

## E.coli Microarray Hybridization Set-up

### NOTES:

- Read this entire protocol before starting.
- The most common problem encountered during hybridization is sample drying to the microarray. This will lead to high background fluorescence that cannot be washed off.
- Drying is best avoided by maintaining high humidity in the hybridization chamber and minimizing time array is exposed to air during hybridization setup and washing.
- Cy dyes are light sensitive. All procedures should be performed in indirect light.

### HYBRIDIZATION SETUP

We have tested two buffers for hybridization. One involves an SDS/SSC hybridization solution (that which is typically reported in the literature) while the second uses a commercial hybridization buffer from Sigma (PerfectHyb Plus™). Both methods are described below. We find that the most common problem encountered during hybridization is drying of the labeled target sample to the array. This will lead to high background, which is typically more pronounced at the edges of the cover slip.

1- In indirect light, set-up hybridization cocktail following one of the two methods below:

#### Sigma PerfectHyb method:

Mix in 0.5ml microfuge tube

x ul labeled target sample 1 & 2 (10-15 ul volume)  
4 ul 10mg/ml salmon sperm DNA (Invitrogen)  
4 ul 4mg/ml yeast transfer RNA (Sigma)  
x ul Sigma Perfect Hyb™ Plus Hybridization Buffer

Total labeled target volume = **80 ul**

#### SSC/SDS method:

Mix in 0.5ml microfuge tube

x ul labeled target sample 1 & 2 (10-15 ul volume)  
y ul milliQ H<sub>2</sub>O  
4 ul 10mg/ml salmon sperm DNA (Invitrogen)  
4 ul 4mg/ml yeast transfer RNA (Sigma)  
14 ul 20X SSC (3.5X final conc.)  
4.8 ul 5% SDS (0.3% final conc.)

Total labeled target volume = **80 ul**

2- Heat target sample mixture to 100°C for 5 minutes

3- Spin 5-10 minutes at 14,000 RPM to pellet any particulate material

#### **\*\*\* Prepare hybridization chamber prior to setting up hybridization**

We add a thin strip of whatman paper, 2.5 inches long, saturated with milliQ H<sub>2</sub>O to the chamber alongside the microarray. Be very careful that this saturated filter paper does not come in contact with the slide in such a way that the target would be absorbed by the filter paper.

- 4- Carefully transfer the target sample/hybridization solution to a new tube avoiding any pelleted material.
- 5- **THE FOLLOWING STEP SHOULD BE PRACTICED UNTIL IT CAN BE DONE RELATIVELY QUICKLY.** Carefully pipette the target/hybridization mix onto the center of a Hybri-Slip. *Gently* and *evenly* lay the microarray, array side down, onto the solution until the slide just touches the hybridization solution. Our E.coli arrays are etched on the back side of the slide to note the array area. Be careful to avoid bubbles; if a few small bubbles are present they will likely go away during hybridization (you will probably cause more problems than you will solve if you try to remove any bubbles). When the solution has diffused out completely under the cover slip, gently and smoothly turn the microarray array side up and **immediately** place in the hybridization chamber. Quickly and carefully seal the chamber and place in 60°C H<sub>2</sub>O bath. Incubate 8-16 hours.

**WASH and SCAN ARRAY** (perform washing in in-direct light)

**\*\* PREPARE ALL WASH CHAMBERS PRIOR TO STARTING THE WASH PROCEDURE**

- 1- Thoroughly dry the exterior of hybridization chamber to remove any excess H<sub>2</sub>O and carefully open the chamber.
- 2- Quickly and carefully remove slide from the chamber and place slide(s) at an angle, array side down, in a washing dish containing 0.2X SSC, 0.1%. Keep slide in this position until the coverslip has fallen off (if no drying has occurred this should happen in <min or so). When the cover slip falls off, transfer the slide to a slide holder and wash with gentle agitation for 2 minutes.
- 3- Transfer **ONLY** the slide(s) to a new slide holder and wash chamber with 0.2X SSC solution. Repeat wash for 2 minutes with gentle agitation.
- 4- Transfer the slide holder with the slide(s) to fresh container of 0.2X SSC and repeat wash.
- 5- Transfer the slide holder with the slide(s) to 0.05X SSC and dip up and down 10X.
- 6- Quickly transfer the slide holder to a microtiter plate carrier and spin for 5 minutes at 500 RPM to dry slide.
- 7- Scan slide immediately.

**Recommended Reagents and Suppliers**

Salmon Sperm DNA 10mg/ul	Invitrogen	Cat# 15632-011
Yeast transfer RNA 4 µg/µl	Sigma	Cat # R8759
PerfectHyb Plus buffer	Sigma	Cat # H7033
Hybridization chambers	Telechem	Cat # AHC-1
24x40 mm Hybri-Slip(GraceBio-Labs)	Sigma	Cat # H-1034

Other reagents: 20X SSC, TE pH7.4, 10% SDS, 500 mM EDTA, 1M NaOH, 1M Tris-HCl pH7.5, sterile dH<sub>2</sub>O and DEPC H<sub>2</sub>O. Filter all solutions.

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 Updated October 14, 04