

Ovation® RNA-Seq System

The first RNA-Seq sample preparation solution for complete transcriptome representation from picogram quantities of total RNA

Highlights of the Ovation RNA-Seq System

- More complete transcriptome representation from picograms of total RNA:** Obtain RNA-Seq data from mRNA and non-polyadenylated transcripts with no 5'/3' positional biases, or added steps of poly(A) selection or rRNA depletion
- Fast and easy protocol based on proven technology:** The Ovation RNA-Seq System yields double-stranded cDNA in 6 hours ready for RNA-Seq library construction, and is powered by Ribo-SPIA® technology, invented by NuGEN®
- A single workflow for multiple applications:** The cDNA may also be used for analysis on microarrays or qPCR, for quality assessment prior to RNA-Seq

Introduction

The arrival of Next Generation Sequencing (NGS) technology has revolutionized the field of genomics and expanded the scope of scientific inquiry to include sequencing Giga bases (Gb) of genomes and transcriptomes in a matter of days. RNA-Seq is a specific technique enabled by NGS, and refers to direct sequencing of cDNAs to permit both sequencing and quantitation of transcript levels simultaneously.

NuGEN's new Ovation RNA-Seq System extends the power and flexibility of this new technology to sample preparation directly from total RNA, with input levels in the range of 500 pg to 100 ng. Researchers can now conduct RNA-Seq research directly from low inputs of total RNA, with uniform read coverage across the full-length of poly(A)+ RNA and non-polyadenylated transcripts.

The digital precision and sensitivity of RNA-Seq is well-suited to the analysis of low-input samples, yet commercially available reagent kits require total RNA above 1.0 microgram (µg). Moreover,

in order to reduce the cost of sequencing and data analysis, many RNA-Seq protocols require additional enrichment steps to select for poly(A)+ RNA and/or reduce the content of ribosomal RNA prior to library construction. Such fractionation procedures can both introduce bias to the relative levels of transcripts, and limit analysis to transcripts having a poly(A) tail. With NuGEN's Ovation RNA-Seq System you now have a more complete picture of the transcriptome, enabling low input preparation of cDNA for RNA-Seq library construction, without the need for rRNA reduction or poly(A) selection.

TABLE 1 Sequencing Performance Metrics from 500 pg of Total RNA

Parameter	Brain 1	Brain 2	Brain 3	Average
Total Reads	21201900	20541551	24854509	22199320
% Unique Reads	48.0	49.1	45.8	47.6
% Mapped Reads	73.0	74.5	69.3	72.3
% rRNA	2.9	3.2	2.8	3.0
# RefSeq Genes	3416939	3449399	3795738	3554025
Parameter	UHR 1	UHR 2	UHR 3	Average
Total Reads	20340793	26163192	26647866	24383950
% Unique Reads	47.5	44.9	44.9	45.8
% Mapped Reads	73.7	69.5	69.4	70.9
% rRNA	4.0	3.3	3.2	3.5
# RefSeq Genes	3831489	4051334	4098186	3993669

Total Reads - Number of unique clusters/lane; % Unique Reads - % of reads uniquely mappable to the hg18 reference using ELAND default parameters; % Mapped Reads - % of reads with at least one match in the hg18 reference; % rRNA - % of reads that map to 18S, 28S or 5.8S ribosomal sequences in the hg18 reference; and # RefSeq Genes - # of reads that uniquely map to the RefSeq annotation of hg18 reference. MAQC Human Brain Reference and Universal Human Reference (UHR) total RNA was processed with the Ovation RNA-Seq System, and sequenced on the Illumina Genome Analyzer IIx with 36 base-pair single-read sequencing.

Simple and fast workflow easily integrated with library construction protocols

As illustrated in **Figure 1**, the Ovation RNA-Seq System protocol yields double-stranded cDNA ready for the construction of RNA-Seq libraries. The protocol can be completed in approximately 6 hours, and yields sufficient cDNA for several sequencing runs.

The size range of the resulting cDNA is approximately 50 bases to 1.5 kb before adapter ligation as measured on the Agilent Bioanalyzer (**Figure 2**), with > 60% of the amplified product falling below 500 bases. The size of amplified cDNA product is optimized for direct integration into the standard library construction protocol provided by NGS suppliers with no further fragmentation required.

