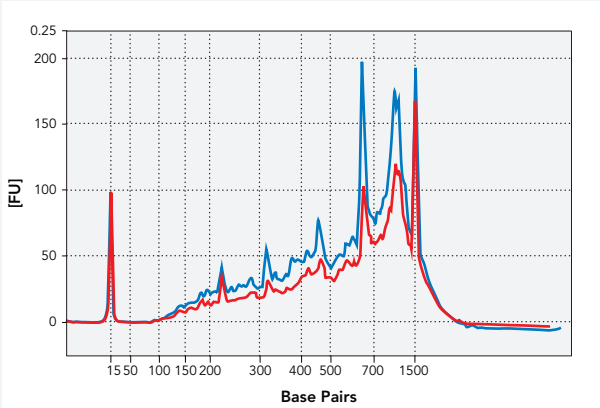
Due to the proprietary combination of enzymes and primers in the Ovation RNA-Seq System V2, non-rRNA sequences are preferentially primed and subsequently amplified, reducing the number of reads from rRNA sequences. Reads mapping to rRNA sequences accounted for about 16% of total reads, with no rRNA depletion or other enrichment techniques used. Similarly, reads mapping to mitochondrial RNA sequences averaged 19%.

These results demonstrate that the Ovation RNA-Seq System V2 provides high-quality cDNA libraries that produce high-quality sequencing metrics, starting from picogram quantities of total RNA. The System also provides significantly higher read coverage in non-polyadenylated regions of the transcriptome compared to libraries made with poly(A) selected sequences without the added cost, time or potential for bias introduced by preprocessing total RNA.

Exon Read Coverage, Sequencing Read Distribution and Positional Bias

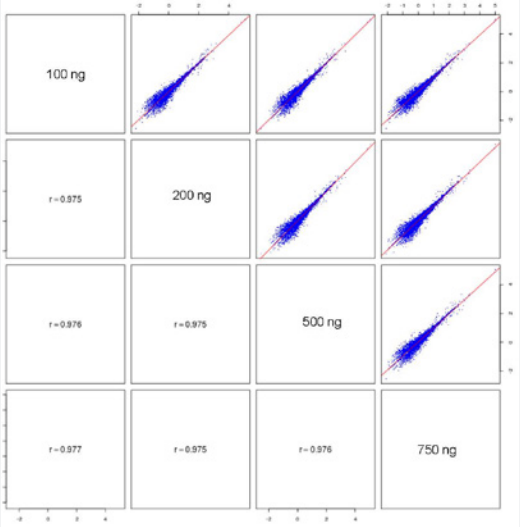
To illustrate the improved exon read coverage and sequencing read distribution provided by the Ovation RNA-Seq System V2, reads from the original Ovation RNA-Seq System and the Ovation RNA-Seq System V2 were compared for three

FIGURE 2 Size Distribution of Amplified cDNA Products



Bioanalyzer trace of SPIA cDNA product obtained from 2 ng of Human Reference Brain (red line) and Universal Human Reference RNA (blue line) using the DNA 1000 Labchip® (Agilent Technologies).

FIGURE 3 Reproducibility of Libraries from Ovation Library Systems



Concordance plots demonstrating reproducibility of the integrated workflow using Ovation RNA-Seq System V2 with NuGEN's Ovation Rapid Library System with differing amounts of SPIA cDNA input to the library construction workflow. RPKM plots average R ≥ 0.9 for different library inputs.

TABLE 1 Sequencing Alignment Metrics with MAQC Samples

Sample	Total Reads	%Total Mapped Reads	%Unique Reads	%Multi Reads	%rRNA Reads	%Mito Reads	#Ref-Seq Genes
Brain	11,575,679	87.3	44.3	43.0	16.3	22.2	16,828
UHR	10,695,770	85.9	43.4	42.5	16.2	16.0	17,043

The data were generated using 2 ng of Total RNA from either Human Brain (MAQC B) or Universal Human Reference (MAQC A) input to the Ovation RNA-Seq System V2 followed by library construction using the Encore NGS Library System I. The resulting libraries were sequenced on the Illumina Genome Analyzer IIx with 40 bp single-read sequencing.

different transcripts — GAPDH, beta actin and GABA receptor-associated protein. As shown in **Figure 4**, use of the Ovation RNA-Seq System V2 produced a more even distribution of sequencing reads across exons.

