P. falciparum cDNA labeling, microarray hybridization AND washing

(01/10/04)

Dye Coupling:

1. Amersham typically ships the succinimidyl esters of the Cy dyes as dried pellets sealed in a foil bag with a small amount of desiccant. If the desiccant material has turned from dark blue to a light pink, this is an indication that moisture has contaminated the sample. Contaminated dye packs should be returned to Amersham for a refund.

2. Resuspend the solid pellet in slightly more than 20 ul (~22) of DMSO to the dye of interest if using cat # PA23001 (Cy3) # PA25001 (Cy5) or slightly more than 10 ul (~12) if using cat # RPN5661. Resuspend very well.

3. Add **1 ul of dye to each of the cDNA samples** (already in 0.1 M NaBicarb) and let sit at **RT in the dark for an hour**. With the remaining dye, aliquot out into eppendorf tubes in 1 ul aliquots, dry down in speed vac and store at 4C in a light-sealed box, preferably under vacuum and in the presence of a large amount of desiccant. These you can use at a later time by adding your sample (in 0.1M NaBicarb) right into the dye tube.

Removal of uncoupled dye material:

Cleanup using Zymo Research DNA Clean and Concentrator-5 (D4004)

- Add 500 ul of Binding Buffer per coupling reaction and mix.
- Load a Zymo column with coupling reaction. Spin for 15 seconds.
- Aspirate off the flow through and add 200 μl Wash Buffer. Spin 15 seconds.
- Wash again with 200 μl Wash Buffer and spin 30 seconds.
- Aspirate the flow through and spin dry 30 more seconds. (THIS IS CRUCIAL TO REMOVE ANY REMAINING ETHANOL!)
- Transfer column to a clean eppendorf tube and add 12.0 ul 10mM Tris, pH 8.5. Let sit 1-2 minutes and then spin for 30 seconds and recover.

Array Hybridization:

1. Prepare probe:

Cy3 + Cy5 samples	**10.0 µl each in 10mM Tris, pH 8.5**
20X SSC	**4.0 µl**
polyA (10mg/ml)	**2.0 µl (optional)**
1M HEPES pH 7.0	**0.67 µl**

At step 4 add 0.6 µl 10%SDS

2. Set slide in hybridization chamber. Put 10µl 3XSSC at end of slide on top of the slide label. This is to ensure a constant humidity in the chamber during hybridizations, if it is on the label, then it will be less likely to spread across the glass and into your sample. **If the 3XSSC is not applied, the array will dry out.** To avoid unpleasant surprises, the concentration of salt in the re-hydration drop and the probe must be the same!

3. **Clean a Lifterslip** with EtOH and distilled water using a Kimwipe to dry. Place slip 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. Use of the Lifterslip greatly improves data quality by preventing non-uniform hybridization.

4. **Add 0.6 ul 10% SDS per and boil probe for 2 min. at 100°C.** Let cool 5 min. at room temperature. Boiling denatures the sample and makes it accessible for hybridization.

5. Slowly **inject the probe** under one corner of the coverslip until the array surface is covered. Continue to apply remaining probe at the other corners. To prevent bubbles make sure there is no debris on the array before laying down coverslip. The arrays may be dusted with compressed air briefly. Bubbles may also be caused by not allowing the solution to completely cover the array before injection at a different corner begins.

6. 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.

7. Allow hybridization to run for **at least** 6 hours. The optimal time for hybridization will depend on sample complexity and concentration. **We typically hybridize 16-20 hours.**

References for Amino allyl Coupling Protocol

1. Randolph, J. B., and Waggoner, A. S. Stability, specificity and fluorescence brightness of multiply-labeled fluorescent DNA probes. (1997) *Nucleic Acids Res* **25**(14)**,** 2923-9.
2. Brumbaugh, J. A., Middendorf, L. R., Grone, D. L., and Ruth, J. L. Continuous, on-line

DNA sequencing using oligodeoxynucleotide primers with multiple fluorophores. (1988) *Proc Natl Acad Sci U S A* **85**(15)**,** 5610-4.
3. Hughes, T. R. et al. Expression profiling using microarrays fabricated by an ink-jet oligonucleotide synthesizer. (2001) *Nat Biotechnol* **19**(4)**,** 342-7.

Array Washing Protocol

1. Prepare wash solutions in glass slide dishes, with each dish having its own rack.

Wash Solution I: **340 ml Milli-Q water**
10 ml 20XSSC
1 ml of 10%SDS

Wash Solution II: **350 ml Milli-Q water**
1 ml 20XSSC

2. Carefully remove hybridization chamber from the water bath, making sure to keep chamber level. Dry chamber with paper towels and attempt to "wick" any water away from chamber seams.

3. 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 the bottom of the chamber.

4. Keep array level when submerging in Wash I. Once submerged, tilt array and allow coverslip to fall away. It may be necessary to lightly swish array under solution to dislodge the slip.

5. Once slip is off and laying on bottom of slide dish, place the array toward the back of the rack and repeat for all additional hybs. Only remove hybs chambers from water bath on a one-by-one basis. This is to prevent non-specific hybridization from occurring on your bench due to cooling. When all chips are in Wash I, plunge rack up and down 10-20 times.

6. Now, individually transfer chips to the slide dish containing Wash II. **DO NOT** transfer entire slide rack, as this will cause too much SDS carryover.

7. Dry array in room temperature table top by spinning at 500 rpm for 5 min.

8. Try to scan the array(s) as soon as possible and definitely within a few hours of washing as the Cy dyes are unstable and will degrade differentially.