A STUDY OF THE FL-OVATION™ cDNA BIOTIN MODULE V2 PERFORMANCE

BACKGROUND

Gene expression studies have benefited from global approaches such as microarray analysis using the Affymetrix GeneChip® array platform and traditional target preparation methods. These studies have, to date, utilized relatively large amounts of RNA and time consuming and laborious procedures.

The requirement for lower RNA input amounts and higher throughput in gene expression projects have significantly increased the demand for simpler, less cumbersome and laborious target preparation approaches. NuGEN's Ovation™ System family of products has met these challenges by enabling the amplification and labeling of minute amounts of total RNA through a fast, simple and automation-friendly process (see diagram on page 2).

The FL-Ovation cDNA Biotin Module V2 is the first of NuGEN's Fragmentation and Labeling (F&L) products, allowing the preparation of up to 5 µg of cDNA for GeneChip array analysis, in less than 2 hours with no need for purification. It is designed for use with cDNA generated using one of two Ovation™ family amplification products: WT-Ovation™ Pico RNA Amplification System (Cat.# 3300), or the Ovation Biotin System (Cat.# 2300).

Although the Ovation Biotin System already contains a set of fragmentation and labeling reagents, for specific applications such as automation and challenging tissue sources with expected low yields the use of the FL-Ovation cDNA Biotin Module V2 is highly advantageous for studies requiring higher sensitivity, shorter hands-on time, elimination of the column step, and facilitation of high throughput sample processing.

Here we describe a set of studies that demonstrate the performance of FL-Ovation cDNA Biotin Module V2 product with cDNA generated with both the WT-Ovation Pico RNA Amplification System (Cat.# 3300-12) as well as the Ovation Biotin System (Cat.# 2300-12).

MATERIALS AND METHODS

In the studies described here, total HeLa cell line RNA was purchased (Ambion, Cat. #7852) and UHR total RNA was purchased (Stratagene, Cat.# 740000). SPIA™ cDNA pools were prepared from 500 pg of HeLa total RNA using the WT-Ovation™ Pico RNA Amplification System, as well as 5 ng of UHR total RNA using the Ovation Biotin System following the procedure outlined in the user guide for each product. Purification and quantitation of the cDNA were also performed following the user guide procedures.

Fragmentation and labeling of all cDNAs were completed using the FL-Ovation cDNA Biotin Module V2 according to the product user guide. Five micrograms of the WT-Ovation Pico System amplified cDNA and 3.75 µg of the Ovation Biotin System generated cDNA in 25 µl were used as input into each F&L reaction. The resulting fragmented and biotin-labeled cDNA targets were in a final volume of 50 µl after a two-step reaction. Array analysis was performed on HG-U133A 2.0 GeneChip arrays (Affymetrix, Cat.#900469). 34 µl of the 50 µl F&L reaction was used to prepare 150 µl of array hybridization solution. This resulted in final cDNA concentration of 22.7 ng/µl for WT-Ovation Pico System amplified cDNA and 17 ng/µl for Ovation Biotin System amplified cDNA. Hybridization, washing and staining protocols outlined in the FL-Ovation cDNA Biotin Module V2 user guide were followed. Array data was analyzed by Affymetrix GCOS software (GeneChip Operating System, 1.4.0.036).

Agilent Bioanalyzer with an RNA 6000 Nano LabChip® (Agilent Cat. #5065-4476) and the Eukaryotic Total RNA Nano program (Nano assay in the Expert 2100 software) were used, according to the manufacturer's instructions.

RESULTS AND CONCLUSIONS

FL-Ovation cDNA Biotin Module V2 utilizes chemical and enzymatic reactions to fragment SPIA™ cDNA. The labeling reaction produces a biotin on 3' end of fragmented cDNA resulting in targets that are suitable for GeneChip array analyses. In **Figure 1**, the typical size distribution of WT-Ovation Pico System cDNA amplified from HeLa RNA is shown before and after fragmentation and labeling. The Bioanalyzer traces vary slightly depending on the source RNA but the post amplification trace for most samples will look very similar to the one shown in Figure 1.

FL-Ovation cDNA Biotin Module V2 reagents can be used to fragment and label SPIA™cDNA generated with

WT-Ovation Pico System and Ovation Biotin System. To demonstrate the performance of the FL-Ovation cDNA Biotin Module V2 with cDNA generated from both systems, we prepared SPIA™cDNA with 500 pg and 5 ng of total RNA, the lowest input amount recommended by NuGEN. cDNA samples generated from each respective system were first pooled then fragmented and labeled, and finally hybridized to HG-U133A 2.0 arrays. Reproducibility of the FL-Ovation cDNA Biotin Module V2 using cDNA generated with the WT-Ovation Pico RNA Amplification System is shown on page 3. **Table 1** shows array performance metrics among three independent F&L reactions with pooled cDNA, demonstrating the high level of array performance and reproducibility among replicates. **Figure 2** shows signal correlations and call concordance between arrays 1 and 2. The two independent fragmentation reactions using WT-Ovation Pico System amplifications of 500 pg HeLa RNA show a signal correlation of 0.993 and a call concordance of 93.4%.

