APPLICATION HIGHLIGHT

Lower Cost, Higher Throughput Library Preparation with the Echo® Liquid Handler and the NuGEN Ovation® Single Cell RNA-Seq System

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Abstract

In this proof of concept study (POC), the Ovation Single Cell RNA-Seq System miniaturization was validated by examining libraries prepared and sequenced at traditional and miniaturized reaction volumes. Additionally, the sample and reagent transfers were automated using the Echo acoustic liquid handling technology. Echo liquid handlers transfer a wide range of fluids without contact of tips or recalibration between fluid types. The industry leading accuracy and precision of Echo liquid handlers at microliter and nanoliter volumes in combination with the NuGEN Ovation Single Cell RNA-Seq System increases library preparation throughput while reducing the costs. This enables a broader application of transcriptome analysis with NGS.

METHODS

Construction of Strand-specific Single Cell Transcriptome Libraries

First-strand synthesis is carried out with selective primers, oligo dT, and the incorporation of a nucleotide analog. cDNA processing degrades the nucleotide analog and the original template RNA, leaving single-stranded antisense cDNA. A random octamer with forward adaptor attaches to the 5' end and is then used to prime the second-strand cDNA synthesis. Following end repair, the reverse adaptor is ligated. Final PCR amplification results in a strand-specific cDNA library enriched for coding and regulatory sequences:

FIGURE 1 ▶

NuGEN Ovation Single Cell RNA-Seq System Workflow

Echo® 525 Liquid Handler

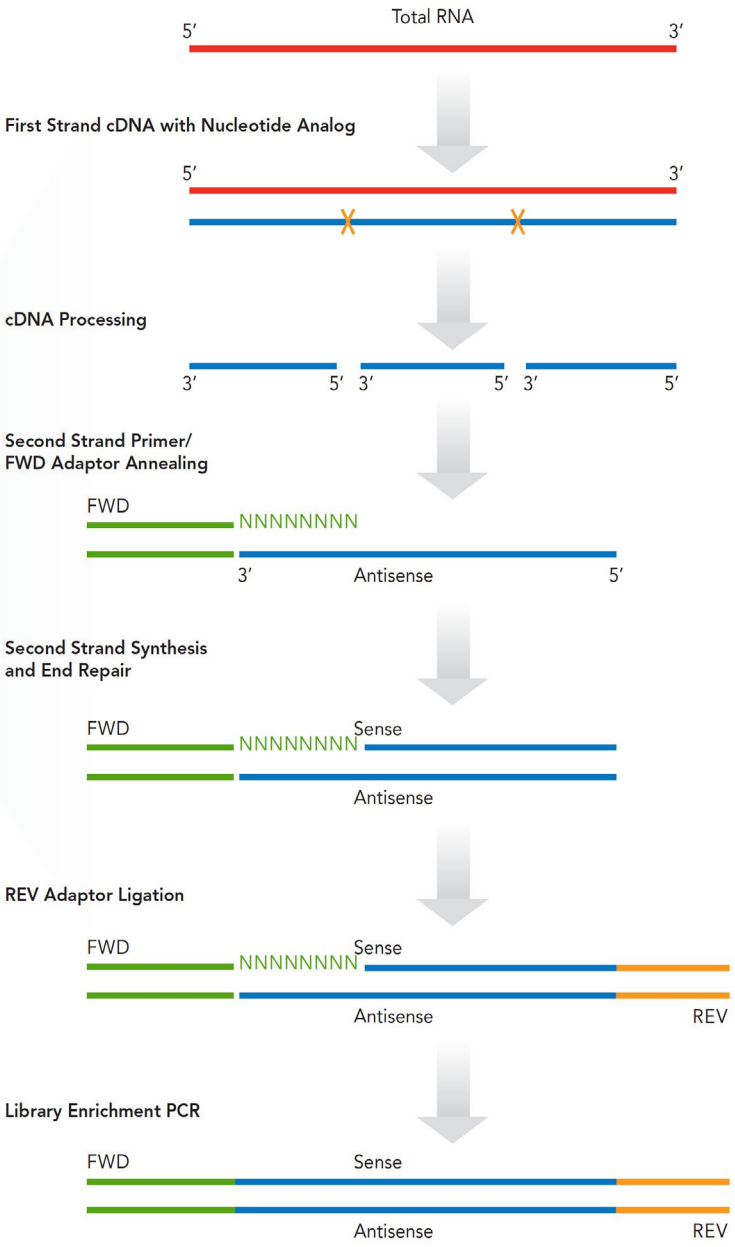

Image courtesy of NuGEN Technologies, Inc.

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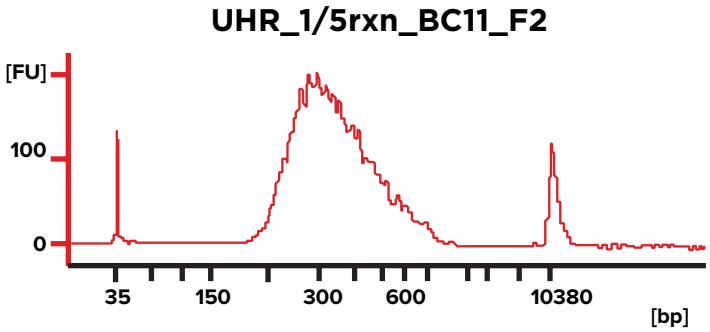
Libraries were generated with the Ovation Single Cell RNA-Seq System using 50 pg of MAQC A and MAQC B RNA. The Echo liquid handler delivered both the standard 40 µl and miniaturized 8 µl total reaction volume. The libraries were sequenced on an Illumina sequencer with 40 bp single read sequencing.

FIGURE 2 ▶

Agilent 2100 Bioanalyzer micrographs of miniaturized reactions (8 µl) for both MAQC A and MAQC B generated quality library preparations



For each of the RNA sources tested, there was a high percentage of aligned reads with minimal contributions from rRNA. Libraries were also enriched for intronic and intergenic sequences that might be of particular interest for noncoding RNAs studies. Good agreement was seen between full reaction volumes of 40 µL and miniaturized reactions at 8 µL and good reproducibility across miniaturized reaction replicates.

TABLE 1 ▶

Sequencing Alignment Statistics for cDNA Libraries

Source of Total RNA 50pg

	Human UHR (MAQC A)				Human Brain (MAQC A)	
Reaction Volume	8µl	8µl	8µl	40µl	40µl	40µl
% of Total RNA						
Not Aligned	14%	13%	18%	36%	24%	37%
Aligned	86%	87%	82%	64%	76%	63%
% of Mapped Reads by Category						
All non-rRNA	82.2%	79.7%	83.2%	97.4%	83.4%	79.9%
All rRNA	17.8%	20.3%	16.8%	2.6%	16.6%	20.1%
Distribution of RefSeq Reads						
Exons	37.4%	41.4%	33.8%	12.6%	26.9%	34.3%
Introns	41.9%	39.4%	37.7%	27.1%	47.6%	46.4%
Intergenic	20.6%	19.2%	28.5%	60.4%	25.5%	19.4%
RefSeq Strand Retention						
Exons	85.7%	84.5%	70.9%	97.5%	98.3%	74.3%
5' UTR	93.5%	94.8%	94.0%	94.9%	97.1%	83.5%
3' UTR	86.8%	81.7%	72.1%	99.1%	98.5%	75.0%

SUMMARY

The Echo 525 Liquid Handler was used to generate sequencing quality libraries with the NuGEN Ovation Single Cell RNA-seq system.

- The sequencing results showed consistent results in both the full reaction and miniaturized reaction for the Human UHR MAQC A and Human Brain MAQC B control RNA preparations.
- The reactions were run in a 384-well format and are automatable enabling higher throughput.
- The reactions were miniaturized resulting in reduced cost per library generated.

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