

## ***Probe generation by nick-translation***

### *Equipment and reagents)*

- E. coli DNA polymerase I, nick translation grade (Roche Molecular Biochemicals)
- 10 mM biotin-16 dUTP (Roche Molecular Biochemicals)
- 10 mM dNTPs set (Roche Molecular Biochemicals)
- $\beta$ -mercaptoethanol (Sigma)
- Bovine serum albumin (BSA) (Sigma)
- Pre-chilled (-20°C) absolute ethanol
- Nuclease-free water
- 10 x nick translation buffer: Add 500  $\mu$ l 1M Tris-HCl (pH 8.0) and 100  $\mu$ l 0.5 M MgCl<sub>2</sub> and 5  $\mu$ l BSA (0.5 mg/ml) into 1.5 ml test tube. Add 395  $\mu$ l water to adjust the reaction volume into 1ml
- Nucleotide stock. To a 1.5 ml test tube add 10  $\mu$ l 10 mM dATP, 10  $\mu$ l 10 mM dCTP, 10  $\mu$ l 10 mM dGTP, 10  $\mu$ l 10 mM biotin-16-dUTP and 4  $\mu$ l 1 M Tris-HCl (pH 7.4). Add 156  $\mu$ l nuclease-free water to adjust the reaction volume to 200  $\mu$ l
- 0.1M  $\beta$ -mercaptoethanol. Into a 1.5 ml test tube add 0.7  $\mu$ l  $\beta$ -mercaptoethanol Add 999.3  $\mu$ l sterile water to adjust the final volume to 1 ml
- Dnase I, RNase-free (10 units,  $\mu$ l) (Roche Molecular Biochemicals: Make a stock solution of 10 mg/ml DNase I in 50 % (v/v) glycerol which can be stored at -20°C. Add 1  $\mu$ l DNase I stock solution into 9  $\mu$ l ice-cold water to make a stock solution (1mg/ml). Add 1  $\mu$ l DNase I (1 mg/ml) stock solution in 100  $\mu$ l ice-cold water immediately before use; discard afterwards

### *Method*

1. Add into a 1.5 ml test tube on ice 10  $\mu$ l 10 x nick translation buffer, 10  $\mu$ l nucleotide stock, 10  $\mu$ l  $\beta$ -mercaptoethanol, 2  $\mu$ g probe DNA, 1  $\mu$ l DNase I (100  $\mu$ g/ml), 1  $\mu$ l DNA polymerase I (10 U/ $\mu$ l). Add 66  $\mu$ l nuclease-free water to adjust the final reaction volume to 100  $\mu$ l<sup>a</sup>
2. Centrifuge the test tube briefly at 4°C

3. Immediately place the test tube into a 16°C water bath. Incubate for 2 h
4. Put the test tube on ice immediately. Take 5 µl of sample from the test tube, add agarose gel loading buffer, denature for 3 min at 95°C, and run on 1% agarose gel<sup>b</sup>.
5. To separate the probe from unincorporated nucleotides by ethanol precipitation add 1 µl 0.1 M EDTA to stop the reaction and add 10 µl 3 M sodium acetate (pH 5.2) and 200 µl of pre-chilled (-20°C) absolute ethanol
6. Let the precipitate form for at least 20 min at -20°C
7. Centrifuge the tube at 13,000 x g for 15 min at 4°C. Very carefully discard the supernatant
8. Dry the pellet under vacuum. Take care not to over dry the sample
9. Resuspend the DNA in 20 µl nuclease-free water for a final concentration of ~ 100 ng/µl.
10. Store at -20°C

<sup>a</sup>The appropriate dilution of DNase I is critical in controlling the size of the labeled probe

<sup>b</sup>The probe should run as a smear between 100-300 bps in size. If the probe is not the correct size, return the test tube to the 16°C water bath for further digestion. If the probe is less than 100 nt in size use less DNase I or shorter incubation periods.