

Hybridisation of labelled material to cDNA microarrays using a Genomic Solutions Hybridisation Station with Ambion Hybridisation Buffer #1

Overview

Samples or samples and controls have been labelled with Cy3-dCTP or Cy5-dCTP and then mixed together with a blocking agent. We now need to add the hybridisation buffer and hybridise the labelled biological material to the cDNA microarrays. This protocol was based on a method from Genomic Solutions (<http://www.genomicsolutions.com>).

Equipment and reagents

- SlideHyb Glass Array Hybridisation Buffer #1 (Ambion; Cat. No. AM8861)
- 1 x SSC + 0.03% SDS
- 0.23 x SSC
- 0.06 x SSC
- 20 x SSC, pH 7.0
- Grant QBT2 hot-block
- Kenair air duster
- Hettich Rotina 35 microtitre plate centrifuge
- Microscope slide box (Merck EuroLab; Cat. No. 406/0286/00)
- Slide staining rack (Philip Harris; Cat. No. B52651)
- Slide staining trough (Philip Harris; Cat. No. B52649)

Procedure

How to set up the hybridisation cassettes:

1. Ensure slide covers are clean. If necessary, dab gently with a tissue soaked in 70% EtOH. Remove any dust by spraying with canned air and make sure the channels are free of liquid. Check that the red O-rings are in position.
2. Ensure that the metal plate is lying flat. If not, loosen screws with thumbnail and re-tighten with the plate in its proper position.
3. Fit black O-rings to slide cover. Do not stretch and avoid dust.
4. Spray slides with canned air and place slides on the black slide holder. Ensure slides are in the correct orientation.
5. Whilst holding the slide holder pointing slightly downwards and to the right, ensure that the slides are correctly positioned
6. Place the slide cover on top of the holder, without dislodging the slides.
7. Hold this 'slide cassette' together and place on the hybridisation station
8. Lower the clamp mechanism into position and screw until finger tight.

Starting the hybridisation:

9. Switch the hybridisation station on and when it has finished booting up insert the floppy disc with the hybridisation protocol.
10. Ensure that the wash solutions are full and the waste is empty and then connect each to the corresponding wash bottle

11. Press Start a run on the LCD field and select 'Floppy disk'. Scroll down to and then load the cDNA hybridisation protocol.
12. Select the slide positions for the run and press continue
13. The protocol will start automatically and prompt you to add the samples after 15 minutes
14. In the mean time prepare the samples that need to be loaded:
 - ◆ Preheat the SlideHyb Glass Array Hybridisation Buffer #1 to 65 °C for 15 to 30 minutes
 - ◆ Heat the labelled sample at 100 °C for 2 minutes on a hot-block
 - ◆ Add 140 µl SlideHyb Buffer to the sample
 - ◆ Centrifuge for 1 minute at 13,000 rpm
 - ◆ Add 135 µl of the labelled sample to the microarray, avoiding any precipitate
15. The instrument will tell you which sample it wants. To add it select the slide position and press probe - a tick should appear and the valve opens.
16. Add sample by pipetting slowly so as to avoid any bubbles. Put a plastic plug in the hole and press finish for this slide. The valve will then close.
17. Repeat procedure with second slide of the block, when finished press finished at the bottom of screen. The hybridisation station will now perform the hybridisation and washes. This will take 16 hours plus 20 minutes per block for the washes.

Hybridisation protocol

Step	Solution	Temperature	Duration	Agitation	Flow	Hold	Cycles
Hybridisation	Sample in SlideHyb	65 °C	16 hours	Yes	-	-	1

After the hybridisation:

18. Unscrew the clamp mechanism and remove the white plugs. Hold the cassette tightly together and then remove it from the hybridisation station.
19. Invert the sandwich and remove the slides by holding them by one edge and lifting away from the slide cover.
20. Now perform a manual wash as described below.

Manual wash:

21. Pre-heat wash solution 1 (0.2 x SSC; 0.2% SDS) and wash solution 2 (0.2 x SSC) to 55 °C using a water bath
22. Gently remove the slides submerged in pre-heated wash solution 1
23. Transfer the slides in a slide staining rack without letting the slides dry
24. Place the rack into a slide staining trough containing pre-heated wash solution 1
25. Mix on an orbital shaker at 50 rpm for 20 minutes at room temperature
26. Transfer the slides to a staining trough containing pre-heated wash solution 2
27. Gently dip the slides up and down for 3 seconds in wash solution 2
28. Transfer the slides to a second staining trough containing fresh pre-heated wash solution 2
29. Gently dip the slides up and down for 3 seconds in wash solution 2
30. Rinse the slides 3 times with fresh deionized water at room temperature (dip the rack in MilliQ water for 3 seconds)
31. Transfer the slides to a clean microscope slide box with tissue at the base and centrifuge at 1000 rpm for 5 minutes
32. The slides are now ready to be scanned.

Cleaning the hybridisation station:

33. Replace the hybridised slides with some blank slides and put the cassettes back on the hybridisation station
34. Place all of the in-flow tubes in 55 °C MilliQ water (helps to remove salt deposits)
35. Go to the main menu and select 'maintenance', followed by 'machine cleaning cycle'
36. Select the appropriate slide positions and start the cleaning cycle
37. Once the cleaning cycle has finished, remove the O-rings and place them in 200 ml boiling MilliQ water
38. Place the cassettes (not the metallic back plates) in a plastic beaker with warm MilliQ water, soak for a couple of minutes
39. Spray the cassettes with 70% ethanol and then leave them to dry at room temperature

B. Fischer (09-07-2008).