

Full Moon Biosystems (FMB) protocol for processing FMB cDNA slides:

Overview

After printing non-modified PCR amplified gene-specific cDNA clones the Full Moon Biosystems (FMB) cDNA slides are then processed to bind the denatured probe DNA to the slide and prevent non-specific hybridisation to the substrate. The outlined protocol is based on the method recommended by Full Moon Biosystems (<http://www.fullmoonbio.com/>).

Equipment and material

- Full Moon Biosystems cDNA slides (Full Moon Biosystems; Cat. No. AS 50)
- Slide staining rack (Philip Harris; Cat. No. B52651)
- Slide staining trough (Philip Harris; Cat. No. B52649)
- UV crosslinker (Ultraviolet products; CL-1000)
- Orbital shaker (Stuart Scientific; mini orbital shaker SO5)
- Hettich Rotina 35 microtitre plate centrifuge
- Microscope slide box (Merck EuroLab; Cat. No. 406/0286/00)
- Horizontal laminar flow work station (Jencons; Cat. No. 566-031)
- Bovine serum albumin (BSA) fraction V (Sigma; Cat. No. A-7906).
- Sodium dodecyl sulfate (SDS), molecular biology grade (Sigma; Cat. No. L-4390)
- Blocking Solution: 4xSSC, 0.1%SDS, 1%BSA
- MilliQ water
- Standard photographic air duster

Protocol

For best results, perform steps 4 onwards just before hybridisation.

1. After arraying, UV cross-link the slides with the cross linker set at 4000 ($\times 100\mu\text{J}$) = 400mJ
2. Allow slides to dry at room temperature for 30 minutes
3. Slides that are not needed for one month or two months can be stored at this stage:
 - ◆ Place the slides in a clean microscope slide box
 - ◆ Then place the microscope slide box in a pastic bag and seal this bag
 - ◆ Store the sealed bag at 2 to 8 °C for 3 to 6 months
4. Meanwhile prepare and preheat the blocking solution to 55 °C in a water bath
5. Pour the blocking solution into a slide staining trough
6. Transfer the slides to a slide staining rack and place the rack into the slide staining trough
7. Place box on shaker at 50 rpm for 20 minutes at room temperature
8. Remove rack, blot off excess solution by placing on a piece of tissue.
9. Place the slides in the staining rack in the plastic box filled with 2.5 L ultra pure water and put the lid on
10. Place the plastic box with the slides on the orbital shaker at 50 rpm for 15 minutes
11. Remove rack, blot off excess solution by placing on a piece of tissue.
12. Repeat steps 8 to 10 twice (three water washes in total)
13. Transfer slides from the rack to a microscope slide box with fresh tissue in the base
14. Centrifuge at 650 rpm for 15 minutes in a microtitre centrifuge to dry the slides
15. Remove any water droplets from the slide using an air duster
16. Store in a clean sealed slide box at room temperature and in the dark (one to two months) until ready to hybridise

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