Systems Virology
of Emerging Respiratory Viruses

T005.0P - 4X44K Hyb Only Protocol

(4/11/10 Lynn Law)

Name:_____ Hybridization:_____ Date:_____
Frag lot #:_____ Blocking Agent. lot#:_____ Hyb. Buff lot#:_____

I. The day before

- Prepare Blocking reagent:

Add 500µl nuclease free H_2O to the vial containing the lyophilized 10x blocking agent (10 hybs) or 1,250 µl nuclease free H_2O (25 hybs). Mix, vortex, and heat to 37°C for 5 min. Centrifuge and store at -20C for up to 2 months.

- Prepare Wash buffer:

Pour 1000 ml of Gene Expression Wash Buffer 2 into a sterile 1000 ml bottle. Put the Gene Expression Wash Buffer 2 into a 37°C water bath. Incubate overnight.

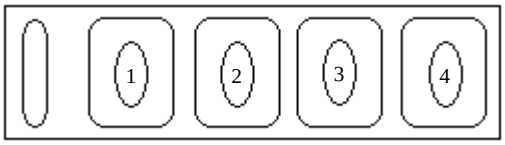
II. Fragmentation

1. Mix together: 1650ng Cy3-cRNA
   11 µl of 10X Blocking Reagent
   Nuclease free H_2O to vol
   Bring volume to 52.8 µl
2. Add 2.2 µl 25X fragmentation buffer
   Total volume = 55.0 µl
3. Mix well but gently by vortexing
4. Incubate at 60°C, 30 min. Protect from light.
5. Add 55 µl 2X Gene Expression Hybridization buffer HI-RPM.
   Total volume = 110 µl
6. Mix well by careful pipetting, do not introduce bubbles.
7. Spin 1 min at RT and 13,000 rpm.
8. Use immediately starting at Step 1 of Hybridization procedure below.

III. Hybridization

1. During the fragmentation incubation:
   a. Turn on Hyb oven to 65°C
   b. Put down a clean piece of foil on bench.
   c. Lay out metal chambers (3 parts each).
   d. Get out slide box and record barcodes. Also write barcodes and slide # on foil.

2. After fragmentation (Do not assemble more than 2 chambers at once) Put a clean Gasket slide (4x44K format has 4 wells per slide) into a metal chamber with the label and gasket facing up.
3. Transfer 100 µl of the fragmented cRNA samples from above onto the center of each of the well without introducing bubbles. (One sample/well)



4. Carefully lower a slide, bar code facing up, onto the bead of liquid. (Agilent to Agilent).
5. Confirm that the edges of the 2 slides are aligned and nothing is ajar.
6. Place the metal chamber cover on top, and then slide the clamp over.
7. Tighten the thumbscrew clockwise until it is hand tight. Don't over tighten.
8. Rotate the chamber vertically to wet the gasket and make sure that all bubbles are mobile.
9. Tap chamber on hand or shake until any adherent bubbles are dispersed.
10. Load the 2 chambers into the hyb oven, set at 65°C and level 10-rotation speed (10 rpm).
11. Repeat until all chambers are loaded.
12. Hybridize at 65°C for 17 hrs.

IV. **<u>Washing</u>**

- Leave Gene Expression Wash Buffer 2 in 37°C water bath until immediately before use.
- Perform all washes in the fume hood.
- Have everything prearranged and organized, any delay can damage the slides.
- Wash a maximum of 8 slides at one time.
- Set up 4 washing dishes with 500 ml of solution each.
    - Three dishes containing 500 ml of Gene Expression Wash Buffer 1
        - Dish 1, for slide disassembly.
        - Dish 2 with a slide rack, used to hold the slides temporarily while disassembling the remaining slides.
        - Dish 3 on a stir plate, setting 4.
    - One dish containing 500 ml of Gene Expression Wash Buffer 2
        - Dish 4 on a stir plate, setting 4.
- Before beginning, wash gloves well with de-ionized water and dry.

1. Remove 2 hybridization chambers from the oven.
2. Pick up 1 slide with your gloved hand, with gasketed coverslip still sandwiched together, and submerge in Dish 1 with Wash 1.
3. Gently pry apart with the plastic tweezers. Swoosh slide in Wash 1 to wash off hyb buffer.
4. Place the slide into the slide rack in the Dish 2 keeping it submerged.
5. Repeat this process with the next slide, making sure there is at least one empty space between each slide in the rack.

6. After 4 slides, turn dish around so that the arrays face toward the center of the dish. Wash no more than 8 slides at once in the rack.
7. Transfer the slide rack to Dish 3 with Wash 1 on the stir plate, level 4. Wash for precisely 1 minute at RT.
8. Pour pre-warmed Gene Expression Wash buffer 2 into Dish four above.
9. Lift rack slowly out of Dish 3 so that the liquid drains uniformly off of the slide. Transfer the slide rack to the Dish 4 with Wash 2 on the stir plate, level 4.
10. Wash precisely for 1 minute.
11. Lift rack **slowly** (10 seconds) so that the liquid drains uniformly off of the slide without spotting. Blot the excess liquid well on a Kimwipe.
12. If there are white particulate on the slide, submerge the slide at once and remove very slowly.
13. Put dry slides into a light tight slide box and scan at once.