Staining DNA microarray

◎*Materials and Reagents*

1 mM ToTo-3 iodide in dimethylformamide (DMSO) (Molecular Probes, Cat. P-3604) or

1 mM PoPo-3 iodide in dimethylformamide (DMSO) (Molecular Probes, Cat. T-3604)

10X PBS (Invitrogen, Cat. 70013-032)

Plastic cover slip (e.g. Hybrislip from Research Products)

Slide rack (Wheaton, Cat. 900234)

glass jar

SpeedVac

Microarray scanner

Wash solution

1%SDS, 10 mM Tris, 1mM EDTA, pH 7.5

1. Thaw the frozen dye (ToTo-3 iodide or PoPo-3 iodide) and then dilute the dye to final concentration of 1μM in1X PBS at room temperature (ToTo-3 staining appears to yield lower background fluorescence).
2. Apply 50 μl of the diluted dye to a plastic lip. Place a slide (array faces down) to the plastic lip. Make sure that the diluted dye immediately covers the whole slide. Alternatively, add appropriate volume of the dye to the center of the array (face up) and immediately cover the array with plastic cover slip. Note: it is critical to quickly apply diluted dye on the array.
3. Incubate the array with the diluted dye for 30 minutes at room temperature in the dark.

4. After the completion of 30 minutes incubation, plunge slide in a rack up and down for two minutes in a glass jar filled with 1X PBS.

5. Dry the slides with SpeedVac for 5 minutes.

6. If the DNA is stained by ToTo-3 iodide, scan the array by using the red laser and Cy5 filter. (ToTo-3 exhibits absorption/emission maximum of 642/660 nm when bound to double stranded DNA.) If the DNA is stained by PoPo-3 iodide, scan the slide by using the green laser and Cy3 filter. (PoPo-3 iodide exhibits absorption/emission maximum of 534/570 nm when bound to double stranded DNA.)