Automation of NuGEN Ovation RNA-Seq System V2 on the Agilent NGS Bravo

Application Note

Authors

Lisa Knapp

Agilent Technologies, Inc.

1362 Kirklees Dr

Carmel, IN 46032

Lin Z Pham

NuGEN Technologies, Inc.

201 Industrial Road, Suite 310

San Carlos, CA 94070

Introduction

The Ovation RNA-Seq System V2 from NuGEN Technologies provides a fast and simple method for preparing amplified cDNA from total RNA for RNA-Seq applications, with enhanced transcript coverage and uniform distribution of sequencing reads. Amplification is initiated at the 3' end as well as randomly throughout the whole transcriptome in the sample. This feature makes the Ovation RNA-Seq System V2 ideal for amplification prior to Next Generation Sequencing, as it allows amplification of both mRNA and non-polyadenylated transcripts.

The Ovation RNA-Seq System V2 is enabled by Ribo-SPIA technology, a rapid, simple, and sensitive RNA amplification process developed by NuGEN. Using Ribo-SPIA technology and starting with as little as 500 pg total RNA, µg quantities of cDNA can be prepared in approximately 4.5 hours.

The Ovation RNA-Seq System V2 (NuGEN part number 7102) provides optimized reagent mixes and a protocol to process eight or 32 total RNA samples, and is also available as an automation solution for processing 96 samples.

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Automated protocols are now available for the Ovation RNA-Seq V2 workflow on Agilent NGS Bravo (part number G5541A) (Figure 1).

Experimental Setup

The NuGEN Ovation RNA-Seq System V2 form-based protocols provide an interactive, visual layout for the end user. The underlying VWorks protocols have been designed to allow laboratory personnel to use the same protocol to run one to 12 columns of samples. Preparation of double-stranded cDNA from total RNA for 96 samples can be accomplished in less than 5 hours. Each protocol has a unique deck layout, which is shown in Figure 2. This is also used to start the protocol and select options such as the number of columns of samples to process.

The labware used in the protocol was selected for optimal performance while being cognizant of minimizing dead volumes when reagent conservation is critical and ease of setup for those reagents where cost is negligible. The Current Tip State selection allows partial boxes of tips to be used to minimize tip waste.

Figure 1. Agilent Bravo platform for NGS.

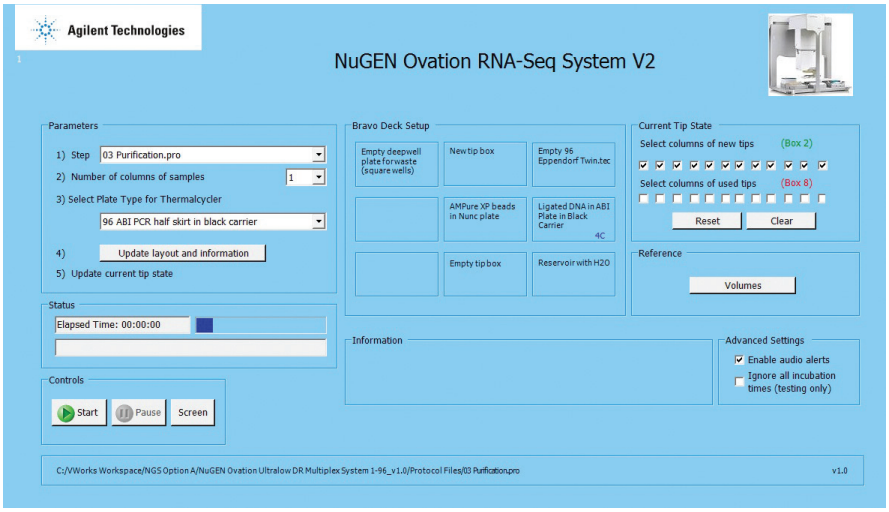


Figure 2. VWorks protocol form.

Links are provided to a spreadsheet, which calculates volumes for all master mixes (Figure 3) and has space provided to enter lot number information or any other information which may be required for documentation within the lab.

