

Hybridisation of labelled material to amino-modified long oligonucleotide microarrays using a Genomic Solutions Hybridisation Station with our hybridisation buffer

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 amino-modified long oligonucleotide microarrays. This protocol was based on a method developed by FlyChip. The hybridisation buffer recipe is from the Ajioka group within the Department of Pathology, University of Cambridge ([Ajioka](#)). The washes are based on a method recommended by Full Moon Biosystems (<http://www.fullmoonbio.com/>).

Equipment and reagents

- Deionised formamide (Sigma; Cat. No. F9037)
- 50 x Denhardt's solution (Sigma; Cat. No. D2532)
- 20 x SSC (Sigma; Cat. No. S6639)
- tetra-Sodium Pyrophosphate (BDH; Cat. No. 102614P)
- Sigma water (Sigma; Cat. No. W 4502)
- 1 M Tris-HCl, pH 7.4 (Sigma; Cat. No. T2663)
- 10% SDS (Sigma; Cat. No. L4522)
- 20 x SSC, pH 7.0
- 0.22 mm filter
- Grant QBT2 hot-block
- Kenair air duster
- Slide staining rack (Philip Harris; Cat. No. B52651)
- Slide staining trough (Philip Harris; Cat. No. B52649)
- Hettich Rotina 35 microtitre plate centrifuge
- Microscope slide box (Merck EuroLab; Cat. No. 406/0286/00)

Procedure

Making the hybridisation buffer:

1. Add the following to 21.78 ml sigma water
 - ◆ 20 ml deionised formamide
 - ◆ 5 ml 50 x Denhardt's solution
 - ◆ 2.5 ml 20 x SSC
 - ◆ 220 µl Sodium Pyrophosphate (1 g tetra sodium pyrophosphate in 1 g in 10 ml Sigma water)
 - ◆ 2.5 ml 1 M Tris-HCL, pH 7.4
 - ◆ 500 µl 10% SDS
2. You should now have a total volume of 50 ml
3. Filter through 0.22 mm filter and store in aliquots at -20 °C

How to set up the hybridisation cassettes:

4. 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.

5. Ensure that the metal plate is lying flat. If not, loosen screws with thumbnail and re-tighten with the plate in its proper position.
6. Fit black O-rings to slide cover. Do not stretch and avoid dust.
7. Spray slides with canned air and place slides on the black slide holder. Ensure slides are in the correct orientation.
8. Whilst holding the slide holder pointing slightly downwards and to the right, ensure that the slides are correctly positioned
9. Place the slide cover on top of the holder, without dislodging the slides.
10. Hold this 'slide cassette' together and place on the hybridisation station
11. Lower the clamp mechanism into position and screw until finger tight.

Starting the hybridisation:

12. Switch the hybridisation station on and when it has finished booting up insert the floppy disc with the hybridisation protocol.
13. Press Start a run on the LCD field and select 'Floppy disk'. Scroll down to and then load the oligo hybridisation protocol.
14. Select the slide positions for the run and press continue
15. The protocol will start automatically and prompt you to add the samples after 15 minutes
16. In the mean time prepare the samples that need to be loaded:
 - ◆ Add 135 µl of hybridisation solution to 4 µl 10% SDS and the labelled mixture
 - ◆ Heat at 100 °C for 2 minutes on a hot-block
 - ◆ Centrifuge for 3 minutes at 13,000 rpm
 - ◆ Add 135 µl of the labelled sample to the microarray, avoiding any precipitate
17. 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.
18. 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.
19. 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, which will take 16 hours.

Hybridisation protocol

Step	Solution	Temperature	Duration	Agitation	Flow	Hold	Cycles
Hybridisation	Sample in Hybridisation buffer	42 °C	16 hours	Yes	-	-	1

After the hybridisation:

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

Manual wash:

23. 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
24. Place the slides in a slide staining rack without letting the slides dry
25. Place the rack into a slide staining trough containing pre-heated wash solution 1

26. Mix on an orbital shaker at 50 rpm for 20 minutes at room temperature
27. Transfer the slides to a staining trough containing pre-heated wash solution 2
28. Gently dip the slides up and down for 1 minute in wash solution 2
29. Repeat steps 27 to 28 twice with fresh 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.

R. Auburn (04-08-2004).