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)
- β-mercaptoethanol (Sigma)
- Bovine serum albumine (BSA) (Sigma)
- Pre-chilled (-20ºC) absolute ethanol
- Nuclease-free water
- 10 x nick translation buffer: Add 500 µl 1M Tris-HCl (pH 8.0) and 100 µl 0.5 M $MgCl_2$ and 5 µl BSA (0.5 mg/ml) into 1.5 ml test tube. Add 395 µl water to adjust the reaction volume into 1ml
- Nucleotide stock. To a 1.5 ml test tube add 10 µl 10 mM dATP, 10 µl 10 mM dCTP, 10 µl 10 mM dGTP, 10 µl 10 mM biotin-16-dUTP and 4 µl 1 M Tris-HCl (pH 7.4). Add 156 µl nuclease-free water to adjust the reaction volume to 200 µl
- 0.1M β-mercaptoethanol. Into a 1.5 ml test tube add 0.7 µl β-mercaptoethanol Add 999.3 µl sterile water to adjust the final volume to 1 ml
- Dnase I, RNase-free (10 units, µl) (Roche Molecular Biochemicals: Make a stock solution of 10 mg/ml DNase I in 50 % (v/v) glycerol which can be stored at –20ºC. Add 1 µl DNase I stock solution into 9 µl ice-cold water to make a stock solution (1mg/ml). Add 1 µl DNase I (1 mg/ml) stock solution in 100 µl ice-cold water immediately before use; discard afterwards

Method

1. Add into a 1.5 ml test tube on ice 10 µl 10 x nick translation buffer, 10 µl nucleotide stock, 10 µl β-mercaptoethanol, 2 µg probe DNA, 1 µl DNase I

(100 μg/ml), 1 μl DNA polymerase I (10 U/μl). Add 66 μl nuclease-free water to adjust the final reaction volume to 100 μl[a]

2. Centrifuge the test tube briefly at 4ºC
3. Immediately place the test tube into a 16ºC waterbath. 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 95ºC, and run on 1% agarose gel[b].
5. To separate the probe from unincorporated nucleotides by ethanol precipititation 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 (-20ºC) absolute ethanol
6. Let the precipitate form for at least 20 min at –20ºC
7. Centrifuge the tube at 13,000 x g for 15 min at 4ºC. 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 –20ºC

[a]The appropriate dilution of DNase I is critical in controlling the size of the labeled probe

[b]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 16ºC waterbath for further digestion. If the probe is less than 100 nt in size use less DNAse I or shorter incubation periods.