P. falciparum cDNA Labeling

3/16/2011

Dye Coupling:

1. Amersham typically ships the Cy dyes *(Cat. No. RPN5661)* as dried pellets sealed in a foil bag with a small amount of desiccant. If the desiccant material has turned from yellow 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 10ul (~12) of DMSO. Resuspend very well.

3. Add 1ul of dye to each of the cDNA samples (already in 0.1M sodium bicarbonate) and let sit at RT in the dark for 1 hour.
    a. With the remaining dye, make 1ul aliquots in eppendorf tubes, label with initials and date, dry down in speed vac and store at 4˚C 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 sodium bicarbonate) directly to the dye tube.

Removal of uncoupled dye material using Zymo DNA Clean and Concentrator Kit:

1. Add 500ul of Binding Buffer to each coupling reaction and mix.
2. Load a Zymo column with the coupling reaction. Spin 30 seconds at max speed.
3. Aspirate off the flow through and add 200ul Wash Buffer. Spin 30 seconds at max speed.
4. Wash again with 200ul Wash Buffer and spin 30 seconds at max speed.
5. Aspirate the flow through and spin dry 30 more seconds at max speed. (THIS IS CRUCIAL TO REMOVE ANY REMAINING ETHANOL!)
6. Transfer column to a clean eppendorf tube and add 12.0ul RNase/DNase-free water (for Agilent arrays) or 12.0ul 10mM Tris, pH 8.5 (for DeRisi-style arrays) directly to column matrix. Let sit 1-2 minutes and then spin for 30 seconds at 2/3 max speed.
    a. Tip: Warming the RNase/DNase-free water to 65˚C prior to elution will enhance recovery.