USER GUIDE

Encore™ Biotin Module

CATALOG NO. 4200-12, 4200-60, 4200-A01

NuGEN®
imagine more from less™

Patents, Licensing and Trademarks

 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 U.S. and other countries. NuGEN, the NuGEN logo, Ovation, SPIA, Ribo-SPIA, WT-Ovation, FL-Ovation, Applause, Encore 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.

The purchase of this product conveys to the buyer the limited, non-exclusive, non-transferable right (without the right to modify, reverse engineer, resell, repackage or further sublicense) under these patent applications and any patents issuing from these patent applications to use this product and methods, accompanying this user guide, for research and development purposes solely in accordance with the intended use described and the written instructions provided in this user guide. No license to make or sell products by use of this product is granted to the buyer whether expressly, by implication, by estoppels or otherwise. In particular, the purchase of this product does not include or carry any right or license to use, develop or otherwise exploit this product commercially and no rights are conveyed to the buyer to use the product or components of the product for purposes including commercial services or clinical diagnostics.

For information on purchasing a license to the NuGEN patents for uses other than in conjunction with this product or to use this product for purposes other than research, please contact NuGEN Technologies, Inc., 201 Industrial Road, Suite 310, San Carlos, CA 94070. Phone 888-654-6544 or 650-590-3600; FAX 888-296-6544 or 650-590-3630.

Warranty

NuGEN warrants that this product meets the performance standards described in the Company's product and technical literature for a period of six months from the date of purchase, provided that the product is handled and stored according to published instructions, and that the product is not altered or misused. If the product fails to meet these performance standards, NuGEN will replace the product free of charge or issue a credit for the purchase price.

NuGEN's liability under this warranty shall not exceed the purchase price of the product. NuGEN shall assume no liability for direct, indirect, consequential or incidental damages arising from the use, results of use or inability to use its products. NuGEN reserves the right to change, alter or modify any product to enhance its performance and design.

NuGEN's products are developed, designed and sold **FOR RESEARCH USE ONLY**. This product is not to be used for diagnostic or drug purposes, nor is it to be administered to humans or animals. Except as expressly set forth herein, no right to modify, reverse engineer, distribute, offer to sell or sell NuGEN's product is conveyed or implied by buyer's purchase of this NuGEN product. The buyer agrees to use NuGEN products accompanying the product insert in accordance with the intended use and the written instructions provided.

Table of Contents

Contents

I. Introduction

A. Background

NuGEN's proprietary fragmentation and labeling process (patent pending) combines enzymatic and chemical processes for fragmentation of amplified single-stranded cDNA to generate labeled targets suitable for hybridization to Affymetrix GeneChip® arrays.

The Encore™ Biotin Module is validated for use with amplified cDNA generated using the following NuGEN products:

- Ovation® RNA Amplification System V2 (Cat. #3100)
- Ovation Whole Blood Solution (Cat. #3100/1300)
- Ovation Pico WTA System (Cat. #3300)
- Ovation PicoSL WTA System (Cat. #3310)
- WT-Ovation™ FFPE System V2 (Cat. #3400)
- WT-Ovation Exon Module (Cat. #2000)
- WT-Ovation One-Direct System (Cat. #3500)
- Applause™ WT-Amp ST and WT-Amp Plus ST (Cat. #5500/5510)
- Applause 3'-Amp System (Cat. #5100)

The resulting fragmented and labeled single-stranded cDNA target generated with the Encore Biotin Module is suitable for hybridization to Affymetrix GeneChip® arrays. Please refer to the amplification system User Guides and www.nugeninc.com for appropriate amplification and labeling system combinations for your application and desired array platform.

B. Fragmentation and Labeling Process

This novel and proprietary two-step fragmentation and labeling process is carried out by a simple "add and incubate" procedure and does not require purification steps.

The first step is a combined chemical and enzymatic fragmentation process that yields single-stranded cDNA products in the 50 to 100 base range. In the second step, this fragmented product is labeled via enzymatic attachment of a biotin-labeled nucleotide to the 3-hydroxyl end of the fragmented cDNA generated in the first step.

C. Performance Specifications

The fragmentation and biotin labeling process is performed in approximately two hours and produces fragmented and labeled single-stranded cDNA ranging from 50 to 100 bases ready for hybridization to GeneChip arrays.

