



# *Eukaryotic Target Hybridization*

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## **This Chapter Contains:**

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- Detailed steps for preparing the eukaryotic hybridization mix containing labeled target and control cRNA.
- Instructions for hybridizing the target mix to a eukaryotic GeneChip® probe array.

After completing the procedures described in this chapter, the hybridized probe array is ready for washing, staining, and scanning, as detailed in Section 2, Chapter 4.

## Reagents and Materials Required

The following reagents and materials are recommendations and have been tested and evaluated by Affymetrix scientists. For supplier phone numbers in the U.S. and Europe, please refer to the Supplier and Reagent Reference List, Appendix A, of this manual. Information and part numbers listed are based on U.S. catalog information. Additional reagents needed for the complete analysis are listed in the appropriate chapters. Appendix A contains a master list of all reagents used in this manual.

- Water, Molecular Biology Grade, BioWhittaker Molecular Applications / Cambrex, P/N 51200
- Acetylated Bovine Serum Albumin (BSA) solution (50 mg/mL), Invitrogen Life Technologies, P/N 15561-020
- Herring Sperm DNA, Promega Corporation, P/N D1811
- GeneChip Eukaryotic Hybridization Control Kit, Affymetrix, P/N 900299 (30 reactions) or P/N 900362 (150 reactions), contains Control cRNA and Control Oligo B2
- Control Oligo B2, 3 nM, Affymetrix, P/N 900301 (can be ordered separately)
- 5M NaCl, RNase-free, DNase-free, Ambion, P/N 9760G
- MES Free Acid Monohydrate SigmaUltra, Sigma-Aldrich, P/N M5287
- MES Sodium Salt, Sigma-Aldrich, P/N M5057
- EDTA Disodium Salt, 0.5M solution (100 mL), Sigma-Aldrich, P/N E7889

### Miscellaneous Reagents

- Surfact-Amps 20 (Tween-20), 10%, Pierce Chemical, P/N 28320

### Miscellaneous Supplies

- Hybridization Oven 640, Affymetrix, P/N 800139 (110V) or 800139 (220V)
- Sterile, RNase-free, microcentrifuge vials, 1.5 mL, USA Scientific, P/N 1415-2600 (or equivalent)
- Micropipettors, (P-2, P-20, P-200, P-1000), Rainin Pipetman or equivalent
- Sterile-barrier pipette tips and non-barrier pipette tips
- Heatblock

## Reagent Preparation

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### 12X MES Stock

(1.22M MES, 0.89M [Na<sup>+</sup>])

**For 1,000 mL:**

70.4g MES-free acid monohydrate

193.3g MES Sodium Salt

800 mL of Molecular Biology Grade water

Mix and adjust volume to 1,000 mL.

The pH should be between 6.5 and 6.7. Filter through a 0.2 µm filter.

**IMPORTANT**

*Do not autoclave. Store at 2°C to 8°C, and shield from light.  
Discard solution if yellow.*

### 2X Hybridization Buffer

(Final 1X concentration is 100 mM MES, 1 M [Na<sup>+</sup>], 20 mM EDTA, 0.01% Tween 20)

**For 50 mL:**

8.3 mL of 12X MES Stock

17.7 mL of 5 M NaCl

4.0 mL of 0.5 M EDTA

0.1 mL of 10% Tween 20

19.9 mL of water

Store at 2°C to 8°C, and shield from light

## Eukaryotic Target Hybridization

Please refer to the table below for the necessary amount of cRNA for appropriate probe array format. These recipes take into account that it is necessary to make extra hybridization cocktail due to a small loss of volume (10-20  $\mu\text{L}$ ) during each hybridization.

- Mix the following for each target, scaling up volumes for hybridization to multiple probe arrays.

