**Microarray Hybridization Protocol**

7-31-03 By Pam Skinner and Hayet Abbassi based on combination of microarray hybridizations described in alternative protocols below.

### Hybridization cocktail

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labeled cDNA</td>
<td>7.8ul</td>
</tr>
<tr>
<td>PolyA (10 mg/ml)</td>
<td>3ul</td>
</tr>
<tr>
<td>SSDNA (10 mg/ml)</td>
<td>3ul</td>
</tr>
<tr>
<td>5% SDS</td>
<td>1.2ul</td>
</tr>
<tr>
<td>20X SSC</td>
<td>15ul</td>
</tr>
<tr>
<td>Formamide</td>
<td>30 ul</td>
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</tbody>
</table>

(for a different final volume, adjust the quantities of the different reagents in the table proportionally).

1) Resuspend dried labeled cDNAs in 7.8ul water
2) Add remaining reagents for Hyb cocktail and mix by pipetting.
3) Heat the probe preparation at 95-100 degrees for 2 minutes. Boiling denatures the sample and makes it accessible for hybridization.
4) Snap cool on ice for 1-3 minutes.
5) Pulse the tubes in the centrifuge to pull down condensation
6) Clean lifterslips with soap and water, then Acetone and dry with Kimwipes. Make sure there are no debris on the array before laying down lifterslip. Place lifterslip on array using either fingers or forceps with dull white strips touching the glass. This creates a platform which allows even distribution of the hybridization solution across the array. Slowly pipette the probe under one corner of the slip until the entire array surface is covered.
7) Put 10-20ul 3XSSC at each little divot at the ends of the Hyb chamber. This is to ensure a constant humidity in the chamber during hybridizations. If the 3XSSC is not applied, the array will dry out.
8) Tightly screw down lid of Hyb chamber and carefully place in incubator. Take caution to keep array completely flat during transfer and hybridizations.
9) Allow hybridization to run for at least 5 hours but not more than 16 hours, in the dark at 42°C.
10) Prepare wash solutions in glass slide dishes
11) Carefully remove array from incubator or water bath, making sure to keep chamber level. Unscrew chamber and remove array.
12) Keep array level and submerge in 2XSSC to get the lifterslip to fall off. Once submerged tilt array and gently dump off coverslip. It may be necessary to lightly swish array under solution to dislodge the slip.
13) Once slip is off and lying on bottom of slide dish, put array in a slide rack and transfer to the first wash solution. Do not allow the chips to dry out at any point during the washes.
14) Wash hybs.
   a) 2X SSC, 0.1% SDS (4ml 10% SDS per 400ml) at 37 or 55°C for 3 minutes with no agitation
b) 2X SSC, RT Let sit 3 minutes in solution then dip up and down 10 times.
c) 0.2X SSC RT Let sit 3 minutes in solution then dip up and down 10 times

15) Immediately dry array in room temperature table top centrifuge at 500 rpm for 6 min.
16) Try to scan array within hours of washing as the Cy dyes are instable and degrade differentially (within 2 days).

Alternative: Essentially as described by Corning
Sample probe preparation
1. Bring probe to 20-45ul total volume in 50% formamide, 5XSSC, 0.1%SDS, 0.5mg/ml PolyA blocker, and 0.5mg/ml E. coli or yeast tRNA. Note 10-30ug Salmon Sperm DNA may be added.
   a. 2ul water
   b. 2 ul polyA (10 mg/ml)
   c. 2ul SSDNA (10 mg/ml)
   d. 4ul 1%SDS
   e. 10ul 20XSSC
   f. 20 ul formamide
2. Heat the probe preparation at 95 degrees for 3-5 minutes.
3. Snap cool on ice for 1-3 minutes.
4. Pulse the tubes in the centrifuge to pull down condensation

Hybridization
1. add probe to slide in hybridization chamber and seal
2. put in 42 degree water bath O/N
Post Hyb washes
1. carefully immerse slide in 2X SSC
2. Wash in 0.1%SDS and 0.1XSSC for 5 minutes
3. repeat wash
4. dry slides in the centrifuge (500 RPM for 5 minutes)

Alternative PROTOCOL FOR ARRAY HYBRIDIZATION from Kapur lab with minor changes by Pam
Essentially as described by Derisi’s lab (http://derisilab.ucsf.edu/microarray/protocols.html) and used in Barbara J. May et. al., Complete genomic sequence of Pasteurella Multocida, PM70. March 13, 2001, PNAS 98(6): 3460-3465