D. Quality Control

Each Encore Biotin Module lot is tested to meet specifications for product size and array performance.

I. Introduction

E. Storage and Stability

The Encore Biotin Module is shipped on dry ice and should be unpacked immediately upon receipt. All components should be stored at –20°C on internal shelves of a freezer without a defrost cycle.

Note: While the Encore Biotin Module is shipped on dry ice, it is critical that it not be stored long term at -80°C as this may result in poor performance.

The Encore Biotin Module has been tested to perform to specifications for up to six freeze/thaw cycles. Kits handled and stored according to the above guidelines should perform to specifications for six months. NuGEN has not yet established long-term storage conditions for the Encore Biotin Module.

F. Material Safety Data Sheet (MSDS)

An MSDS for this product is available from NuGEN Technical Service by calling 888-654-6544 or by sending an email to: techserv@nugeninc.com.

II. Kit Components

A. Reagents and Supplies Provided

Table 1. cDNA Fragmentation and Biotin Labeling Reagents

COMPONENT	4200-12 PART NUMBER	4200-60 PART NUMBER	4200-A01 PART NUMBER	VIAL CAP	VIAL NUMBER
Fragmentation Buffer Mix	S01182	S01182	S01177	Orange	FL1
Fragmentation Enzyme Mix	S01175	S01183	S01178	Orange	FL2
Labeling Buffer Mix	S01184	S01184	S01179	Orange	FL3
Biotin Reagent	S01172	S01185	S01180	Orange	FL4
Labeling Enzyme Mix	S01173	S01186	S01181	Orange	FL5

B. Additional Equipment, Reagents and Labware

Required Materials

- **Equipment**
  - Microcentrifuge for individual 1.5 mL and 0.5 mL tubes
  - Microcentrifuge for 0.2 mL individual and 8 X 0.2 mL strip PCR tubes (e.g., PGC #16-7009-70/72 or similar)
  - 0.5–10 µL pipette, 2–20 µL pipette, 20–200 µL pipette, 200–1000 µL pipette
  - Vortexer
  - Thermal cycler with 0.2 mL tube heat block, heated lid, and 100 µL reaction capacity
  - Appropriate spectrophotometer and cuvettes, or Nanodrop® UV-Vis Spectrophotometer
- **Labware**
  - Nuclease-free pipette tips
  - 1.5 mL and 0.5 mL RNase-free microcentrifuge tubes
  - 0.2 mL individual thin wall PCR tubes or 8 X 0.2 mL strip PCR tubes
  - Appropriate spectrophotometer cuvettes
  - Disposable gloves
  - Kimwipes
  - Ice bucket

II. Kit Components

Optional Equipment

- Agilent 2100 bioanalyzer or other equipment for electrophoretic analysis of RNA
- Real-time PCR system

III. Planning the Experiment

A. Input RNA Requirements

1. cDNA Source

The most important requirement for achieving successful results with the Encore Biotin Module is to use cDNA generated with one of NuGEN's Ovation System or Applause System amplification products that have been validated for use with this module.

Note: The Encore Biotin Module is designed solely for use with cDNA prepared using the NuGEN products listed below. It is not designed for use with cDNA from other sources.

- Ovation RNA Amplification System V2 (Cat.#3100)
- Ovation Whole Blood Solution (Cat.#3100/1300)
- Ovation Pico WTA System (Cat.#3300)
- Ovation PicoSL WTA System (Cat. #3310)
- WT-Ovation FFPE System V2 (Cat.#3400)
- WT-Ovation Exon Module (Cat.#2000)
- WT-Ovation One-Direct System (Cat.#3500)
- Applause WT-Amp ST and WT-Amp Plus ST (Cat.#5500/5510)*
- Applause 3'-Amp System (Cat.#5100)*

***Note:** Fragmentation and Labeling protocols for cDNA generated using the Applause products are found in the respective User Guides.

The unlabeled cDNA product may be stored at –20°C, with minimum freeze/thaw cycles prior to fragmentation and labeling. For recommendations on the input cDNA quality assessment, see Appendices B and C of this user guide. You may also choose to qualify the starting cDNA by performing qPCR assays as recommended in the appropriate NuGEN Amplification System user guides.

