TECHNICAL REPORT

Encore® Biotin Module Performance

Introduction

Microarray analysis using approaches such as traditional target preparation methods and the Affymetrix GeneChip® array platform have been beneficial to gene expression studies. To date, however, these studies have required relatively large amounts of RNA and time-consuming, laborious procedures.

Additional gene expression studies are possible if the required RNA input amount is lower, throughput is higher and target preparation approaches are simpler. NuGEN products meet these challenges by enabling the amplification and labeling of minute amounts of total RNA through a fast, simple and automation-friendly process (**Figure 1**).

The Encore Biotin Module is the first of NuGEN's fragmentation and labeling products. It allows researchers to prepare up to 5 µg of fragmented, 3´-biotinylated cDNA suitable for GeneChip array analysis in fewer than 2 hours, with no need for purification. The Encore Biotin Module was designed for use with cDNA generated using Ovation Amplification Systems.

Here we describe a set of studies that demonstrate the performance of the Encore Biotin Module with cDNA generated with both the Ovation Pico WTA System (PN 3300-12) and the Ovation Biotin System (Note: both these systems have been replaced by Ovation Pico WTA System V2, Cat. #3302).

FIGURE 1. **The Encore Biotin Module uses a simple add and incubate process.**

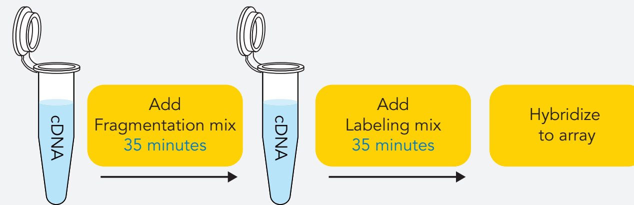
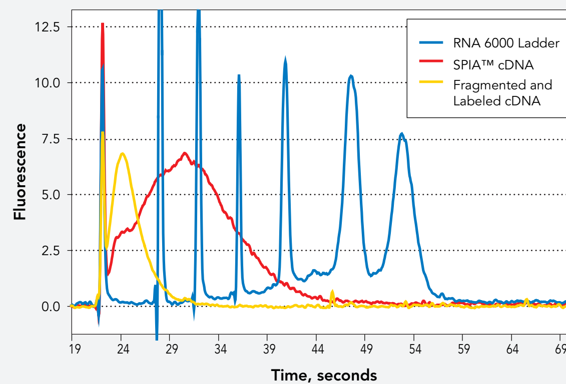


FIGURE 2. **Agilent Bioanalyzer trace of amplified, unfragmented and fragmented cDNA product.**



HeLa RNA amplified with the Ovation Pico WTA System (PN 3300-12, SPIA cDNA) was processed with the Encore Biotin Module, (fragmented and labeled cDNA), and analyzed on the Agilent Bioanalyzer.

Materials and Methods

In the studies described here, both HeLa cell line total RNA (Life Technologies, Cat.# AM7852) and Stratagene Universal Human Reference (UHR) total RNA (Agilent Technologies, Cat.# 740000) were purchased. One SPIA® cDNA pool was prepared from 500 pg of HeLa total RNA using the Ovation Pico WTA System. Another pool was prepared from 5 ng of UHR total RNA using the Ovation Biotin System. Both pool preparations were performed following the procedure outlined in the user guide for the respective product. Purification and quantitation of the cDNA were also performed following user guide procedures.

Fragmentation and labeling of all cDNAs were completed using the Encore Biotin Module according to the product user guide. Of the cDNA generated by the Ovation Pico WTA System, 5 µg was used in a 25 µL total volume as input into each Encore Biotin Module reaction. Of the cDNA generated using the Ovation Biotin System, 3.75 µg in a 25 µL total volume was used as input into each 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).

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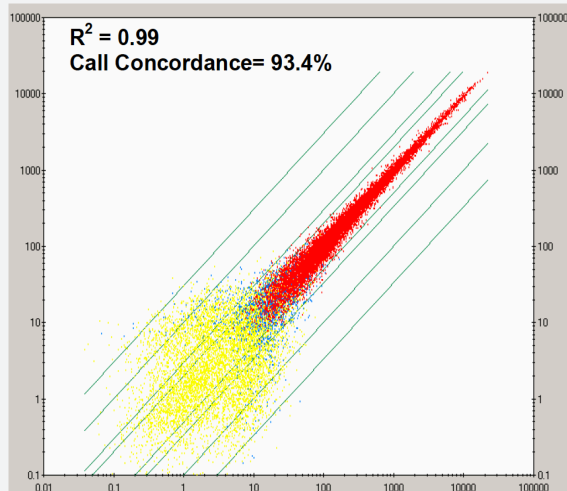

FIGURE 3. Signal correlation for two independent Encore Biotin Module reactions using pooled cDNA generated from 500 pg of HeLa RNA and analyzed on GeneChip arrays, show a high level of signal correlation and call concordance (arrays 1 and 2 from Table 1).

