QUICK PROTOCOL

Encore® Biotin Module (4200)

Enter the following information to automatically calculate the volumes needed to prepare each reaction. The calculated volumes include an appropriate overfill in excess of the nominal volume requirements to allow for volume loss due to handling. Simply print this document to create a working guide for your experiment, which can be kept as a record.

Operator's Name: _____ Date: _____

Ovation WGA System Lot No.: _____ Encore Kit Lot No.: _____

Number of Samples:* _____

Thermal Cycler Programs	
Program 1: cDNA Fragmentation	37°C – 30 min, 95°C – 2 min, hold at 4°C
Program 2: Biotin Labeling	37°C – 60 min, 70°C – 10 min, hold at 4°C

Fragmentation of SPIA® cDNA			
Thaw the **Fragmentation and Labeling Reagents (orange)**, then place on ice.			
Invert **FL2** vial to mix and spin. Vortex **FL1**, **FL3** and **FL4**, spin and place on ice. Place 25 µL of SPIA cDNA into a 0.2 mL PCR tube on ice.			
Make **Fragmentation Master Mix**. Per sample combine: 5 µL Fragmentation Buffer Mix **FL1** + 2 µL Fragmentation Enzyme Mix **FL2**. Mix well by pipetting, spin and place on ice.	**No. of Samples**	**FL1**	**FL2**
	1	5 µL	2 µL
	0	0	0
Add 7 µL of the **Fragmentation Master Mix** to each sample, mix, vortex and spin.			
Place the tubes in a thermal cycler running Program 1 (37°C – 30 min, 95°C – 2 min, hold at 4°C).			
Once the thermal cycler reaches 4°C, remove tubes, spin and place on ice.			

** Number of samples field ties into embedded logic to calculate master mix volumes, number of reactions.*

Biotin labeling of SPIA cDNA

Invert FL5 vial, mix and spin.

Make **Labeling Master Mix**. Per sample combine:
15 µL Labeling Buffer Mix FL3 + 1.5 µL Biotin Reagent FL4 +1.5 µL Labeling Enzyme Mix FL5.
Mix by pipetting, spin and place on ice.

No. of Samples	FL3	FL4	FL5
1	15 µL	1.5 µL	1.5 µL
0	0	0	0

Add 18 µL of the **Labeling Master Mix** to each fragmented cDNA sample tube, mix, vortex, spin and place on ice.

Place the tubes in a pre-warmed thermal cycler running Program 2 (37°C – 60 min, 70°C – 10 min, hold at 4°C).

Once the thermal cycler reaches 4°C, spin and place tubes on ice.

Proceed to array hybridization or store fragmented and labeled cDNA at –20°C.

GeneChip® Array Hybridization

Mix the hybridization cocktail as outlined in the appropriate table below.

Follow manufacturer's recommendations for prehybridization conditions.

Meanwhile, denature the hybridization cocktail for 2 minutes at 99°C.

Heat the cocktail for 5 min at 45°C, then spin for 5 minutes at maximum speed.

Load the cocktail onto arrays and hybridize for 18 hours ±2 hours at 45°C.

Use fluidics protocols specified in the appropriate table below.

Analyze array data following standard Affymetrix protocols.

Hybridization Mix, Cocktail Assembly and Fluidics Protocols for Single GeneChip® Arrays using Affymetrix HWS Kit				
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 oligonucleotide B2 (3 nM)	3.7 µL	2.5 µL	1.9 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
Total Volume	**220 µL**	**150 µL**	**110 µL**	
Array Loading Volume	200 µL	130 µL	90 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 of the Encore Biotin Module User Guide for cDNA input requirements into fragmentation and labeling reactions and final hybridization cocktail concentrations.

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