1) Prepare probe as described at end of RT-labeling protocol.
   20ul works well for standard 22mm X 22mm coverslip. 60ul is a good volume for 25X60mm lifterslips.
   2) Boil probe for 2 min. at 100O C. let cool 5-10 min. at room temp (not on ice because ice will cause the SDS to precipitate). Boiling denatures the sample and makes it accessible for hybridization.
   3) Label slide with diamond tip pen and put in a hybridization chamber. Put 10ul-30ul 3XSSC at end of slide or in little divot of chamber in drop. This is to ensure a constant humidity in the chamber during hybridizations. If the 3XSSC is not applied, the array will dry out.
4) Clean a lifterslip or coverslip with EtOH/Acetone and Kimwipes. To prevent bubbles make sure there is no debris on the array before laying down lifterslip. The arrays may be dusted with compressed air briefly.
   a) Place lifterslip on array using either fingers or forceps. Slip should be applied with dull white strips on the long axis of the chip touching the glass. This creates a platform which allows even distribution of the hybridization solution across the array. Slowly pipette the probe under one corner of the slip until the entire array surface is covered. Use of the lifterslip may greatly improve data quality by preventing non-uniform hybridization. (Kapur lab: You may also use a regular coverslip - the lifterslips help to avoid bubbles and are easier to use)
   b) Coverslip: Put the probe onto one edge of the array in a drop (avoiding bubbles). The coverslip should be placed on the array starting at the edge next to the probe while you slowly lower the other end onto the slide - practice with water first to avoid trapping bubbles under the coverslip. If bubbles do get trapped under the coverslip, most will move out once the slide is placed in the water bath.
5) Tightly screw down chamber lid and carefully place chamber in a 63°C water bath. Take caution to keep array completely flat during transfer and hybridizations.
6) Allow hybridization to run for at least 5 hours but not more than 16 hours. Other systems may benefit by different temperatures and times.

ARRAY WASHING

- It is recommended that all wash solutions be filtered before using.
- Prepare wash solutions in glass slide dishes, with each dish having its own rack.

**Wash Soln. I Wash Soln. II**
340 mL Millipore water 350 mL Millipore water
10 mL 20X SSC (final 0.6XSSC) 1 mL 20X SSC (0.06XSSC)
1 mL 10% SDS (final 0.3%SDS)

1) Carefully remove array from water bath, making sure to keep chamber level. Dry array with paper towels and attempt to "wick" any water away from chamber seems.
2) Unscrew chamber and remove array. Some water may enter chamber and pool under slide at this time. If so, it is helpful to have a pair of forceps to pry array away from chamber.
3) Keep array level when submerging in Wash I. Once submerged tilt array and gently dump off coverslip. It may be necessary to lightly swish array under solution to dislodge the slip.
4) Once slip is off and laying on bottom of slide dish, put array in rack and remove any additional hybs from water bath. When all chips are in Wash I, plunge rack up and down 10-20 times (at least 1 minute). Do Not allow the chips to dry out at any point during the washes.
5) Individually transfer chips to slide dish containing Wash II, do not transfer entire slide rack as this will cause too much SDS carryover. plunge 10-20 times again.
6) Dry array in room temperature table top centrifuge at 600 rpm for 5 min.
7) Try to scan array within hours of washing as the Cy dyes are instable and degrade differentially. Do not use a coverslip. Fluorophore preservative??

**Alternative Hybridization protocol (based on Packard BioScience application Note PBT-004)**

- apply MH Research double adhesive slide frame to microarray (heat it to get good seal and do well in advance) Use the biggest ones for BMAP arrays
- Mix up Hyb in ClonTech's ExpressHyb solution
  - 20ul labeled cDNA
  - 3 ul 20X SSC
  - 3 ul Poly A
  - 3 ul ssDNA
  - heat 95 degrees for 2 minutes then ice
  - add 271 ul Express Hyb (pre-warmed to 68 degrees)
  - cover Hyb solution with chamber cover and leave an air bubble the size of a 20 pt letter O.
• insert slide into a Falcon 50 ml tube and put in rotating Hyb chamber at 65 degrees at 6 rpm for 1.5 hours to O/N.
  • wash hybs
    o 2X SSC, 0.1% SDS (4ml 10% SDS per 400ml) 55 degrees
    o 2X SSC, RT
    o 0.2X SSC RT
  • Air dry

• scan within 2 days.