Reproducible sequencing results without rRNA removal directly from 500 pg of total RNA

In order to evaluate the performance of RNA-Seq using material generated with the Ovation RNA-Seq System, libraries were constructed using total RNA from Human Brain Reference and Universal Human Reference (UHR) MAQC samples, amplified by three independent operators, and sequenced by single-read sequencing with 36 base-pair reads on the Illumina Genome Analyzer IIx platform.

As shown in **Table 1**, total RNA inputs of 500 pg for each sample generated an average of approximately 20 to 26 million total reads per flow cell lane, with approximately 46% unique reads (mapped only once in the reference sequence), and 72% mapped reads (all reads that are mappable to the reference genome). Each of these aggregate sequencing metrics was similar across replicate determinations, and generally the same between brain and UHR samples. These parameters do not vary significantly from previously published RNA-Seq results using poly(A) selected material from higher starting amounts of unamplified total RNA.

FIGURE 1 Schematic of the Ovation RNA-Seq Process

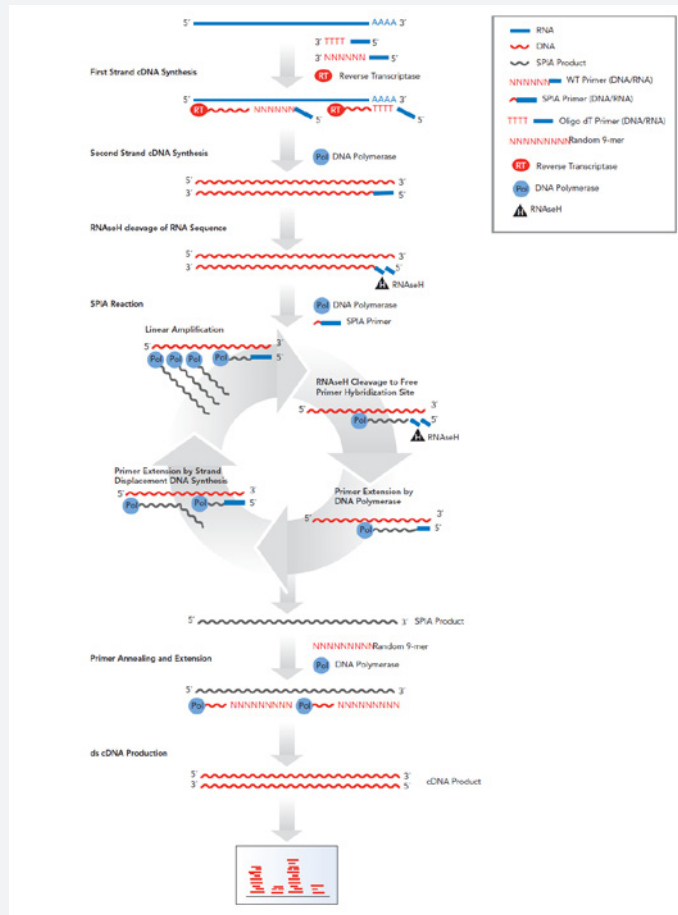
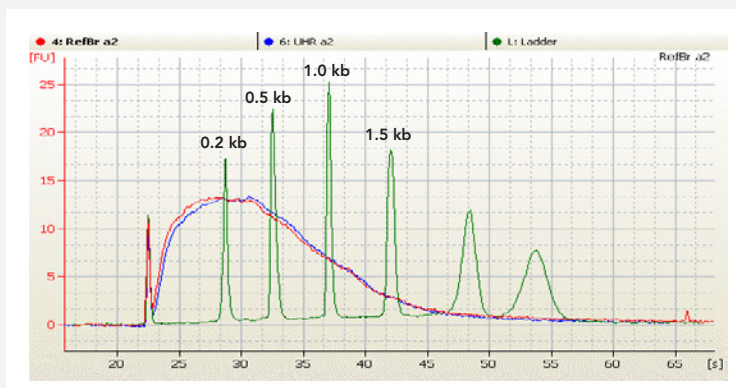


FIGURE 2 Size Distribution of Amplified cDNA Products



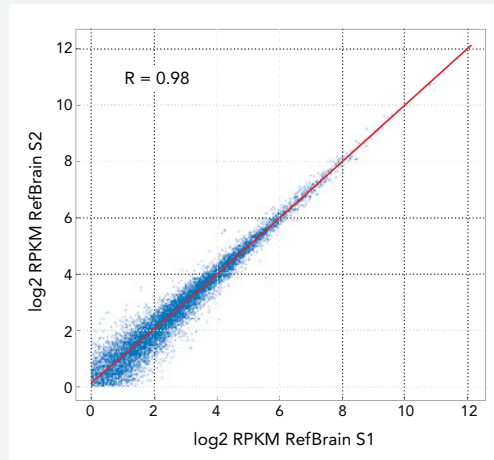
Bioanalyzer trace of amplified cDNA obtained from 10 ng of total Human Reference Brain RNA (red line) and Universal Human Reference RNA (blue line). Results were obtained using an RNA 6000 Nano LabChip® (Agilent Technologies). The positions of the RNA 6000 size ladder are shown in green.

