E.coli MICROARRAY HYBRIDIZATION

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 2 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. The commercial buffer may mitigate this somewhat due to its viscous nature.

Sigma PerfectHyb method:

Mix in 0.5ml microfuge tube

x ul labeled target sample
4 ul 10mg/ml salmon sperm DNA (BRL)
4 ul 4mg/ml yeast tRNA (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
y ul milliQ H_2O
4 ul 10mg/ml salmon sperm DNA
4 ul 4mg/ml yeast tRNA
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 probe mixture to 100°C for 5 minutes
3- Spin for 10 minutes at 14,000 RPM to pellet any particulate material

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445 Henry Mall
Madison, WI 53716

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

To prevent arrays from drying during hybridization, pipette 20-25 ul of H_2O into the wells located on either end of the hybridization chamber. **OPTIONAL** (Add a thin strip of whatman paper, 2.5 inches long, saturated with miliQ H_2O (60-70ul), to the chamber alongside the microarray. Be very careful that this filter paper does not come in contact with the slide in such a way that the probe would be absorbed by the filter paper.)

4- Carefully transfer the probe/hybridization solution to a new tube avoiding any pelleted material.

5- **THE FOLLOWING STEP SHOULD BE PRACTICED UNTIL IT CAN BE DONE REALATIVELY QUICKLY.** Carefully pipette the probe/hybridization mix onto the center of a Hybri-Slip. *Gently* and *evenly* lay the microarray, array side down, onto the probe solution until the slide just touches the hybridization solution. The spotted array is located between the two etch marks on the backside of the slide. Be careful to avoid bubbles, but if a few are present they should go away during hybridization (you will probably cause more problems than you will solve if you try to remove any bubbles). When the target sample has diffused out completely under the cover slip, gently and smoothly turn the microarray array side up and **immediately** place in the hybridization chamber. Spot 10 ul of milliQ H_2O at the ends of the slide as far away from the coverslip as possible.

6- Quickly and carefully seal the chamber and place in 60°C H_2O bath. Incubate 8-16 hours.

WASH and SCAN ARRAY

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

1- Thoroughly dry the exterior of hybridization chamber to remove any excess H_2O

2- Carefully open hybridization chamber to avoid H_2O entering the chamber

3- Quickly and carefully remove slide from the chamber and place the slide, array side down, in a washing dish containing 0.2X SSC, 0.1% SDS and a slide holder. Position the slide, upside down and at a slight angle, so the coverslip falls away from the slide. Keep slide in this position until the coverslip has fallen off (if no drying has occurred this should happen in a minute or so). When cover slip falls off, place slide in the slide holder and dip up and down for 2 minutes. **Minimize time the array is exposed to air!**

4- Transfer ONLY the slide to a glass wash chamber with 0.2X SSC containing a clean slide holder. Dip up and down gently for 2 minutes trying to minimize time slide is out of the wash solution

5- Transfer the slide holder with the slide to fresh 0.2X SSC and repeat.

6- Transfer the slide holder with the slide to 0.05X SSC and dip up and down 10X

7- As quickly as possible transfer the slide and slide holder to a microtiter plate carrier and spin for 5 minutes at 500 RPM to dry slide

8- Scan slide

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Reagents and Suppliers

Note- The GEC sells NEN Cy3 and Cy5 - dUTP at a discounted price (please inquire)

Cy3-dUTP:	1 mM	Amersham	Cat # PA53022
Cy5-dUTP:	1 mM	Amersham	Cat # PA55022
SuperScript II:	200 U/µl	GIBCO-BRL	Cat # 18064-014
RNAsin	20-40 U/µl	Promega	Cat # N2515
Yeast tRNA	4 µg/µl*	Sigma	Cat # R8759
100 mM dNTP set	10X**	Pharmacia	Cat # 27-2035-01
$pd(N)_6$ (Hexamer)	5mg/ml*	Amersham	Cat#27-2166-01
Microcon YM-30 columns		Amicon	Cat # 42410
Hybridization Chambers		Telechem	Cat # AHC-1
Perfecthyb Plus buffer		Sigma	Cat # H7033
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 dH2O and DEPC H2O. Filter all solutions.

***** comes lyophilized, must be resuspended at specified concentration

******for 10X stock: 5 mM each of dA, dG, dC and 2 mM of dT in DEPC H2O

Prepared by:

Sandra Splinter BonDurant
UW Gene Expression Center
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Please direct any questions concerning this protocol to:

Sandra Splinter BonDurant (sandra@genome.wisc.edu)