



# Institute of Food Research

## Protocol from the *Salmonella* group



### Microarray Hybridisations

1. To 10  $\mu$ l of dried down labelling reactions add:
  - a) 1.5  $\mu$ l of 50 X Denhardt's solution
  - b) 2.25  $\mu$ l of 20 X SSC
  - c) 1.125  $\mu$ l of *E. coli* tRNA (10  $\mu$ g/ $\mu$ l) (Sigma, Cat: R8759)
  - d) 0.375  $\mu$ l of 1M HEPES, pH 7.0.

**NOTE:** The hybridisation components can be mixed together as a stock solution, either from a fresh or frozen stock, and added to labelling reactions.

2. Add 0.375  $\mu$ l of 10% SDS to the mixture
3. Incubate 100 °C for 2 min. Let stand on the bench for 5 - 10 min. **Do not** place the reaction on ice because the SDS will precipitate.
4. Spin in a microfuge at full speed for 5 min, transfer supernatant to clean tube and repeat spin.
5. Place a microarray slide in a metal hybridisation chamber ([Photo 3](#)).
6. Pipette the hybridisation solution towards one edge of an array.
7. Place edge of a clean (washed with 70% ethanol), dry, coverslip on the edge of the array.
8. Position fine nosed forceps (VWR, Cat: 406/0073/04) under the coverslip.
9. Using the forceps slowly lower the coverslip down onto the hybridisation solution and the array ensuring no air bubbles are present under the coverslip. If air bubbles are present it is sometimes possible to move them to the edge of the coverslip by pressing gently with the forceps.
10. Apply 20  $\mu$ l of 3 X SSC around the 4 corners of the slide using a pipette (approximately 5  $\mu$ l per corner). This will maintain the correct humidity in the hybridisation chamber.
11. Screw the lid onto the chamber and place at the bottom of a water bath at 63°C overnight.
12. Remove the slides from the hybridisation chambers and place them in a plastic slide rack ([Photo 4](#)). The slides are then placed into a 63°C wash solution containing 2 X SSC, 0.1% SDS and agitated for 5 mins. (We use a 2L beaker with a stirrer bar in

the bottom and a platform for the slide rack to sit on for this, **Photos 5 & 6**). It may be necessary to tap the slide rack on the platform to make sure that the cover slips are removed. We use a thermocouple attached to a hot-plate stirrer for this step. Repeat the washing step.

Note: SDS fluoresces so you should avoid carrying it over into the washes in steps 13 and 14.

13. Transfer slide rack to a solution of 1 X SSC at room temperature and agitate for 5 min. Repeat. (We use black tissue boxes often used for histology for this)

14. Wash in 0.2 X SSC for 5 min. Repeat.

15. Spin dry in a centrifuge at 1200 rpm for 5 min at room temp in an enclosed slide chamber (with blotting paper lining the bottom of the chamber).

16. Scan.

**NB** All solutions are filter sterilised (0.1 µm) and made up with Sigma water (molecular biology reagent, Sigma, Cat: W4502).