These results highlight the uniform exon read coverage and lack of 3′ bias found in reads based on the Ovation RNA-Seq System V2. This lack of bias was confirmed at the whole transcriptome level by amplifying total RNA from the Human Brain Reference using both versions of the Ovation RNA-Seq System and the Ovation 3′-DGE System. Libraries were constructed using the Encore NGS Multiplex System I and 40-base-pair single reads were obtained using the Illumina Genome Analyzer IIx platform.

Figure 5 shows the distribution of sequencing reads in the 5′ to 3′ direction across RefSeq transcripts that are 1.5 to 3.0 Kb in length. The graph demonstrates the expected 3′ bias of the Ovation 3′-DGE System and the relative lack of bias found with both versions of the Ovation RNA-Seq System and a publicly available poly(A)+ reference library.

High Concordance with qPCR Results for Differential Expression Analysis

The Ovation RNA-Seq System V2 produces differential expression data comparable to that generated by quantitative PCR (qPCR). MAQC Human Brain Reference and UHR samples amplified using either the Ovation RNA-Seq System V2 or qPCR using the TaqMan® assay to compare the differential expression data generated by each method.

As shown in **Figure 6**, the differential expression fold changes produced by the Ovation RNA-Seq System V2 and qPCR are concordant and without significant data compression, as evidenced by the R-value and the slope, respectively. These results demonstrate that differentially expressed genes can be quantified by RNA-Seq, using material amplified with the Ovation RNA-Seq System V2, to produce results similar to those obtained by qPCR.

FIGURE 4A GAPDH

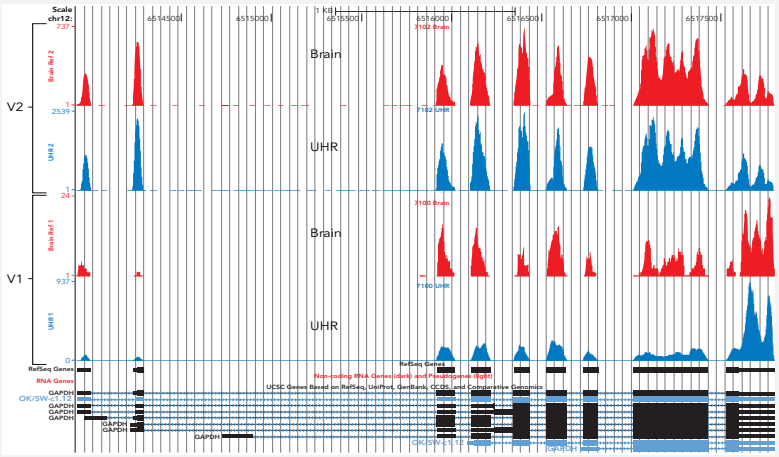


FIGURE 4B Beta Actin

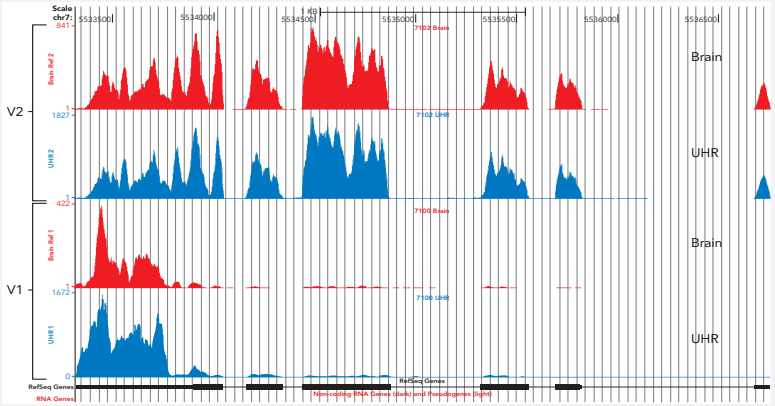
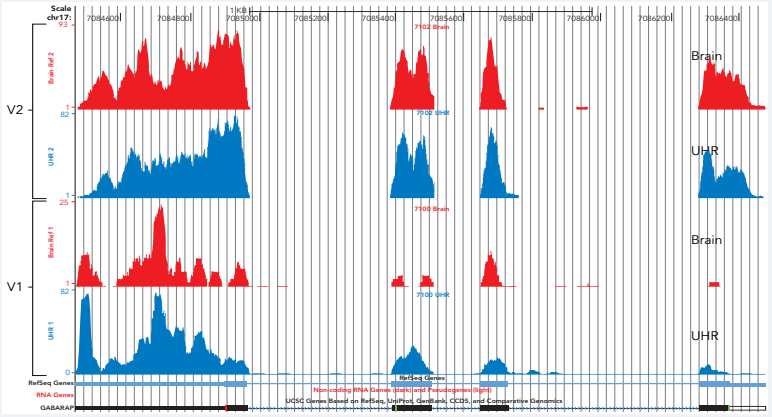