2. cDNA Purity

The cDNA used with the Encore Biotin Module must be purified using the purification methods recommended in the user guides of the NuGEN Amplification System products. The adjusted 260/280 absorbance ratio of the purified SPIA cDNA must be >1.8.

B. Using Nuclease-free Techniques

Nuclease contamination from equipment and work environment will lead to experimental failure. Follow these guidelines to minimize contamination:

- Wear disposable gloves and change them frequently.
- Avoid touching surfaces or materials that could introduce DNases.
- Use only the reagents provided and recommended.
- Prior to initiating protocol, clean and decontaminate work areas and instruments, including pipettes, with commercially available decontamination reagents.
- Use only new DNase-free pipette tips and microcentrifuge tubes.

C. Amplified Input cDNA Storage

The unlabeled cDNA product generated by NuGEN Amplification System products may be stored at –20°C for up to six months prior to fragmentation and labeling.

D. Fragmented and Labeled cDNA Storage

The fragmented and biotin-labeled cDNA product can be used immediately after preparation, or stored at –20°C.

IV. Protocol

A. Overview

The cDNA fragmentation and biotin labeling is performed in two stages:

1. cDNA fragmentation	0.5 hours
2. Biotin attachment	1.25 hours
Total time to fragment and label amplified cDNA	**1.75 hours**

B. Protocol Notes

- This protocol should be carried out in a post-amplification workspace designated for handling SPIA cDNA amplification products using dedicated post-amplification equipment and consumables. Care should be exercised to avoid the introduction of SPIA cDNA into workspaces used to set up SPIA amplification reactions. For more information on this topic please refer to the NuGEN RNA amplification product user guide or contact NuGEN Technical Services.
- Thaw only components used in each step and immediately place them on ice.
- Always keep thawed reagents and reaction tubes on ice unless otherwise instructed.
- After thawing and mixing buffer mixes, if any precipitate is observed, re-dissolve it completely prior to use. You may gently warm the buffer mix for two minutes at room temperature followed by brief vortexing. Do not warm any enzyme mixes.
- FL3 labeling buffer may appear to have pink coloration, this is normal.
- Spin down labeling master mix briefly at low speed. High speed spins for long periods can cause formation of a precipitate.
- The reagent volumes recovered greatly depend on the number of batches processed with each kit. Set up no fewer than three reactions at a time with the 4200-12 kit, no fewer than 10 reactions at a time with 4200-60, and no fewer than 48 reactions at a time with 4200-A01. The A01 kit has been designed for use with an automation protocol requiring large batch sizes. For information on automation solutions contact NuGEN Technical Services.
- When placing small amounts of reagents into reaction mix, gently pipet up and down several times to ensure complete transfer.
- When instructed to pipet mix, gently aspirate and dispense at least half of total reaction mix volume. Repeat a minimum of five times to ensure complete mixing.
- Allow thermal cycler to reach incubation temperature before placing samples in the block.
- When working with more than one sample, excess master mix may be needed.
- Components of this NuGEN product should not be used or combined with any other types of Ovation Systems or Applause products and vice versa.

C. Preparing cDNA Samples

The amount of amplified cDNA required for each fragmentation and labeling reaction depends on the method of cDNA generation. NuGEN's various amplification systems

validated for use with the Encore Biotin Module and the required cDNA input from each are listed in Table 2. Ensure that the correct input is used in section IV.E., step 6 of the Fragmentation Protocol.

Table 2. cDNA Input Requirements for Fragmentation and Labeling Reactions and Final Hybridization Cocktail Concentrations

NUGEN AMPLIFICATION SYSTEM (CAT. #)	cDNA INPUT PER REACTION	FINAL HYB COCKTAIL CONCENTRATION
WT-Ovation One-Direct RNA Amplification System (Cat. #3500)	5–6 µg*	23-27 ng/µL
WT-Ovation FFPE System V2 (Cat. #3400)	4–5 µg*	18-23 ng/µL
Ovation Pico WTA System (Cat. #3300)	5 µg*	23 ng/µL
Ovation PicoSL WTA System (Cat. #3310)	2.5 µg**	23 ng/µL
Ovation RNA Amplification System V2 (Cat. #3100)	3.75 µg*	17 ng/µL
Whole Blood Solution and the Ovation WB Reagent (Cat. #1300)	4.4 µg*	20 ng/µL
WT-Ovation Exon Module (Cat. #2000)	5 µg*	23 ng/µL
Applause WT-Amp ST and WT-Amp Plus ST (Cat. #5500/5510)	See Appropriate Applause User Guide	See Appropriate Applause User Guide
Applause 3'-Amp System (Cat. #5100)	See Appropriate Applause User Guide	See Appropriate Applause User Guide

