Processing Poly-L-Lysine coated glass microscope slides

Overview

After printing non-modified PCR amplified gene-specific cDNA clones the poly-l-lysine coated glass microscope slides are processed to bind denatured probe DNA to the slide and prevent non-specific binding during hybridisation. The outlined protocol is based on the method recommended by BioRobotics (http://www.genomicsolutions.com/).

Equipment and reagents

- Slide staining rack (Philip Harris; Cat. No. B52651)
- Slide staining trough (Philip Harris; Cat. No. B52649)
- Hotplate (Stuart Scientific)
- UV crosslinker (Ultraviolet products; CL-1000)
- Succinic anhydride (Sigma; Cat. No. S-7626)
- 1-methyl-2-pyrrolidinone (Sigma; Cat. No. M-6762)
- Boric acid (Sigma; Cat. No. B-7660)
- MilliQ water
- Orbital shaker (in fume hood) (Stuart Scientific; mini orbital shaker SO5)
- Microwave oven (Panasonic; NN-T559W)
- 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)
- 96% Ethanol

Procedure

1. After printing bake the Poly-L-Lysine slides for 15 minutes at 80 °C on a hotplate. Then UV cross-link the array to the slides using the UV Crosslinker set to 650 (x100 µJ). During both of these steps make sure the arrayed surface is facing up. Transfer the slides into a staining rack and place each rack into a separate staining trough.
2. Make the Blocking solution. This step must be performed in the fume hood. Once in solution, the blocking agents do not remain active for long, so it is important to prepare fresh solutions and use them immediately. Dissolve 0.75 g succinic anhydride completely in 125 ml 1-methyl-2-pyrrolidinone. Immediately mix in 125 ml of 0.2M boric acid pH 8.0.
3. This step must be performed in the fume hood. Plunge the slides up and down vigorously several times (5 to 10 times) in the Blocking Solution and then leave in the solution for 20 minutes on an orbital shaker (or magnetic stirrer with constant agitation). Vigorous agitation is essential so that background caused by localized re-attenuation of excess DNA material can be avoided.
4. This step should be performed in the fume hood. Plunge the slides up and down vigorously into 95 °C ddH2O for 2 minutes. The 95 °C ddH2O can be easily prepared by boiling ddH2O in a beaker in a microwave oven. Plunge the slides into the water once it has stopped bubbling. This step will denature the dsDNA.
5. Plunge the slides into 96% ethanol for 1 minute, without agitation, then take to Laminar Flow Workstation. Transfer slides into microscope slide boxes, with plenty of tissue at the base, and centrifuge for 5 minutes at 650 rpm in a microtitre plate centrifuge. The slides can either be used immediately in a hybridization reaction, or stored in a cool, dry and dust-free cupboard for at least a month.

R. Auburn (07-06-2004).