Institute of Food Research

Protocol from the *Salmonella* group

Labelling protocol for reduced amounts of RNA

(This method will label down to 0.35 µg of RNA in our laboratory)

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

a) 5 µg (or maximum amount) of total RNA
b) 5 µg of random hexamers (Invitrogen, Cat: 48190011)
c) In a total volume of 14.4 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 0.1M DTT
   c) 1.6 µl of **C50** X dNTP's (**25mM** dCTP, dATP, dGTP, dTTP)

3) Add RT reaction mix to RNA (5.6 µl per reaction).

4) Add 4 µl of reverse transcriptase

   The total reaction volume is 24 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) Clean up using Qia-quick PCR purification kit (Qiagen, Cat: 28104). 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. This generates cDNA from the RNA.

9) Label the cDNA using the Direct Labelling of DNA protocol.