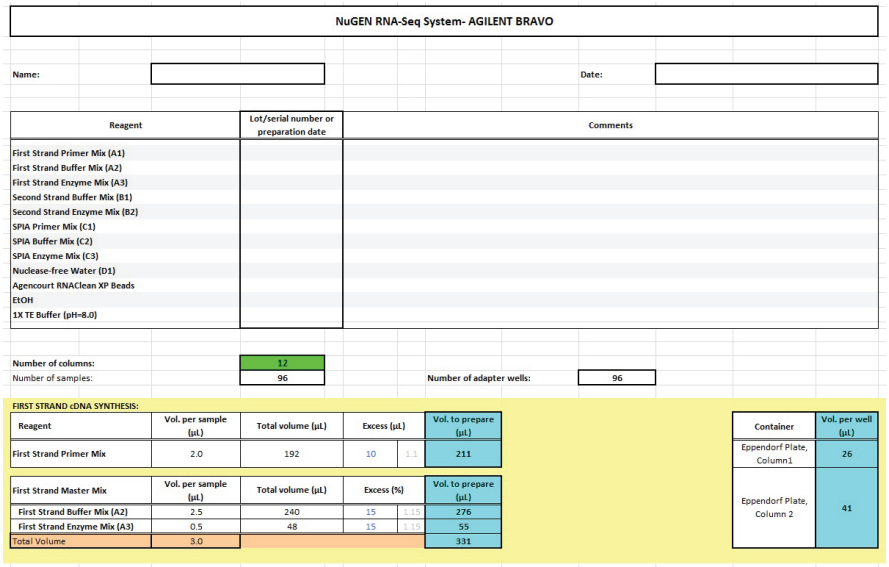
Results and Discussion

Two nanograms of Universal Human Reference total RNA (MAQC A - part number 740000) and Brain total RNA (MAQC B) were used to generate cDNA using the Ovation RNA-Seq System V2 (part number 7102) on Agilent NGS Bravo. One hundred nanograms of the resulting cDNA was used to produce sequencing libraries using the NuGEN Ovation Ultralow Library System on the Agilent NGS Bravo.

Table 1 shows the sequencing alignment metrics with the expected level of uniquely mapped reads, low levels of rRNA reads, and good detection of RNA-Seq transcripts, as compared to the control data from manual preps.

Conclusion

Automated protocols of Ovation RNA-Seq System V2 generated similar technical performance as compared to the manual process. With automation, up to 96 samples can be processed simultaneously. This is a marked improvement in throughput over the manual method without comprising the integrity of the experiment. The Ovation RNA-Seq System V2 is available from NuGEN in a 96 reaction size (part number 7102-A01).



NuGEN RNA-Seq System- AGILENT BRAVO

Name: Date:

Reagent	Lot/serial number or preparation date	Comments
First Strand Primer Mix (A1)		
First Strand Buffer Mix (A2)		
First Strand Enzyme Mix (A3)		
Second Strand Buffer Mix (B1)		
Second Strand Enzyme Mix (B2)		
SPIA Primer Mix (C1)		
SPIA Buffer Mix (C2)		
SPIA Enzyme Mix (C3)		
Nuclease-free Water (D1)		
Agencourt RNAClean XP Beads		
EtOH		
1X TE Buffer (pH=8.0)		

Number of columns: 12
Number of samples: 96 Number of adapter wells: 96

FIRST STRAND cDNA SYNTHESIS:

Reagent	Vol. per sample (µL)	Total volume (µL)	Excess (µL)		Vol. to prepare (µL)
First Strand Primer Mix	2.0	192	10	1.1	211

First Strand Master Mix	Vol. per sample (µL)	Total volume (µL)	Excess (%)		Vol. to prepare (µL)
First Strand Buffer Mix (A2)	2.5	240	15	1.15	276
First Strand Enzyme Mix (A3)	0.5	48	15	1.15	55
Total Volume	3.0				331

Container	Vol. per well (µl)
Eppendorf Plate, Column1	26
Eppendorf Plate, Column 2	41

Figure 3. Volume tables and data sheet.

Table 1. Sequencing alignment metrics.

Sample	Method	Total reads	% Mapped reads	% Unique reads	% rRNA reads	# Ref-Seq genes
Brain	Bravo	14,895,950	86	61.89	38.30	15,970
UHR	Bravo	13,238,807	87	56.59	35.40	16,037
UHR_rep1	Manual	13,531,223	66	40.33	36.10	15,544
UHR_rep2	Manual	14,051,431	67	41.05	37.10	15,500

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