TABLE 1. Array metrics for triplicate fragmentation and labeling 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

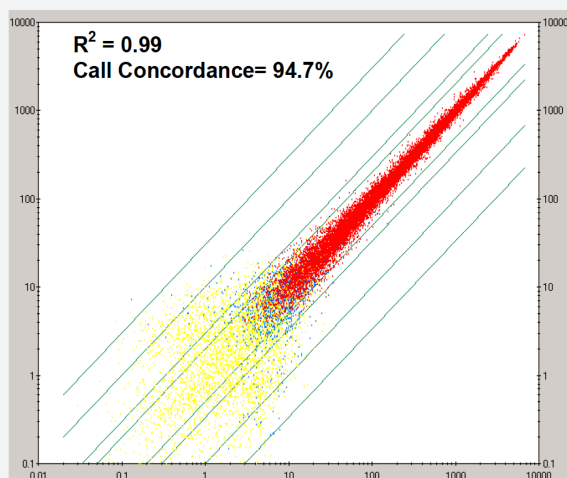

FIGURE 4. Signal correlation for two independent Encore Biotin Module reactions using pooled cDNA generated from 5 ng of UHR RNA and analyzed on GeneChip arrays, show a high level of signal correlation and call concordance, (arrays 2 and 3 from Table 2).

TABLE 2. Array metrics for triplicate fragmentation and labeling 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

Of the 50 µL Encore Biotin Module reaction, 34 µL was used to prepare 150 µL of array hybridization solution, resulting in a final cDNA concentration of 22.7 ng/µL for the Ovation Pico WTA System amplified cDNA and 17 ng/µL for the Ovation Biotin System amplified cDNA. Hybridization, washing and staining protocols outlined in the Encore Biotin Module user guide were followed. Array data was analyzed by Affymetrix GCOS software (GeneChip Operating System, 1.4.0.036).

Analysis was performed using an 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), according to the manufacturer's instructions.

Results

The Encore Biotin Module uses chemical and enzymatic reactions to fragment SPIA cDNA. In **Figure 2**, the typical size distribution of Ovation Pico WTA 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 should look similar to **Figure 2**.

To demonstrate the performance of the Encore Biotin Module with cDNA generated from both the Ovation Pico WTA System and the Ovation Biotin System, we prepared SPIA cDNA with 500 pg and 5 ng of total RNA, respectively, 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 Encore Biotin Module using cDNA generated with the Ovation Pico WTA System is shown in **Figures 3** and **4**. **Table 1** shows array performance metrics among three independent Encore Biotin Module reactions with pooled cDNA, demonstrating the high level of array performance and reproducibility among replicates. **Figure 3** shows signal correlations and call concordance between arrays 1 and 2. The two independent fragmentation reactions using the Ovation Pico WTA System amplifications of 500 pg of 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 4** for cDNA generated with the Ovation Biotin System and the Encore Biotin Module. **Table 2** shows the array performance metrics for three independent Encore Biotin Module reactions performed with pooled cDNA. Reproducibility between arrays from the independent fragmentation and labeling reactions is high with a signal correlation of 0.994 and a call concordance of 94.7%.

We recruited individuals without prior experience running Encore Biotin Module procedures to further demonstrate the high level of reproducibility and robustness of the protocol in **Figure 5**. Pooled, amplified cDNA, generated from individual HeLa RNA amplifications using the Ovation Pico WTA System, was used with a 5 µg cDNA input per 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 in intra-operator comparisons, with an average R^2 of 0.993 + 0.004 (average + SD) and a call concordance of 92.9 + 0.3%. The 12 inter-operator comparisons also showed very high

FIGURE 5. **Encore Biotin Module reproducibility among different operators.**

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

Pooled, amplified cDNA generated from individual 500 pg HeLa RNA amplifications with the Ovation Pico WTA System was used at a 5 µg input per Encore Biotin Module 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.

FIGURE 6. **Lot-to-lot performance of Encore Biotin Module.**

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

Pooled, amplified cDNA generated from individual 500 pg HeLa RNA amplifications with the Ovation Pico WTA System was used at a 5 µg input per Encore Biotin Module reaction. Reactions using three independent assembled kit lots were run 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 those of duplicate arrays 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.

reproducibility with an average R^2 of 0.993 + 0.002 and call concordance of 92.9% + 0.3. The correlations and concordance calls of inter-operator arrays were identical to those of duplicate arrays from a single operator, indicating a robust protocol.

Next, we obtained data from three different lots of the Encore Biotin Module to demonstrate robust and consistent kit performance. Pooled, amplified cDNA, generated from individual 500 pg HeLa RNA amplifications with the Ovation Pico WTA System, was used as a 5 µg cDNA input per Encore Biotin Module reaction.

Reactions using three independent kit lots were run 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%, as shown in **Figure 6**. Signal correlation and call concordance are very high in the three intra-lot comparisons, with an average R^2 of 0.993 + 0.001 and call concordance of 93.3% + 0.2. The 12 inter-lot comparisons also showed very high reproducibility with an average R^2 of 0.992 + 0.002 and call concordance of 93.3% + 0.2. The correlations and concordance calls of inter-lot arrays were as high as those of duplicate arrays from a single kit lot indicating highly consistent reagent performance.

Conclusions

The results shown here strongly demonstrate the high reproducibility of the Encore Biotin Module for GeneChip array target preparation. The Module offers a combination of a high level of sensitivity, high labeling efficiency, and ease of use, making 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, the Encore Biotin Module is an ideal tool for high-throughput sample processing.

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