Post-processing of DNA microarrays printed with long oligos

This protocol describes the post processing of DNA microarrays that have been printed with long 70-mer oligonucleotides. It is based on the Brown lab DNA protocol for cDNA arrays and differs primarily by omitting the boiling step.

1. Cue up slides
   Select one rack full of slides for post-processing (usually 20-30 slides).

2. Label slides
   Make sure slides all have identical orientation. A diamond pen can be used to etch a number in the corner of the slide. Alternatively use laminated labels.

3. Mark array area
   Using the diamond pen, make an etch on the back of the slide denoting a guide for the cover slip.

4. Rehydrate the slides and snap dry.
   Place the slides face down over a humid chamber containing 3X SSC that has been warmed slightly above room temperature (37 C works well). The surface of the slide will fog up with condensation. Typical times are 1-5 minutes. The spots should be re-hydrated and become glistening but not so much that they run together. Once the surface has been hydrated the slides should be immediately placed array side up on a hot plate set between 100-140 C. At the right angle of light, one can observe the condensation rapidly disappearing from the slide surface, this is referred to as snap drying. It usually takes less than 5 seconds.

5. UV crosslink the DNA to the slides with 65 mJ of energy (optional).

6. Place the slides back in the rack.

Succinic anhydride blocking:

1. Measure out a 15 ml aliquot of 1M NaBorate pH 8.

2. Combine 335 ml 1-methyl-2-pyrrolidinone and 5.5 g succinic anhydride in a dry, clean pyrex dish with a stir bar. Make sure the level of the liquid is deep enough to submerge a slide rack.

3. As soon as the last flake of succinic anhydride dissolves mix in the NaBorate.

4. Quickly plunge the slide rack full of slides up and down in the solution. Plunge quickly and evenly for 30 seconds. Let the slides sit in the solution with gentle agitation on an orbital shaker for 15 minutes.

5. Remove the slide rack and plunge the the slides up and down in a slide dish with room temperature distilled water to rinse off the blocking solution. Incubate 1 minute.

6. Transfer the slide rack to a dish containing 95% ethanol and plunge up and down several times.

7. Dry the slides by spinning in a tabe top centrifuge at 500 RPM for 5 minutes.

8. Store the slides at room temperature in a dust free box until ready for use.

Notes:

Since the arrayed spots will disappear upon processing, a couple of etch marks on the back of the slide can guide coverslip placement. This is easily accomplished by placing two plastic pipet tip boxes close together as an etch support, and placing the slide upside down across the two boxes such that the area of the spots is facing down between the boxes and is thus untouched.

For re-hydration and snap dry the slides are processed basically one at a time or a few in parallel with a few seconds between each manipulation.

Re-hydration - placing a desk lamp over the humid chamber helps add heat to the liquid and fosters condensation on the cooler glass surface. The light also helps one visualize the re-hydration. The purpose of this step is to redistribute the DNA uniformly throughout the spots and then dry it quickly to maintain that pattern.

Set things up for efficiency and avoid awkward transitions. A slide can be grabbed from the humid chamber and placed on the heat block in one smooth quick step. Notice how your hand can rotate with your forearm and keep this in mind while setting up the chamber and the heat block, placing things in ways that take advantage of how your body moves.

Move rapidly during the dunking in succinic anhydride solution. The half life of succinic anhydride is very short, on the order of minutes. The dunking can be tricky, as this is the step where "comet tails" can be introduced.

1M NaBorate is prepared from solid Boric Acid and pH adjusted to 8.0 with NaOH pellets or liquid.

A low cost label maker with laminated labels can be used for slide labeling. The labels will survive all aspects of slide processing. The "Brother" labeller can print a series of labels with spaces so that slides can be easily labelled while sitting on the arrayer.

Materials:

1-methyl-2-pyrrolidinone	Aldrich	32,863-4 (1 Liter)
Succinic Anhydride	Aldrich	23,969-0
Boric Acid	Fisher	A73-500
Humid Chamber	Sigma	H6644
Diamond Pen	VWR	52865-005

This protocol is based on the Array post-processing protocol found in the MGuide written by Joe DeRisi and Pat Brown.
http://cmgm.stanford.edu/pbrown/mguide
http://www.microarrays.org/

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