Agilent Array Hybridization and Washing

3/16/2011

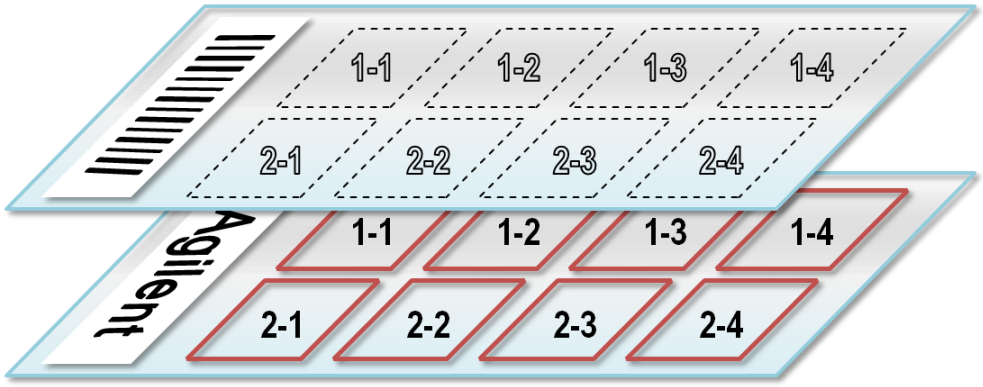
Hybridization

Start with purified Cy-labeled cDNA that has been cleaned up using a Zymo DNA clean and concentrator kit and eluted in 12ul of RNase/DNase-free water.

1. Using the Nanodrop Microarray function, select "SS-DNA 33" and measure 1ul for cDNA concentration and Cy3 & Cy5 fluorescence (blank with water).
    a. The 260/280 reading should be between 1.8 and 2.0.
    b. You should see a Cy3 peak at 550 and Cy5 peak at 650.
2. Combine equal total ng of reference cDNA and sample cDNA (250-1000ng) in an eppendorf tube. Check that the corresponding total pmol of dye are similar for both channels, not to exceed 20pmol.
3. Adjust total volume to 20.9ul with RNase/DNase-free water.
4. Add the following:

    5.5µl 10x Gene Expression Blocking Agent *(Agilent Cat. No. 5188-5281)*
    28.6µl 2x Hi-RPM Hybridization Buffer *(Agilent Cat. No. 5190-0403)*

    55.0µl Total Volume
5. Centrifuge at max speed for 30 seconds to remove bubbles. If bubbles persist, put sample in 65˚C water bath for <30 seconds and centrifuge again.
6. Check log book to find unused array, then choose appropriate gasket for loading your sample. Record information in array log book.
7. Place fresh gasket (Agilent label facing up) inside of the metal Agilent array chamber.
8. Pipette hybridization mixture into appropriate gasket well (try to not introduce any bubbles into mixture). Aim for 50ul per array.
   **Note: it is important to NOT wet the rubber gasket ring so that, when sealed, there will not be any leakage of the hybridization mixture.
9. Slowly place array (Agilent label facing down and barcode facing up) on top of gasket.



10. Place lid of chamber on top of array sandwich, slide seal around chamber and tighten.
    a. Check to see that there is only one large air bubble per array. If smaller bubbles are present, try tapping the chamber on the bench to combine bubbles or place chamber in the 65˚C hybridization oven for 5 minutes and then tap to combine. It is very important to dislodge any stationary bubbles.
11. Slide array + chamber into an empty slot in the 65°C hybridization oven and incubate for ~17hrs.
    a. Rotate chamber at 10-15 rpm.
    b. Be sure to balance your array chamber with an empty chamber.
    c. Make note of the labeled number on the chamber that you are using because multiple labs use the hybe ovens.

Washing Array

1. Home-made wash buffers: Mix 1L of each in RNase-free bottles, adding components in the order listed.
    a. Wash Buffer A (1L)
        i. 700ml Milli-Q water
        ii. 300ml 20X SSPE
        iii. 0.25ml 20% N-laurylsarcosine
    b. Wash Buffer B (1L)
        i. 997ml Milli-Q water
        ii. 3ml 20x SSPE
        iii. 0.25 ml 20% N-laurylsarcosine
    c. You will also need approximately 500ml of Acetonitrile.
2. Prepare washing apparatus
    a. You will need three wash chambers, 1 slide rack, 3 1”stir bars, and 1 tweezers.
    b. Arrange apparatus as shown below.
        i. Your array “sandwich” should be separated while immersed in Buffer A.
        ii. Proceed to wash the array slide as follows: