Hybridization with cDNA targets

Overview

This protocol describes our microarray hybridization and washing procedures for cDNA targets and has been optimized to produce images with a high signal to noise ratio. This protocol is based on a 2X SSC, non formamide hybridization buffer and works well with cDNA target prepared using direct or indirect labeling methods. While the protocol below works for cRNA targets, it does result in a higher background and for this reason we are recommending an alternative protocol for cRNA targets which is posted at www.maizearrays.org. The Telechem hybridization cassettes and washing station are easy to use and produce consistent results though hybridization cassettes and washing stations produced by other manufacturers would work as well. Microarray hybridization and washing are critical steps in the overall process of obtaining consistent expression data values and one or more practice runs using the procedure below are advised before scaling up to do larger numbers of hybridizations.

Materials

Microarray holder and wash station (Telechem International Cat# HTW)
10 mL disposable pipette
50 mL tubes
Sterile measuring cylinder
Extra-deep Hybridization Cassettes (Telechem International Cat# AHCXD)
LifterSlip (Erie Scientific Cat#24X60I-2-4733, ordered through VWR or Fisher Scientific)
Hybridization oven set to 55°C
Liquid Blocking Reagent (Amersham; Cat # RPN3601)
2% SDS
20XSSC (Invitrogen Ultrapure, Cat# 15557-044)
DEPC treated H_2O

Procedure

DNA Probe Immobilization:

Re-hydration and UV cross linking can be done well in advance before microarray hybridization, and the slides can be stored at room temperature for several months. However we do not recommend storage of washed microarray slides for extended periods of time.

1. Mark the corner boundaries of the array on a separate glass slide.
    a. Once spots have been immobilized and the slide is washed, the spots will not be visible (the spots are only visible due to the presence of SSC crystals).
    b. One needs to know the boundaries of the array in order to correctly place coverslip over array during hybridization.
2. Re-hydrate slide over a 50°C water bath for 5 seconds.
    a. Hold slide label side down over the water vapor.
    b. Watch spots carefully so that they do not over-hydrate and begin to merge together. In humid environments this is particularly important.
3. Snap dry the slide on a 45°C heating block for 5 seconds.
    a. Place slide label side up on heating block.
    b. Allow slide to cool for 1 minute or cool with compressed air by blowing over the **back** of the slide.
4. Repeat steps 1-3 a total of four times.

5. UV cross-link the slides by exposing them in batches, label side up, to 180 mJ in a commercial cross-linker (we employ a Stratalinker).
6. Wash the slide in 1% SDS for 5 minutes at RT on a shaker or agitate by hand.
7. Remove SDS by dipping the slides ten times into DDH2O.
8. Immediately transfer the slides to 100% ethanol, dip five times, then incubate for three minutes with shaking.
9. Spin dry slide in centrifuge at no more than 200 x g for 2-4 minutes.
    a. Pack bottom of 50 mL centrifuge tube with Kimwipes.
    b. Using forceps carefully place slide into tube with label at the bottom.
    c. Repeat spin if any liquid is remaining on the slide surface.
10. Repeat the ethanol wash if any visible streaks remain after spin dry.
11. Store slide in a lint-free light-proof box at RT with low humidity.

Hybridization Setup:

Hybridization Mix (160 µl is enough to hybridize both the MOA and MOB slides)

1. Mix the following in a microfuge tube:

20X SSC	16.0 µl
Liquid Block	4.8 µl
2% SDS	6.4 µl
Both Labeled Targets*	µl
H_2O	to 150 µl

*We have obtained the most consistent results using 2-3 ug of each target per slide. Therefore, a 160 µl hybridization mix which can be hybridized to both the MOA and MOB slides will have 4 to 6 ug of each Cy labeled target. It may be necessary to optimize the number of ug of target on each array to your individual laboratory setting but the above values provide a good starting point. We have found that loading by picomoles of target often produces unbalanced images.

2. Denature labeled target by incubating tube at 65^{o} C for 5 min.
3. Apply on to the slides directly (preferred) or place on ice.
4. Rinse ArrayIt™ Hybridization Cassette with distilled water and dry thoroughly.
5. Make sure flexible rubber gasket is seated evenly in gasket channel.
6. Add 15. µl water to the lower groove inside the cassette chamber.
7. Insert the microarray (1" x 3" or 25mm x 75mm slide) into cassette chamber, DNA side up.
8. Place the lifter slip over the microarray slide (make sure the white stripe of the lifterslip is at the lower side)
9. Apply the PRE-HEATED sample slowly to the one end of the lifterslip and let it disperse. Use 60 to 70µl of sample for each slide being careful not to introduce air bubbles.
10. Quickly place the clear plastic cassette lid on top of the cassette chamber.
11. Apply downward pressure and manually tighten (clockwise) the four sealing screws.
12. Check all four screws again to confirm a tight seal.
13. Place the cassette into a hybridization oven set at 55C.
14. Allow the hybridization reaction to proceed for 12 to 14 hours.
15. After hybridization, remove cassette, manually loosen the four sealing screws (counterclockwise) and remove lid.

16. Remove the microarray slide from the cassette chamber using forceps and place the slides into the washing buffer.

Microarray Washing

1. Wash slide in the following solutions for 5 min each:
   2x SSC, 0.1% SDS @ 55°C
   0.5x SSC @ RT (second wash)
   0.1x SSC @ RT (third wash)
2. Washing is done by immersing the slides in a glass the Telechem wash station (Cat# HTW) containing approximately 400 ml of wash buffer, followed by placing it on a magnetic stir plate set at ~120 rpm. Pre-heat the first wash solution to 55°C, and make sure the slides are completely immersed in wash buffer. To ensure even washing, rotate the slide holder 90 degrees mid way through each wash.
3. After completion of the washes, plunge the slide holder five times in RT 0.1X SSC and spin dry the slide in the centrifuge at no more than 1000 rpm for 2-4 min.
   a. Pack bottom of 50 mL plastic disposable centrifuge tube with Kimwipes.
   b. Using forceps, carefully place slide into tube with label at the bottom.
   c. Repeat spin if any liquid remains on the slide.

   Note: Washing is a critical step and care needs to be taken not to over or under wash your slides. For consistent results it is advisable to wash the slides the same way each time.
4. Scan slide immediately, or store in a light proof box @ room temp under dry conditions. Save the image as .TIFF file. Immediate scanning is recommended. However, we have observed that properly stored slides (light protected-dry- RT) can retain the signal up to a month. Some reports indicate environmental pollutants (ozone) can drastically affect fluorescence. Examine the scanned images immediately to determine the number of elements that are near zero or are saturated (for a 16-bit scanner, this represents a value of 65,400). The proportion of these elements should be acceptably low, since information is lost in either case. It is much more preferable to rescan with altered gain settings on the scanner than to proceed with analysis of images containing large proportions of zero or saturated elements.