

Quality control of printed microarrays by hybridisation to an alexa-488 panomer 9 random oligo

Overview

After printing microarrays using PCR amplified cDNA clones or long oligonucleotide probe DNA, a random sample of microarrays is stained and then scanned so that we can assess the print quality and constancy. These checks include substrate defects, sub-grid and meta-grid positioning on the substrate, checking that all spots have been printed and spot morphology. Print batches that fail these quality control test are either used for teaching or internal development.

Equipment and Reagents

- Between 2 and 8 microarrays from the printing batch to be tested
- 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)
- Horizontal laminar flow work station (Jencons; Cat. No. 566-031)
- QC wash solution 1: 1x SSC, 0.03% SDS
- QC wash solution 2: 0.06x SSC
- 24 x 60 mm microscope slide cover slips (Menzel Gläser; Cat. No. BB024060A1)
- ArrayHyb Hybridisation Solution (Sigma; Cat. No. A7718)
- Hettich micro 20 centrifuge
- Panomer 9 random oligo Alexa flour 488 conjugate (Molecular Probes; Cat No. P-21680)

Procedure (per slide)

1. Add 2 μ l Alexa 488 random oligo (100 μ M stock solution) to 40 μ l hybridisation buffer
2. Incubate the oligo-buffer mixture at 90 °C for 2 minutes, then centrifuge at 13000 rpm for 1 minute
3. Add 40 μ l of the oligo-buffer mixture to a cover slip, carefully place the printed microarray onto the cover slide, then invert so that the cover slip is on top
4. Incubate for 5 minutes at room temperature
5. Transfer the slide(s) to a slide staining rack and then place in a slide staining trough containing QC wash solution 1
6. Rinse the slide by gently plunging up and down ten times
7. Transfer the slide staining rack to a slide staining trough containing QC wash solution 2
8. Rinse the slide(s) by gently plunging up and down ten times
9. Transfer the slide(s) to a microscope slide box with tissue paper at the base
10. Centrifuge at 650 rpm for between 5 to 10 minutes to dry the slide(s)

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