* Concentration as measured using 33 µg/mL per O.D. unit

** Sufficient amount for use with Affymetrix Array Plates only. NuGEN recommends using the Encore Biotin Module fragmentation and labeling procedure for Applause WT-Amp ST cDNA targets found in Chapter V., sections D, E and F of the Applause WT-Amp ST User Guide.

IV. Protocol

D. Programming the Thermal Cycler

Use a thermal cycler with a heat block designed for 0.2 mL tubes, equipped with a heated lid, and with a capacity of 100 µL reaction volume. Prepare the 2 programs shown in Table 3, following the operating instructions provided by the manufacturer. For thermal cyclers with an adjustable heated lid, set the lid temperature at 100°C. For thermal cyclers with a fixed temperature heated lid (e.g., ABI GeneAmp® PCR 9600 and 9700 models) use the default settings (typically 100 to 105°C).

Table 3. Thermal Cycler Programming

PROGRAMMING DETAILS	
Program 1 cDNA Fragmentation	37°C – 30 min, 95°C – 2 min, hold at 4°C
Program 2 Labeling	37°C – 60 min, 70°C – 10 min, hold at 4°C

E. Fragmentation Protocol

1. Obtain the Fragmentation Buffer Mix (Orange: FL1) and Fragmentation Enzyme Mix (Orange: FL2) from the product box stored at -20°C.

   Note: You may thaw all reagents at once. See Labeling protocol section for thawing and mixing instructions for the Labeling reagents.

2. Thaw FL1 at room temperature and mix by vortexing for 2 seconds, spin in a microcentrifuge for 2 seconds, place on ice.

3. Mix FL2 by inverting the tube 3 times, spin tube in microcentrifuge for 2 seconds, place on ice.

4. Make Fragmentation Master Mix by combining FL1 and FL2 in a 0.5 mL capped tube, according to the volumes shown in Table 4.

Table 4. Fragmentation Master Mix (volumes listed are for a single reaction)

FRAGMENTATION BUFFER MIX (ORANGE: FL1)	FRAGMENTATION ENZYME MIX (ORANGE: FL2)
5 µL	2 µL

5. Mix by pipetting and spin down the master mix briefly. Place on ice. Use master mix immediately.

! *Use Fragmentation Master Mix immediately after preparation.*

6. Place 0.2 mL PCR tube(s) in a rack on ice.
7. For each reaction, pipet 25 µL (refer to Table 2 to determine the amount of required amplified cDNA input) of the purified SPIA cDNA into a PCR tube. Add water, if necessary, to bring up the volume of samples to 25 µL.
8. Add 7 µL of the Fragmentation Master Mix to each sample.
9. Mix well by pipetting up and down 8 to 10 times.
10. Cap tubes; vortex and spin for 2 seconds to ensure thorough mixing.
11. Place tubes in a pre-warmed thermal cycler programmed to run Program 1 (cDNA Fragmentation, see Table 3):

    37°C – 30 min, 95°C – 2 min, hold at 4°C
12. Remove tubes from the thermal cycler and spin for 2 seconds to collect condensation, then place on ice. Proceed immediately to the Labeling step.

F. Labeling Protocol

1. Obtain the Labeling Buffer Mix (Orange: FL3), Labeling Reagent (Orange: FL4) and the Labeling Enzyme Mix (Orange: FL5) from the product box stored at –20°C.
2. Place all reagents immediately on ice.
3. Thaw FL3 and FL4 at room temperature and mix by vortexing for 2 seconds, spin in a microcentrifuge for 2 seconds, place on ice.
4. Mix FL5 by inverting the tube 3 times, spin tube in microcentrifuge for 2 seconds, place on ice.
5. Make Labeling Master Mix as outlined below:

Table 5. Labeling Master Mix (volumes listed are for a single reaction)

LABELING BUFFER MIX (ORANGE: FL3)	LABELING REAGENT (ORANGE: FL4)	LABELING ENZYME MIX (ORANGE: FL5)
15 µL	1.5 µL	1.5 µL

Use Labeling Master Mix immediately after preparation.

