



Institute of Food Research

Protocol from the *Salmonella* group



Direct labelling of DNA

- 1) In a sterile microfuge tube add 2 µg of chromosomal DNA isolated using the Qiagen 'Genomic DNA' Kit (Cat: 19060 for the buffer kit; 10243 for the columns) and bring volume to 21µl with Sigma ultra-pure water (mol. biol. reagent, Cat: W4502).
- 2) Add 20 µl of 2.5X Random primer/reaction buffer mix from the Invitrogen BioPrime® DNA Labeling System (18094011). Boil 5 min, then put on ice for 5 min. Fragmentation of DNA (sonication or digestion with restriction enzymes) is recommended when using high complexity genomic DNA such as human.
- 3) On ice, add:
 - a) 5 µl of 10X dNTP mix (10X dNTP mix: 1.2 mM each dATP, dGTP, dTTP; 0.6 mM dCTP; 10 mM Tris pH8.0; 1 mM EDTA; **Do not use the dNTP mix from the kit**).
 - b) 3 µl of Cy5 dCTP or Cy3 dCTP (1 mM stock, GE Healthcare Lifesciences, Cat: PA55321)
 - c) 1 µl of Klenow enzyme from the kit.
- 4) The total reaction volume is 50 µl.
- 5) Spin briefly and incubate the reaction mixture at 37°C overnight protected from light.
- 6) If using labelled genomic DNA as the reference in a type II experiment (DeRisi *et al*, Science, 1997, **278**, 680-686), this protocol supplies sufficient labelled DNA for five hybridisations. Mix a 1/5 volume (i.e. 10 µl) of the labelled DNA with the labelled cDNA.
- 7) Use a Qia-quick PCR purification kit (Qiagen Cat: 28104) to remove unincorporated/quenched Cy dyes. Elute twice using 50 µl of Sigma water to maximise recovery.