

## Affymetrix GeneChip Resource

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### Preparation of Hybridization Cocktail

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Please do not make copies of or distribute this protocol.

A. Required reagents:

DEPC-treated water (Ambion)

Fragmented cRNA

Control oligonucleotide B2 (Affymetrix) (Stored at  $-20^{\circ}\text{C}$ )

Herring sperm DNA (Promega) (Stored at  $-20^{\circ}\text{C}$ )

Acetylated BSA (Invitrogen) (Stored at  $4^{\circ}\text{C}$ )

2X Hybridization buffer (Stored at  $4^{\circ}\text{C}$ )

20X Eukaryotic hybridization controls (BioB,C,D, and Cre) (Affymetrix) (Stored at  $-20^{\circ}\text{C}$ )

DMSO (Sigma-Aldrich)

B. Equipment and supplies:

Microcentrifuge with 1.5 ml rotor

Micropipettors

Aerosol-barrier tips

Vortex mixer

Powder-free gloves

1.5 ml microcentrifuge tubes

Heat Block adjusted to  $65^{\circ}\text{C}$

**C. HYBRIDIZATION COCKTAIL PREPARATION**

\* Use adjusted fragmented cRNA amount when starting from total RNA.

\* Completely thaw all the reagents stored at  $-20^{\circ}\text{C}$ . Mix by gentle vortexing and spin down.

\* It is imperative that frozen stocks of 20X Eukaryotic hybridization controls and fragmented cRNA (if stored at  $-20^{\circ}\text{C}$ ) be heated to  $65^{\circ}\text{C}$  for 5 min and followed by brief vortexing to completely resuspend the cRNA.

\* Unless cRNA amount is limiting, always make hybridization cocktail for standard array.

1. Please refer to the table below for the necessary amount of cRNA for appropriate probe array format. These recipes accommodate for the small loss of volume (10 to 20 $\mu$ l) during each hybridization.
2. Mix the following for each target, scaling up volumes for hybridization to multiple probe arrays.

Component	Micro/Mini Array	Midi Array	Standard Array	Final Concentration
Fragmented cRNA	5 $\mu$ g	10 $\mu$ g	15 $\mu$ g	0.05 $\mu$ g/ $\mu$ l
Control Oligonucleotide B2 (5nM)	1.7 $\mu$ l	3.3 $\mu$ l	5 $\mu$ l	50 pM
20 X Eukaryotic Hybridization Controls (BioB, BioC, BioD, Cre)	5 $\mu$ l	10 $\mu$ l	15 $\mu$ l	1.5, 5, 25 and 100 pM respectively
Herring Sperm DNA (10mg/ml)	1 $\mu$ l	2 $\mu$ l	3 $\mu$ l	0.1 mg/ml
Acetylated BSA (50 mg/ml)	1 $\mu$ l	2 $\mu$ l	3 $\mu$ l	0.5 mg/ml
2 X Hybridization Buffer	50 $\mu$ l	100 $\mu$ l	150 $\mu$ l	1 X
DMSO	10 $\mu$ l	20 $\mu$ l	30 $\mu$ l	
DEPC-Water	To final volume of 100 $\mu$ l	To final volume of 200 $\mu$ l	To final volume of 300 $\mu$ l	
Final Volume	100	200	300	

3. If the hybridization mix is not used immediately, it should be kept frozen at  $-20^{\circ}$ C.