The same type of data is shown in **Table 2** and **Figure 3** for cDNA generated with the Ovation Biotin System and the FL-Ovation cDNA Biotin Module V2. Table 2 shows the array performance metrics for three independent F&L reactions performed with pooled cDNA. Reproducibility between arrays from the independent F&L reactions is high with a signal correlation of 0.994 and a call concordance of 94.7%.

We recruited individuals without prior experience in running the FL-Ovation cDNA Biotin Module V2 procedures to further demonstrate the high level of reproducibility and robustness of the protocol in **Figure 4**. Pooled amplified cDNA generated from individual HeLa RNA amplifications with the WT-Ovation Pico System was used with a 5 µg cDNA input per F&L reaction. Three individuals ran independent reactions according to the standard NuGEN protocol. Two reactions from each user were analyzed on HG-U133A 2.0 arrays. Among the six arrays, the pair-wise signal correlations had an average R^2 value of 0.993 and average pair-wise call concordance of 92.9%. Signal correlation and call concordance are very high between three intra-operator comparisons, with an average R^2 of 0.993 $\pm$ 0.004 (average $\pm$ SD) and a call concordance of 92.9 $\pm$ 0.3%. The 12 inter-operator comparisons also showed very high reproducibility with an average R^2 of 0.993 $\pm$ 0.002 and call concordance of 92.9% $\pm$ 0.3. The correlations and concordance calls of inter-operator arrays were identical to that of duplicate arrays from a single operator indicative of the robustness of the protocol.

We next obtained data from three different lots of the FL-Ovation cDNA Biotin Module V2 to demonstrate robust and consistent kit performance. Pooled amplified cDNA generated from individual 500 pg HeLa RNA amplifications with the WT-Ovation Pico System was used with a 5 µg cDNA input per F&L reaction. Reactions using three independent kit lots were ran according to the standard NuGEN protocol. Two reactions from each kit lot were analyzed on HG-U133A 2.0 arrays. Among the six arrays, the pair-wise signal correlations had an average R^2 value of 0.992 and average pair-wise call concordance values of 93.2%, shown in **Figure 5**. Signal correlation and call concordance are very high between the three intra-lot comparisons, with an average R^2 of 0.993 $\pm$ 0.001 and call concordance of 93.3% $\pm$ 0.2. The 12 inter-lot comparisons also showed very high reproducibility with an average R^2 of 0.992 $\pm$ 0.002 and call concordance of 93.3% $\pm$ 0.2. The correlations and concordance calls of inter-lot arrays were as high as that of duplicate arrays from a single kit lot indicating highly consistent reagent performance.

In conclusion, the results shown here strongly demonstrate the high reproducibility of the FL-Ovation cDNA Biotin Module V2 product for GeneChip array target preparation.

The combination of the high level of sensitivity, high labeling efficiency, and the ease of use offered by this product, can make a significant impact on the overall quality and efficacy of gene expression studies. Because this assay is a simple, mix, add, and incubate approach, without cumbersome purification steps, this product is ideal tool for high throughput sample processing.