The Ovation RNA-Seq System produced high quality libraries, and as expected, significantly higher read coverage in non-polyadenylated regions of the transcriptome in comparison to libraries made with poly(A) selected sequences.

In addition, inter-user reproducibility was very good as shown in **Figure 3**, where a high correlation was observed between RNA-Seq data obtained from two independent operators ($R=0.98$). These results demonstrate the consistent performance of the Ovation RNA-Seq System for NGS.

With the proprietary combination of reverse transcriptase and primers in the Ovation RNA-Seq System, non-rRNA sequences are preferentially primed and subsequently amplified, reducing the number of reads from rRNA sequences. Reads mapping to rRNA sequences were in the range of 2.9% to 4%, without the use of procedures for rRNA depletion or other enrichment techniques. These results illustrate that high-quality sequencing metrics are obtained using picograms quantities of total RNA amplified with the Ovation RNA-Seq System, without the added cost, time and potential for bias introduced by preprocessing total RNA.

FIGURE 3 Inter-user Reproducibility



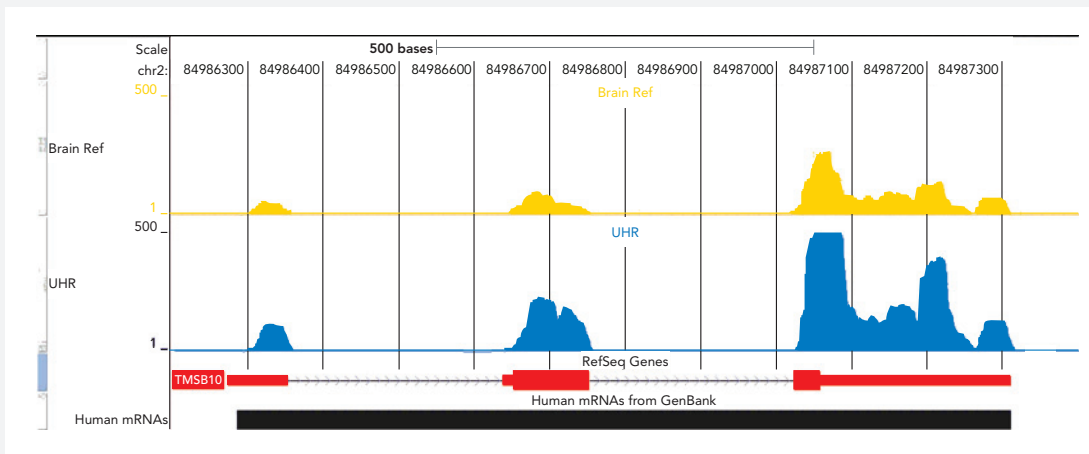
Scatterplot of the log₂ RPKM values for two Human Reference Brain samples (designated as S1 and S2) prepared using 10 ng of total RNA and amplified by two independent operators with the Ovation RNA-Seq System. RPKM stands for Reads Per Kilobase of exon model per Million mapped reads. The RPKM measure 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 Ix platform.

An example of a differentially expressed gene detected in these samples is shown in **Figure 4**, where a 2.75-fold higher level of expression was observed in UHR relative to human brain based on reads mapped to three exons in TMSB10 (Thymosin, beta 10). The density of reads across each exon is

even, illustrating the lack of positional bias of the read coverage.

Taken together, these results demonstrate that amplified cDNA produced from total RNA using the Ovation RNA-Seq System generates high-quality data using Next Generation Sequencing technology.

FIGURE 4 Differential Expression of TMSB10 (Thymosin, beta 10)



Data from UCSC genome browser demonstrating differential expression of the TMSB10 gene in Human Brain Reference vs. Universal Human Reference (UHR) MAQC samples. The average # reads/base is 190 for UHR and 69 for Brain ref, indicating 2.75-fold difference in expression levels.

