

Hybridisation and Washing

Oligonucleotide Slides

1.) Prior to hybridization each microarray is immersed in 50 ml of hot Milli-Q water (80-95C) and agitated gently for 5 min. The slide is then dried by centrifugation at 750rpm for 5min.

2.) The labeled cDNA is mixed with 0.64 μ L of 25mg/mL yeast tRNA, 4 μ L of 2mg/mL poly A and 20 μ L of 1mg/mL Cot-1 DNA. The mix is dried under reduced pressure then dissolved in the appropriate volume of formamide and 6.25 X SSC (*Table 1*):

Array	Coverslip size	Formamide	6.25 X SSC
Mouse 22K	60 x 24 mm	16 μ L	16 μ L
Human 20K	50 x 24 mm	14 μ L	14 μ L
Zebrafish 16K	50 x 24 mm	14 μ L	14 μ L

Table 1

The mixture is heated to 100C for 3min., transferred directly to ice then 0.5 μ L of 10% SDS is added before the solution is applied to the centre of the cover slip. The array is lowered onto the cover slip then incubated at 42C overnight in a humidified chamber.

3.) Following incubation, the array is immersed in solution A Solution A (0.5 X SSC, 0.01% SDS) until the coverslip disengages the surface. After the coverslip is discarded the array is washed in Solution A for 5 min., Solution B (0.5 X SSC) for 5 min then Solution C (0.2 X SSC) for 3 min. The slide is dried in a centrifuge at 750 rpm for 5 min and stored in the dark prior to scanning.

Hybridisation and Washing

cDNA Slides

- 1.) Prior to hybridization each microarray is incubated in pre-hybridisation solution (10mg/mL BSA, 25% formamide, 5 X SSC and 0.1% SDS) for 60 min at 42C then rinsed in Milli-Q water and dried by centrifugation at 750rpm. for 5 min.

- 2.) Labeled cDNA is mixed with 0.64 μ L of 25mg/mL yeast tRNA, 4 μ L of 2mg/mL poly A and 20 μ L of 1mg/mL Cot-1 DNA. The mix is dried under reduced pressure then dissolved in 14 μ L of formamide and 14 μ L of 6.25 X SSC. The mixture is heated to 100C for 3min., transferred directly to ice then 0.2 μ L of 10% SDS is added before the solution is applied to the center of a 50 x 24mm cover slip. The array is lowered onto the cover slip then incubated at 42C overnight in a humidified chamber.

- 3.) Following 'hybridisation' the slide is washed for 1min. in Solution A (0.5 X SSC, 0.01% SDS), 3min. in Solution B (0.5 X SSC), 3min. in Solution C (0.06 X SSC) then finally rinsed 5 X in MilliQ water. The slide is then dried in a centrifuge at 800rpm. for 5min. and stored in the dark prior to scanning.