Systems Specifications

Cat No.: 4200-12, 12 reactions

Input: 3.75 - 5 µg cDNA

Yield: Fragmented and biotin-labeled cDNA

A Simple Add & Incubate Process

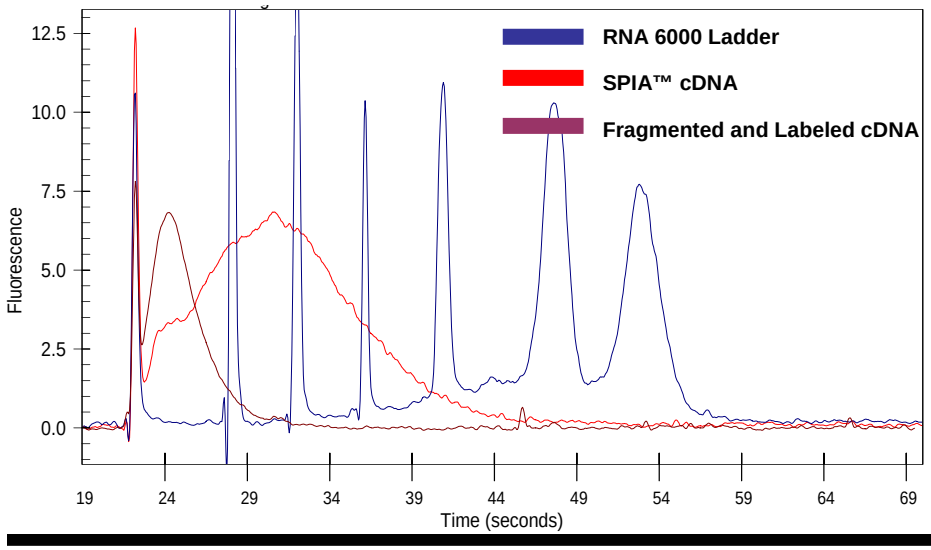

Figure 1. Agilent Bioanalyzer trace of amplified, un-fragmented and fragmented cDNA Product. HeLa RNA amplified with the WT-Ovation Pico System (cat.# 3300-12, SPIA™cDNA) was processed with the FL-Ovation cDNA Biotin Module V2, (fragmented and labeled cDNA), and analyzed on the Bioanalyzer.

Using cDNA generated by WT-Ovation™ Pico System

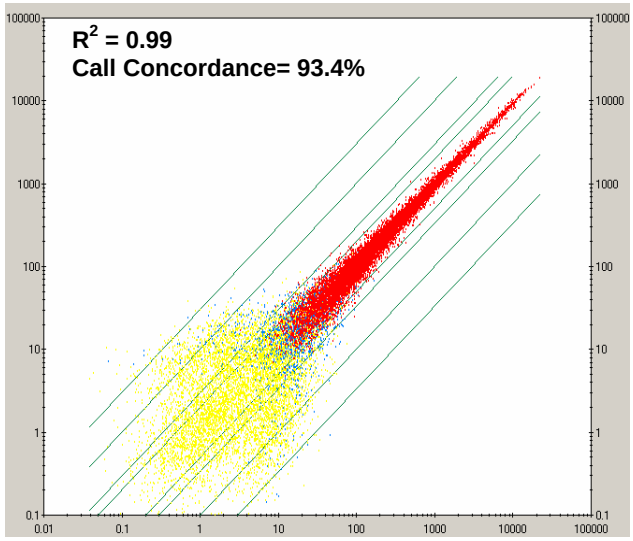

Table 1. Array metrics for triplicate F&L reactions.

Arrays	Raw Q	Scaling Factor	Back-ground	% Present	(3'/5') GAPDH	(3'/5') Actin
1	0.82	1.8	28.2	61.2	1.15	7.6
2	0.89	1.6	30.7	61.2	1.14	7.7
3	0.91	1.4	31.9	62.8	1.26	8.7
Avg	0.87	1.6	30.3	61.7	1.18	8.0
SD	0.05	0.2	1.91	0.92	0.07	0.62

Figure 2. Signal Correlation for two independent FL-Ovation cDNA Biotin Module V2 reactions using pooled cDNA generated from 500 pg HeLa RNA, analyzed on GeneChip arrays, show a high level of signal correlation and call concordance, (arrays 1 and 2 from Table 1 above).

Using cDNA generated by Ovation™ Biotin System

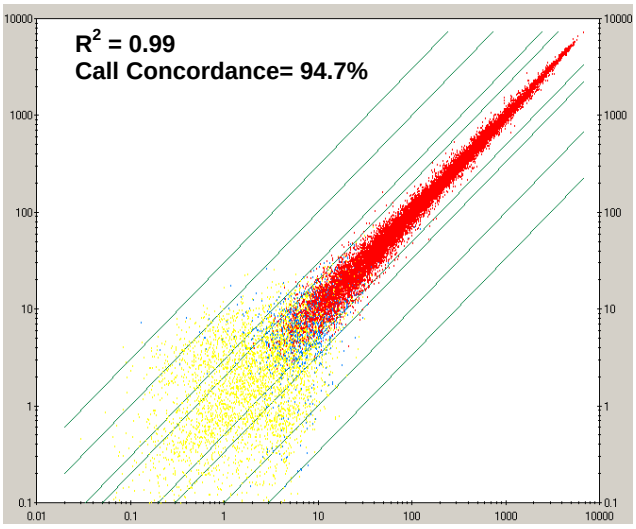

Table 2. Array metrics for triplicate F&L reactions.