6. Mix by pipetting and spin down the master mix briefly at low speed, place on ice. Use master mix immediately.
7. Place 0.2 mL PCR tube(s) in a rack on ice.
8. Add 18 µL of the Labeling Master Mix to each fragmented cDNA sample tube.
9. Mix well by pipetting up and down 8 to 10 times.
10. Cap tube, vortex and spin for 2 seconds to ensure thorough mixing.

11. Place tubes in a pre-warmed thermal cycler programmed to run Program 2 (Labeling, see Table 3):

    37°C – 60 min, 70°C – 10 min, hold at 4°C

12. After completion, remove tubes from thermal cycler and spin for 2 seconds to collect condensation.

13. The fragmented and labeled cDNA may be processed immediately for array hybridization or stored at –20°C. For recommendations on array hybridization, see Appendix A.

V. Technical Support

For Technical Support, please contact NuGEN at (U.S. only) 888.654.6544 (Toll-Free Phone) or 888.296.6544 (Toll-Free Fax) or email techserv@nugeninc.com.

In Europe contact NuGEN at +31(0)135780215 (Phone) or +31(0)135780216(Fax) or email at europe@nugeninc.com.

In all other locations, contact your NuGEN distributors Technical Support team.

VI. Appendix

A. Target Preparation for Affymetrix GeneChip® Eukaryotic Array Analysis

Using Affymetrix Hybridization Wash Stain (HWS) Kit for 3′ and ST Arrays

In general, cDNA targets labeled using the Encore Biotin Module are prepared for analysis on GeneChip Gene 1.0 ST and Exon 1.0 ST arrays according to the Affymetrix GeneChip Whole Transcript (WT) Sense Labeling Assay User Manual (P/N 701880 Rev. 5) unless otherwise noted below.

To prepare target for a single array, use a 1.5 mL microcentrifuge tube and mix at room temperature the amount of target cDNA and volumes of hybridization cocktail components indicated in Table 6 below. Heat denature the hybridization cocktail at 99°C for 2 minutes (not 5 minutes as specified by Affymetrix), then follow the Affymetrix standard protocol (45°C in a heat block for 5 minutes then centrifuge at maximum speed for 1 minute just prior to loading). For the 49 and 169 format GeneChip arrays, use 200 µL and 90 µL hybridization volumes, respectively. We recommend a hybridization time of 18 hours ± 2 hours. Hybridization for 16 to 20 hours yields comparable results. For Affymetrix GeneChip ST Exon Arrays and ST Gene Arrays, use fluidics protocols FS450_0001 and FS450_0007, respectively, on the GeneChip Fluidics Station 450 (See Table 6).

Special note when using target prepared using the WT-Ovation One-Direct System: We recommend extending the array hybridization time to 40 hours when using the WT-Ovation One-Direct System to maximize detection sensitivity with the exceedingly small samples used.

VI. Appendix

Table 6. Hybridization, Cocktail Assembly and Fluidics Protocols for Single GeneChip® Arrays using Affymetrix HWS kit (Affymetrix P/N 900720)

COMPONENT	STANDARD ARRAY (49 or 64 FORMAT)	MIDI ARRAY (100 FORMAT)	MINI ARRAY (169 FORMAT)	FINAL CONCENTRATION
Fragmented, biotin-labeled amplified cDNA	50 µL	34 µL	25 µL	Depends on sample type and amplification method*
Control oligo-nucleotide B2 (3 nM)	3.7 µL	2.5 µL	1.8 µL	50 pM
20X Eukaryotic hybridization controls (bioB, bioC, bioD, cre)	11 µL	7.5 µL	5.5 µL	1.5, 5, 25 and 100 pM, respectively
2X Hybridization buffer	110 µL	75 µL	55 µL	1X
100% DMSO	22 µL	15 µL	11 µL	10%
Water	23.3 µL	16 µL	11.6 µL	N/A
Final Volume	**220 µL**	**150 µL**	**110 µL**	
FLUIDICS PROTOCOLS				
For 3' arrays	FS450_0004	FS450_0002		
For ST arrays	FS450_0001 (Exon arrays)		FS450_0007 (Gene arrays)	

*Refer to Table 2 for cDNA input requirements into fragmentation and labeling reactions and final hybridization cocktail concentrations.