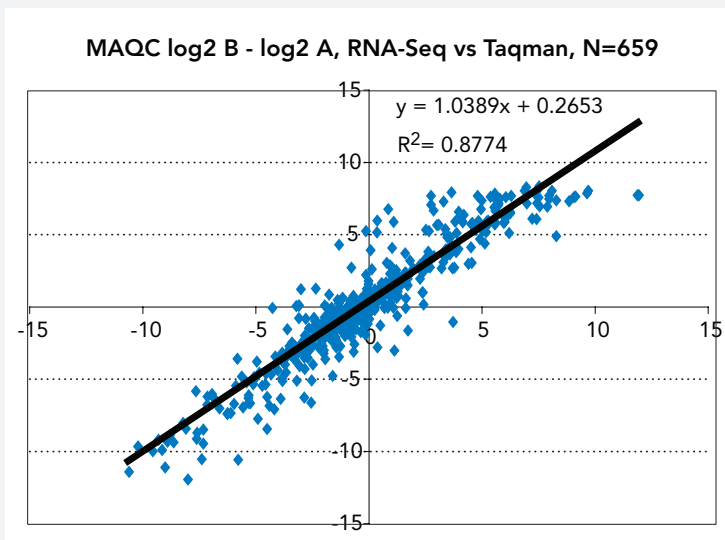
High concordance with qPCR results for differential expression analysis

MAQC Human Brain Reference and UHR samples amplified with the Ovation RNA-Seq System were analyzed by RNA-Seq to compare with the differential expression data generated by quantitative PCR (qPCR) using the TaqMan® assay. As shown in **Figure 5**, differential expression fold changes are concordant with this reference expression assay (R²) without significant data compression as evidenced by the slope. These results demonstrate that differentially expressed genes can be quantified by RNA-Seq using material amplified with the Ovation RNA-Seq System in a manner consistent with results obtained by qPCR.

Conclusion

The Ovation RNA-Seq System is the first commercial solution to enable Next Generation Sequencing directly from picogram quantities of total RNA, without the use of fractionation steps that could limit complete sequencing of the transcriptome. The System provides for a simple protocol that can be completed in approximately 6 hours, without the need for rRNA reduction or poly(A) selection. The same amplified material may also be used for qPCR or microarray analysis. Based on NuGEN's proven Ribo-SPIA technology, the

FIGURE 5 Comparison of Ovation RNA-Seq and qPCR



Differential expression data was generated for MAQC A (Human Universal Reference) and MAQC B (Human Brain Reference) by both RNA-Seq and qPCR. RNA-Seq data from 10 ng of total RNA is plotted on X-axis, qPCR data plotted on Y-axis. 659 TaqMan probes that uniquely map to the RefSeq annotations used in the RPKM calculations are represented. The qPCR results were originally reported in Shi, L. et al. Nature Biotechnology (2006) 24(9): 1151-1161.

Ovation RNA-Seq System yields several micrograms of cDNA ready for RNA-Seq library construction. This new kit 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 the complete investigation of the transcriptome with the power of RNA-Seq.

ORDERING INFORMATION

Catalog No.	No. of Reactions
Ovation RNA-Seq System	
7100-08	8 reactions
Technical Documents	
Ovation RNA-Seq System User Guide Ovation RNA-Seq System Quick Protocol	

NuGEN Technologies, Inc.

Headquarters USA

201 Industrial Road, Suite 310
San Carlos, CA 94070 USA
Toll Free Tel: 888.654.6544
Toll Free Fax: 888.296.6544
custserv@nugeninc.com
techserv@nugeninc.com

Europe

P.O. Box 149,
6680 AC Bommel,
The Netherlands,
Tel: +31-13-5780215
Fax: +31-13-5780216
europe@nugeninc.com

For our international distributor's contact information, visit our website

www.nugeninc.com



© 2009, NuGEN Technologies Inc. The Ovation® System family of products and methods is covered by U.S. Patent Nos. 6,692,918, 6,251,639, 6,946,251 and 7,354,717, and other issued and pending patents in the US and other countries. NuGEN, Ovation, SPIA, Ribo-SPIA, WT-Ovation, FL-Ovation, Encore, Applause and Imagine More From Less are trademarks or registered trademarks of NuGEN Technologies, Inc. Other marks appearing in these materials are marks of their respective owners.