**Table 2.3.1**  
Hybridization Cocktail for Single Probe Array\*

Component	49 Format (Standard) / 64 Format Array	100 Format (Midi) Array	400 Format (Micro) Array / 169 Format (Mini) Array	Final Concentration
Fragmented cRNA **	15 $\mu\text{g}$	10 $\mu\text{g}$	5 $\mu\text{g}$	0.05 $\mu\text{g}/\mu\text{L}$
Control Oligonucleotide B2 (3 nM)	5 $\mu\text{L}$	3.3 $\mu\text{L}$	1.7 $\mu\text{L}$	50 pM
20X Eukaryotic Hybridization Controls ( <i>bioB</i> , <i>bioC</i> , <i>bioD</i> , <i>cre</i> )	15 $\mu\text{L}$	10 $\mu\text{L}$	5 $\mu\text{L}$	1.5, 5, 25 and 100 pM respectively
Herring Sperm DNA (10 mg/mL)	3 $\mu\text{L}$	2 $\mu\text{L}$	1 $\mu\text{L}$	0.1 mg/mL
Acetylated BSA (50 mg/mL)	3 $\mu\text{L}$	2 $\mu\text{L}$	1 $\mu\text{L}$	0.5 mg/mL
2X Hybridization Buffer	150 $\mu\text{L}$	100 $\mu\text{L}$	50 $\mu\text{L}$	1X
H <sub>2</sub> O	to final volume of 300 $\mu\text{L}$	to final volume of 200 $\mu\text{L}$	to final volume of 100 $\mu\text{L}$	
<b>Final volume</b>	<b>300 <math>\mu\text{L}</math></b>	<b>200 <math>\mu\text{L}</math></b>	<b>100 <math>\mu\text{L}</math></b>	

\*Please refer to specific probe array package insert for information on array format.

\*\*Please see Section 2, Chapter 1, page 2.1.20, for amount of adjusted fragmented cRNA to use when starting from total RNA.

### IMPORTANT

*It is imperative that frozen stocks of 20X GeneChip Eukaryotic Hybridization Control is heated to 65°C for 5 minutes to completely resuspend the cRNA before aliquotting.*

- Equilibrate probe array to room temperature immediately before use.

### Note

*It is important to allow the arrays to equilibrate to room temperature completely. Specifically, if the rubber septa are not equilibrated to room temperature, they may be prone to cracking, which can lead to leaks.*

- Heat the hybridization cocktail to 99°C for 5 minutes in a heat block.
- Meanwhile, wet the array by filling it through one of the septa (see **Figure 2.3.1** for location of the probe array septa) with appropriate volume 1X Hybridization Buffer using a micropipettor and appropriate tips (**Table 2.3.2**).

### Note

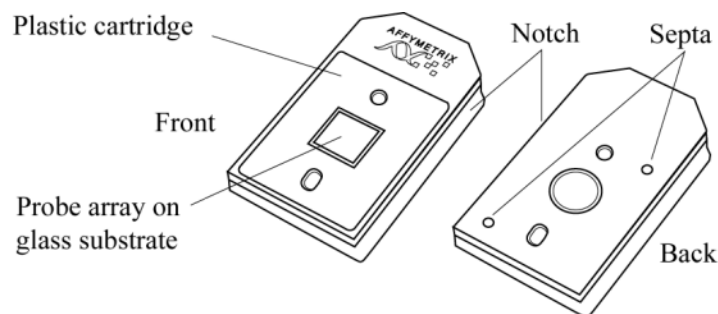
*It is necessary to use two pipette tips when filling the probe array cartridge: one for filling and the second to allow venting of air from the hybridization chamber.*

- Incubate the probe array filled with 1X Hybridization Buffer at 45°C for 10 minutes with rotation.

**Table 2.3.2**  
Probe Array Cartridge Volumes

Array	Hybridization Volume	Total Fill Volume
49 Format (Standard)	200 $\mu$ L	250 $\mu$ L
64 Format	200 $\mu$ L	250 $\mu$ L
100 Format (Midi)	130 $\mu$ L	160 $\mu$ L
169 Format (Mini)	80 $\mu$ L	100 $\mu$ L
400 Format (Micro)	80 $\mu$ L	100 $\mu$ L

6. Transfer the hybridization cocktail that has been heated at 99°C, in step 3, to a 45°C heat block for 5 minutes.
7. Spin hybridization cocktail(s) at maximum speed in a microcentrifuge for 5 minutes to remove any insoluble material from the hybridization mixture.
8. Remove the buffer solution from the probe array cartridge and fill with appropriate volume (**Table 2.3.2** on page 2.3.8) of the clarified hybridization cocktail, avoiding any insoluble matter at the bottom of the tube.
9. Place probe array into the Hybridization Oven, set to 45°C.  
Avoid stress to the motor; load probe arrays in a balanced configuration around the axis. Rotate at 60 rpm.
10. Hybridize for 16 hours.  
During the latter part of the 16-hour hybridization, proceed to Section 2, Chapter 4 to prepare reagents required immediately after completion of hybridization.



**Figure 2.3.1**  
GeneChip® Probe Array