

Quality control of printed microarrays by staining with Syto 61

Overview

After printing microarrays with 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)
- TE buffer: 10 mM Tris-Cl (pH 7.5), 1 mM EDTA
- QC wash solution: 2 x SSC; 0.5% SDS
- 24 x 60 mm microscope slide cover slips (Menzel Gläser; Cat. No. BB024060A1)
- Syto 61 dye (Molecular Probes; Cat. No. S-11343)
- Standard photographic air duster
- MilliQ water

Procedure (2 slides)

1. Dilute 1 μ l Syto 61 in 100 μ l TE buffer
2. Incubate each slide with 45 μ l of this mix under a cover slip for 5 minutes in the dark, i.e. under a staining trough
3. Place the slides in a staining rack and then transfer to a staining trough containing QC wash solution 1
4. Remove the cover slip
5. Wash the slides for 5 minutes in QC wash solution 1 in a staining trough at room temperature on the orbital shaker at 50 rpm
6. Remove rack, blot off excess solution by placing on a piece of tissue
7. Repeat the QC wash solution 1 once more
8. Remove rack, blot off excess solution by placing on a piece of tissue
9. Plunge rack up and down 30 times in a slide staining trough containing MilliQ water
10. Remove rack, blot off excess solution by placing on a piece of tissue
11. Plunge rack up and down a further 30 times in a second slide staining trough containing fresh MilliQ water
12. Remove rack, blot off excess solution by placing on a piece of tissue
13. Plunge rack up and down a further 30 times in a third slide staining trough containing fresh MilliQ water
14. Remove rack, blot off excess solution by placing on a piece of tissue
15. Transfer slides to a microscope with fresh tissue in the base
16. Centrifuge at 650 rpm for 15 minutes
17. Remove any water droplets from the slide using an air duster
18. Scan using the cy5-channel of a CCD or dual laser scanner

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