FIGURE 4C GABA Receptor Associated Protein



Total RNA from Human Brain (MAQC B, 2.0 ng) was amplified using either the original Ovation RNA-Seq System or the Ovation RNA-Seq System V2 and libraries constructed using the Encore NGS Multiplex System I. Single-read sequencing results were obtained using the Illumina Genome Analyzer IIx platform with 40 bp reads. The data shown in Figures 4A, B and C show examples of the sequencing read distribution across the indicated transcripts and illustrate the improved exon read coverage with Ovation RNA-Seq System V2.

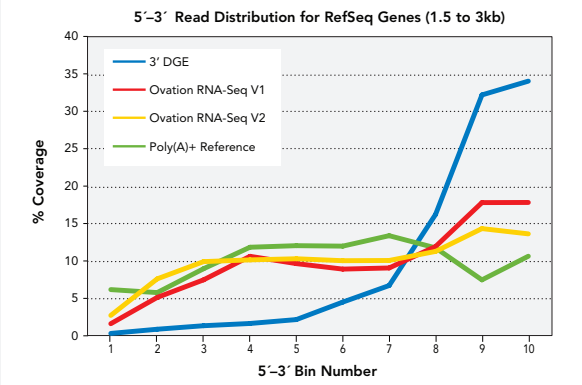
Conclusion

Based on NuGEN's proven Ribo-SPIA technology, the Ovation RNA-Seq System V2 yields several micrograms of cDNA ready for RNA-Seq library construction from just picograms of total RNA. The System includes a simple protocol that can be completed in approximately 4.5 hours, without the need for rRNA reduction or poly(A) selection protocols that can limit complete sequencing of the transcriptome. The same amplified material may also be used for qPCR or microarray analysis. This system brings the same degree of sensitivity and reproducibility to RNA-Seq applications that researchers have come to expect from NuGEN's sample preparation products and enables complete investigation of the transcriptome using the power of RNA-Seq.

ORDERING INFORMATION

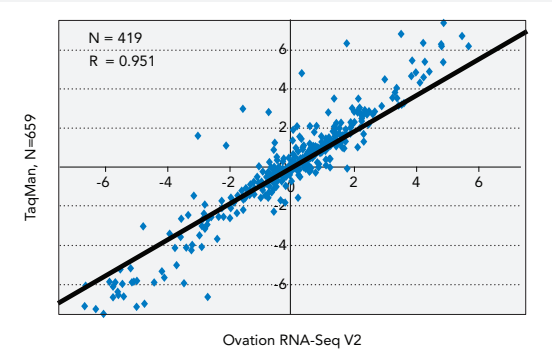
Part No.	Product Name
7102	Ovation® RNA-Seq System V2

FIGURE 5 Comparison of 3′-Bias Between Different RNA-Seq Protocols



Total RNA from Human Brain (MAQC B, 2.0 ng) was amplified using either the Ovation RNA-Seq System, Ovation RNA-Seq System V2 or Ovation 3′-DGE System and libraries constructed using the Encore NGS Multiplex System I. Single-read sequencing results were obtained using the Illumina Genome Analyzer IIx platform with 40 bp reads. The graph represents the distribution of sequencing reads in the 5′ to 3′ direction for RefSeq transcripts that are 1.5 to 3.0 Kb in length. The track labeled Poly(A)+ Reference is from a publicly available RNA-Seq data generated using isolated mRNA as input to library construction.

FIGURE 6 Differential Expression Using Ovation RNA-Seq System V2 and qPCR



Differences between Log_2 transformed expression values for MAQC A and MAQC B samples, RNA-Seq data using 2 ng total RNA as input are plotted on the X axis, qPCR data are plotted on the Y axis. 659 TaqMan probes that uniquely map to the RefSeq annotations used in the RPKM calculations are represented. RPKM stands for **R**eads **P**er **K**ilobase of exon model per **M**illion 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.

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