



# Institute of Food Research

## Protocol from the *Salmonella* group



### Direct labelling of RNA

Set up random priming reactions in 1.5 ml microfuge tubes. Add:

- a) 10 µg of total RNA (this may require concentrating using a speed vac)
- b) 5 µg of random hexamers (Invitrogen, Cat: 48190011)
- c) In a total volume of 9.4 µl of Sigma ultra-pure water (mol. biol. reagent, Cat: W4502).
- d) Incubate 70°C for 5 min protected from light then chill on ice for 10 min. Spin briefly in microfuge.

2) Using the Stratagene AffinityScript multi-temperature Reverse Transcriptase (Cat: 600109) Prepare RT reaction mix (sufficient for one labelling reaction)

- a) 2.0 µl of 10 X RT buffer
- b) 2.0 µl of 0.1 M DTT
- c) 0.6 µl of 50 X dNTP's \*

3) Add RT reaction mix to RNA (4.6 µl per reaction)

4) Finally add:

- a) 2 µl of Cy3 or Cy5-dCTP (1mM stock, GE Healthcare Lifesciences, Cat: PA55321)
- b) 4 µl of reverse transcriptase.

The total reaction volume is 20 µl.

5) Mix and incubate at 25°C for 10 mins.

6) Incubate overnight at 42°C.

7) Add 15 µl of freshly prepared 0.1M NaOH and hydrolyse the RNA at 70°C for 10 minutes. Add 15 µl of 0.1M HCl to neutralise the alkali.

8) If performing a type I experiment follow section 'a' or for a type II follow section 'b'

- a) If performing a type I experiment (RNA verses RNA) (DeRisi *et al*, Science, 1997, **278**, 680-686), combine reactions and clean up using Qia-quick PCR purification kit (Qiagen, Cat: 28104) to remove unincorporated/quenched cy dyes. Elute twice using 50 µl Sigma water as final eluant to maximise recovery. Speed vac on medium setting and redissolve pellet in 10 µl Sigma water (mol. biol. reagent, Cat: W4502). Proceed to 'Microarray Hybridisations' protocol.
- b) If performing a type II experiment (RNA verses genomic DNA), mix labelled cDNA with labelled genomic DNA (see 'Direct labelling of DNA' protocol). Clean up using Qia-quick PCR purification kit (Qiagen, Cat: 28104) to remove unincorporated/quenched cy dyes. Elute twice using 50 µl Sigma water as final eluant to maximise recovery. Speed vac on medium setting and redissolve pellet in

10  $\mu$ l Sigma water (mol. biol. reagent, Cat: W4502). Proceed to 'Microarray Hybridisations' protocol.

\*To prepare 50 X dNTP's, mix 25 mM dATP, dGTP, dTTP and 10 mM dCTP, (25  $\mu$ l of dA, dG, dT and 10  $\mu$ l of dCTP from 100 mM stock dNTP kit (GE Healthcare Lifesciences, Cat: 27-2035-02). Add Sigma water to 100  $\mu$ l.

NB: Sigma water (molecular biology reagent, Cat: W4502) was used to prepare all solutions