VI. Appendix

Using User-prepared Hybridization, Washing and Staining Reagents for 3′ Arrays

Components and supply sources used in the hybridization cocktail are as specified in the Affymetrix GeneChip® Expression Analysis Technical Manual (P/N 702232 Rev. 2, pp. 193–228), however there are a few exceptions and minor differences in the protocol that are outlined below.

To prepare target for a single standard array, use a 1.5 mL microcentrifuge tube and mix at room temperature the amount of target cDNA and volumes of hybridization cocktail components indicated in Table 7 below. Heat denature the hybridization cocktail at 99°C for 2 minutes (not 5 minutes as specified by Affymetrix), then follow the Affymetrix standard protocol (45°C in a heat block for 5 minutes then centrifuge at maximum speed for 5 minutes just prior to loading). In the meantime, incubate the probe array filled with 1X Hybridization Buffer at 45°C for 10 minutes with rotation. For a standard GeneChip 3′ array use a 200 µL volume and for a Midi 3′ array use 130 µL. NuGEN recommends hybridization time of 18 hours ± 2 hours. Hybridization for 16 to 20 hours yields comparable results.

Special note when using target prepared using the WT-Ovation One-Direct System: We recommend extending the array hybridization time to 40 hours when using the WT-Ovation One-Direct System to maximize detection sensitivity with the exceedingly small samples used.

Use protocol EukGE-WS2v4_450 for standard arrays or the Midi_Euk-2v3_450 for Midi arrays on the GeneChip Fluidics Station 450.

VI. Appendix

Table 7. Hybridization, Cocktail Assembly and Fluidics Protocols for Single GeneChip® Arrays using user-prepared Hybridization, Wash and Staining Reagents

COMPONENT	STANDARD ARRAY (49 or 64 FORMAT)	MIDI ARRAY (100 FORMAT)	MINI ARRAY (169 FORMAT)	FINAL CONCENTRATION
Fragmented, biotin-labeled amplified cDNA	50 µL	34 µL	Depends on sample type and amplification method*	Depends on sample type and amplification method*
Control oligonucleotide B2 (3 nM)	3.7 µL	2.5 µL	50 pM	50 pM
20X Eukaryotic hybridization controls (bioB, bioC, bioD, cre)	11 µL	7.5 µL	1.5, 5, 25 and 100 pM respectively	1.5, 5, 25 and 100 pM, respectively
Herring sperm DNA (10 mg/mL)	2.2 µL	1.5 µL	0.1 mg/ml	1X
Acetylated BSA (50 mg/ml)	2.2 µL	1.5 µL	0.5 mg/ml	10%
2X Hybridization buffer	110 µL	75 µL	1X	
100% DMSO	22 µL	15 µL	10%	
Water	19 µL	13 µL	N/A	N/A
Final Volume	**220 µL**	**150 µL**		
FLUIDICS PROTOCOLS				
For 3' arrays	FS450_0004	FS450_0002		
For ST arrays	FS450_0001 (Exon arrays)		FS450_0007 (Gene arrays)	

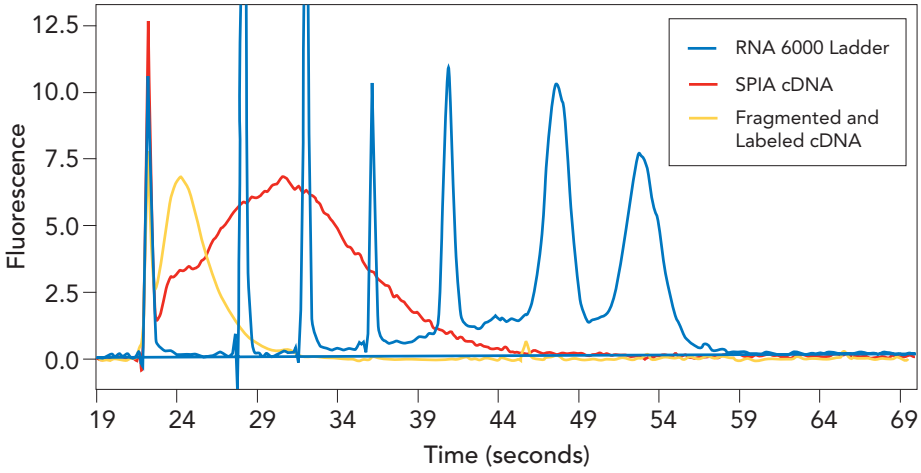
*Refer to Table 2 for cDNA input requirements into fragmentation and labeling reactions and final hybridization cocktail concentrations.

