

Hybridisation of labelled material to amino–modified long oligonucleotide microarrays using a Genomic Solutions Hybridisation Station with the Ocimum 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 washes are based on a method recommended by Full Moon Biosystems (<http://www.fullmoonbio.com/>).

Equipment and reagents

- 10% SDS (Sigma; Cat. No. L4522)
- Ocimum hybridisation buffer (Biosolutions; Cat. No. 1180–200000)
- 20 x SSC, pH 7.0
- 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

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. Press Start a run on the LCD field and select 'Floppy disk'. Scroll down to and then load the oligo hybridisation protocol.
11. Select the slide positions for the run and press continue
12. The protocol will start automatically and prompt you to add the samples after 15 minutes

13. In the mean time prepare the samples that need to be loaded:
 - ◆ Add 140 µl of Ocimum hybridisation buffer to 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
14. 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.
15. 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.
16. 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

Microarray	Step	Solution	Temperature	Duration	Agitation	Flow	Hold	Cycles
<u>FL001</u>	Hybridisation	Sample in Hybridisation buffer	50 °C	16 hours	Yes	–	–	1
<u>FL002</u>	Hybridisation	Sample in Hybridisation buffer	51 °C	16 hours	Yes	–	–	1

After the hybridisation:

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

Manual wash:

20. 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
21. Place the slides in a slide staining rack without letting the slides dry
22. Place the rack into a slide staining trough containing pre-heated wash solution 1
23. Mix on an orbital shaker at 50 rpm for 20 minutes at room temperature
24. Transfer the slides to a staining trough containing pre-heated wash solution 2
25. Gently dip the slides up and down for 1 minute in wash solution 2
26. Repeat steps 24 to 25 twice with fresh wash solution 2
27. Rinse the slides 3 times with fresh deionized water at room temperature (dip the rack in MilliQ water for 3 seconds)
28. Transfer the slides to a clean microscope slide box with tissue at the base and centrifuge at 1000 rpm for 5 minutes
29. The slides are now ready to be scanned.

Cleaning the hybridisation station:

30. Rinse the cassettes with running reverse osmosis water, to remove the salt deposits
31. Replace the hybridised slides with some blank slides and put the cassettes back on the hybridisation station

Starting the hybridisation:

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