Arrays	Raw Q	Scaling Factor	Back-ground	% Present	(3'/5') GAPDH	(3'/5') Actin
1	1.09	0.5	33.8	79.1	1.25	3.1
2	0.99	0.6	35.1	77.9	1.21	3.4
3	1.07	0.7	35.7	76.9	1.27	3.6
Avg	1.05	0.6	34.9	78.0	1.24	3.4
SD	0.05	0.1	0.99	1.10	0.03	0.26

Figure 3. Signal Correlation for two independent FL-Ovation cDNA Biotin Module V2 reactions using pooled cDNA generated from 5 ng UHR RNA, analyzed on GeneChip arrays, show a high level of signal correlation and call concordance, (arrays 2 and 3 from Table 2 above).

R^2	1	2	3	4	5
2	0.992				
3	0.996	0.990			
4	0.995	0.991	0.994		
5	0.993	0.988	0.992	0.995	
6	0.995	0.990	0.996	0.994	0.992

Call Concordance	1	2	3	4	5
2	93.1				
3	93.3	93.1			
4	93.0	93.0	93.2		
5	92.4	92.6	92.6	92.6	
6	93.1	92.9	93.1	93.3	92.4

Figure 4. FL-Ovation cDNA Biotin Module V2 reproducibility among different operators.
Pooled, amplified cDNA generated from individual 500 pg HeLa RNA amplifications with the WT-Ovation Pico System was used with 5 µg cDNA input per F&L reaction. Three individuals ran independent reactions according to the standard NuGEN protocol. Two reactions from each user were analyzed on HG-U133A 2.0 arrays. Among the six arrays, the pair-wise signal correlations had average R^2 values of 0.993 and average pair-wise call concordance of 92.9%. Arrays 1 and 4 were processed by operator 1, arrays 2 and 5 by operator 2, and arrays 3 and 6 by operator 3.

R^2	1	2	3	4	5
2	0.993				
3	0.991	0.992			
4	0.990	0.993	0.993		
5	0.991	0.992	0.994	0.991	
6	0.990	0.994	0.993	0.996	0.992

Call Concordance	1	2	3	4	5
2	93.2				
3	93.4	93.3			
4	93.0	93.2	92.9		
5	93.4	92.8	93.4	93.4	
6	93.1	93.4	93.2	93.6	92.9

Figure 5. Lot-to-lot performance of FL-Ovation cDNA Biotin Module V2.
Pooled, amplified cDNA generated from individual 500 pg HeLa RNA amplifications with the WT-Ovation Pico System was used with 5 µg cDNA input per F&L reaction. Reactions using three independent assembled kit lots were ran according to the standard NuGEN protocol. Two reactions from each kit lot were analyzed on HG-U133A 2.0 arrays. Among the six arrays, the pair-wise signal correlations had average R^2 values of 0.992 and average pair-wise call concordance of 93.2%. The correlations and concordance calls of inter-kit lot arrays were as good as that of duplicate array from a single kit lot. Arrays 1 and 2 represent lot #1, arrays 3 and 4 represent lot #2, and arrays 5 and 6 represent lot #3.

NuGEN Technologies, Inc.
Headquarters USA
821 Industrial Road, Unit A San Carlos, CA 94070 USA, Toll Free Tel: 888.654.6544 Toll Free Fax: 888.296.6544 www.nugeninc.com
custserv@nugeninc.com techserv@nugeninc.com

Canada
MJS BioLynx Inc.
P.O Bag 1150, 300 Laurier Blvd.
Brockville, ON K6V 5W1
Canada
Toll Free: 1-888-593-5969
Tel: (613) 498-2126
Fax: (613) 342-1341
sales@biolynx.ca or tech@biolynx ca
www.biolynx.ca/contact-biolynx.html

Europe
NuGEN Technologies,
Inc. P.O. Box 149,
6680 AC Bemmel
The Netherlands
Tel: +31(0)13 5780215
Fax: +31(0)13 5780216
europe@nugeninc.com
www.nugeninc.com

Asia
MediBIC.
Daido Seimei Kasumigaseki
Building 8F, 1-4-2
Kasumigaseki, Chiyoda-ku,
Tokyo 100-0013, Japan
Tel: +81-3-5510-2313
Fax: +81-3-5510-2312
info@medibic com
www.medibic com

Australia
Integrated Sciences Pty. Ltd.
2 McCabe Place
PO Box 731
Willoughby NSW 2068 Australia
Tel: 02 9417 7866 or
1 800 252 204 (Australia only)
Fax: 02 9417 5066
tech@integratedsci com au
www.integratedsci.com.au/contactus asp

Israel
ZOTAL Biologicals
& Instrumentation
4 Habarzel Street
Tel Aviv 69710, Israel
Tel:+972.3.6492444
Fax:+972.3.6496664
sales@zotal.co.il
www.zotal co il