VI. Appendix

B. Quality Control of Amplified, Fragmented and Labeled cDNA Product

The fragmentation success and the size distribution of the final fragmented and biotinylated product may be viewed on an Agilent Bioanalyzer by loading 100 ng of each sample before and after the fragmentation and labeling process on an RNA 6000 Nano LabChip® (Agilent Cat. #5065-4476) using the mRNA Smear Nano program following the manufacturer's instructions. Product that is not sufficiently fragmented has been shown to yield poor results on GeneChip arrays. For good results on GeneChip arrays, 80% or greater of the fragmented cDNA product should be smaller than 200 bases in length. For examples of Bioanalyzer traces of unfragmented and fragmented cDNA product, see Figure 1.

Figure 1. Bioanalyzer Trace of Amplified, Un-fragmented and Fragmented cDNA Product. HeLa RNA amplified with the Ovation Pico WTA System (Cat. #3300) was processed with the Encore Biotin Module, and analyzed on an Agilent Bioanalyzer.



C. Input cDNA Analysis: Measuring Concentration and Purity

1. Before using the Encore Biotin Module, it is highly recommended to determine the concentration of your sample to ensure sufficient cDNA input for the fragmentation and labeling process.
2. Mix the sample by brief vortexing and spinning prior to checking the concentration.
3. Measure the absorbance of the amplified cDNA product at 260, 280 and 320 nm. You may need to make a 1:20 dilution of the cDNA in water prior to measuring the absorbance.
4. Purity: Subtract the A320 value from both A260 and A280 values. The adjusted

   (A260 – A320 / A280 – A320) ratio should be >1.8.

VI. Appendix

5. Yield: Assume 1 absorbance unit at 260 nm of single-stranded DNA = 33 µg/mL.

   To calculate:
   (A260 – A320 of diluted sample) X (dilution factor) X 33 (concentration in µg/mL of a 1 absorbance unit solution) X 0.03 (final volume in mL) = total yield in micrograms

6. Alternatively you may measure the concentration and purity of cDNA with a Nanodrop, using 1 absorbance unit at 260 nm of single-stranded DNA = 33 µg/mL as the constant.

VI. Appendix

D. Frequently Asked Questions (FAQs)

Q1. What materials are provided with the Encore Biotin Module?
The Module provides all necessary buffers and enzymes for fragmentation and labeling of cDNA generated with a validated NuGEN Amplification System.

Q2. What equipment is required or will be useful?
Required equipment includes a microcentrifuge, pipettes, vortexer, a thermal cycler and a UV/Vis spectrophotometer. An Agilent Bioanalyzer or a similar instrument may be used for quality control.

Q3. What additional reagents are required for the Encore Biotin Module?
No additional reagents are required.

Q4. What type of cDNA should I use with the Encore Biotin Module?
You must use SPIA cDNA generated with one of the following NuGEN products with the Encore Biotin Module :

- Ovation RNA Amplification System V2 (Cat.#3100)
- Ovation Whole Blood Solution (Cat.#3100/1300)
- Ovation Pico WTA System (Cat.#3300)
- Ovation PicoSL WTA System (Cat.#3310)
- WT-Ovation FFPE System V2 (Cat.#3400)
- WT-Ovation Exon Module (Cat.#2000)
- WT-Ovation One-Direct System (Cat.#3500)
- Applause WT-Amp ST and WT-Amp Plus ST (Cat.#5500/5510)
- Applause 3'-Amp System (Cat.#5100)

Q5. How much labeled cDNA should I hybridize to a GeneChip array?
We recommend using the entire 50 µL of the Fragmentation and labeling reaction for a standard GeneChip array and 34 µL for a Midi format array hybridization, see Appendix A of product user guide.

Q6. Can I vary the amount of cDNA input to fragmentation and labeling?
The cDNA input amounts range from 3.75 to 6 µg, depending on your sample type and amplification kit used. Please see Table 2 in the Protocol section of the Encore Biotin Module user guide. It is very important that the amount of cDNA input is kept consistent across all samples for each experiment.

Q7. Can I use any cDNA as starting material in the Encore Biotin Module?
No, the cDNA must be generated using a validated NuGEN Amplification System. Use of other cDNAs will result in poor performance.

Q8. How much fragmented and labeled cDNA yield can I expect?
Since this module does not require any purification, the total yield is equal to the input cDNA.

VI. Appendix

Q9. What is the size range of fragmented and labeled cDNA generated by the Encore Biotin Module?
As measured with an Agilent Bioanalyzer, 80% of product falls below 200 bases with an average peak at 85 bases.

Q10. Has NuGEN performed reproducibility studies on the Encore Biotin Module?
Yes, our studies have included sample-to-sample, lot-to-lot and operator-to-operator reproducibility. See Encore Biotin Module Technical Report #1 for some of these studies.

Q11. Can the Encore Biotin Module be used for fragmentation and labeling of RNA?
No.

Q12. Should I purify the cDNA before hybridization?
No. Purification of the fragmented and labeled product is not necessary.

Q13. What are the recommended storage conditions for the fragmented and labeled cDNA?
The fragmented and labeled cDNA is to be stored at –20°C. Ensure the vials are well sealed and avoid multiple freeze/thaw cycles.

Q14. What types of arrays work with the Encore Biotin Module cDNA?
The Encore Biotin Module has been validated on Affymetrix 3′ Expression and GeneChip ST arrays.

Q15. Are the array hybridization reagents included in the Encore Biotin Module?
No. We only provide the reagents necessary for fragmentation and labeling of cDNA. We do provide a recommended procedure for hybridization. See Appendix A of product user guide.

Q16. What hybridization and wash protocols do you recommend for Affymetrix GeneChip applications?
We recommend the same methods as the Affymetrix protocol with the following adjustments:

a. Heat denature the hybridization cocktail at 99°C for 2 minutes, instead of 5 minutes

b. Hybridize arrays for 16 to 20 hours (40 hours if using WT-Ovation One-Direct System cDNA).

c. Use the appropriate Affymetrix fluidics script. See Appendix A and B of product user guide.

VI. Appendix

Q17. What are the Encore Biotin Module incubation temperatures for each step?
cDNA Fragmentation: 37°C for 30 minutes, then 95°C for 2 minutes, then hold at 4°C.

cDNA Labeling: 37°C for 60 minutes, then 70°C for 10 minutes, then hold at 4°C.

Q18. Where can I safely stop in the fragmentation and labeling protocol?
We do not recommend stopping at any step of the protocol.

Q19. How do I determine fragmentation success?
If you choose to determine the success of fragmentation, you may use the Agilent Bioanalyzer to inspect the size distribution of samples before and after fragmentation. See Appendix B of product user guide.

Q20. How should I qualify my cDNA for use with the Encore Biotin Module?
You must use cDNA generated with a validated NuGEN Amplification System product. The concentration of starting cDNA must be determined to ensure adequate input into the F&L reaction and, therefore, onto the arrays. Please see Table 2 in the Protocol section of the Encore Biotin Module user guide for cDNA input requirements. You may choose to further qualify the starting cDNA by performing qPCR assays as recommended in the appropriate NuGEN Amplification System user guides.

Q21. Which protocol do I use for cDNA produced using Applause 3′-Amp, Applause WT-Amp ST and WT-Amp Plus ST RNA Amplification Systems?
The Fragmentation and Labeling protocols for cDNA amplified with Applause 3′-Amp, Applause WT-Amp ST and WT-Amp Plus ST RNA are found in the respective user guide for each product.

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 Bemmel
The Netherlands
Tel: +31-13-5780215
Fax: +31-13-5780216
europe@nugeninc.com

For our international distributors contact information, visit our website

www.nugeninc.com

M01111 v4