

Labelling of probes for FISH by nick translation

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Equipment and reagents

- ◆ MicroSpin G50 columns (Amersham Pharmacia Biotech)
- ◆ Purified DNA, whole chromosome library, or PCR amplified chromosomes
- ◆ 10 x nick translation buffer: 0.5 M Tris-HCl pH 7.5, 50 mM MgCl₂, 0.5 mg/ml nuclease-free bovine serum albumin (BSA)
- ◆ 1 mM biotin-16-dUTP, or 1 mM digoxigenin (dig)-11-dUTP (Boehringer Mannheim)
- ◆ 100 mM dithiothreitol (DTT) (Sigma)
- ◆ dNTP mix: 0.5 mM each dATP, dCTP, dGTP, and 0.1 mM dTTP (Roche Diagnostics)
- ◆ DNase I (200,000 U) (RNase-free, Grade I pure, Roche Diagnostics)
- ◆ DNase I dilution buffer: 50% glycerol, 0.15 M NaCl, 20 mM sodium acetate pH 5
- ◆ 10 U/μl DNA polymerase I (New England BioLabs)
- ◆ *E. coli* tRNA (10 mg/ml) (Roche Diagnostics)
- ◆ Salmon sperm DNA (5 mg/ml, sonicated to 200–500 bp) (Sigma)
- ◆ TE: 10 mM Tris-HCl pH 7.5, 1 mM EDTA
- ◆ Gel loading buffer (5 x bromophenol blue): 10% (w/v) Ficoll, 0.1 M Na₂EDTA, 0.5% (w/v) sodium dodecyl sulfate (SDS), 0.1% (w/v) bromophenol blue
- ◆ Electrophoresis buffer (10 x TBE): 108 g Tris base (89 mM), 55 g boric acid (89 mM), 40 ml 0.5 M EDTA pH 8 (2 mM) per litre
- ◆ *PhiX174/HaeIII*, size marker (BRL Life Technologies)

Method

- 1 Add the following (in order) to a 1.5 ml Eppendorf tube on ice:
 - 1 μg probe DNA
 - 1.2 μl 1 mM biotin-16-dUTP or dig-11-dUTP
 - 5 μl dNTP mix
 - 5 μl 10 x nick translation buffer
 - 5 μl 100 mM dithiothreitol (DTT)
 - sterile distilled water to make up to a final volume of 50 μl
 - tested volume 100 U/ml DNase I ^a

- 1 μl 10 U/ μl DNA polymerase I
- 2 Mix well.
- 3 Incubate at 15 °C for 90 min.
- 4 Stop reaction by placing tubes on ice.
- 5 Check the size of the labelled products by running an aliquot on a 2% agarose gel (in TBE and containing 5 μl of 5 mg/ml ethidium bromide per 100 ml) as follows:
 - 5 μl labelled probe (approx. 100 ng)
 - 4 μl gel loading buffer (5 x bromophenol blue)
 - 11 μl sterile distilled water
- 6 Run at 50 V for 1–1.5 h with *PhiX174/HaeIII* (20 μl = 250 ng) as a size marker.
- 7 View on transilluminator and photograph. The optimal size range for *in situ* hybridization is 50–500 bp. A smear of products from 100–300 bp (corresponding to the six smallest bands of *PhiX174*) is suitable. If the size range is larger than this, add a further 3–5 μl DNase I, place at 15 °C for a further 30–60 min, and run another aliquot on a gel to test the size.
- 8 Purify to remove unincorporated nucleotides by passing the labelled probe through a MicroSpin G50 column (designed for biotinylated probes) according to the manufacturer's instructions.
- 9 Measure the volume of eluate then ethanol precipitate the purified, labelled probe by adding:
 - 50 μg *E. coli* tRNA
 - 50 μg salmon sperm DNA
 - 0.1 vol. 3 M sodium acetate pH 5.6
 - 2–2.25 vol. ice-cold ethanolMix well and place at –70 °C for 1–2 h or –20 °C overnight.
- 10 Centrifuge in a microcentrifuge for 15–25 min at 4 °C. Pour off the supernatant and dry the pellet (either air dry or in a vacuum desiccator). Resuspend the pellet in 20 μl TE pH 8 to give a final concentration of 50 ng/ μl . Allow the DNA to dissolve at room temperature for 1–2 h or at 4 °C overnight with occasional mixing. Purified, labelled probes are stable for several years when stored at –20 °C.

Notes

- a To determine the volume of DNase for each new batch: Add 1 ml DNase dilution buffer to make 200,000 U/ml stock. Dilute this 1/2000 (in ice-cold H₂O) just before

use. Carry out a series of digestions using 1 µg probe DNA, 10 x nick translation buffer, DTT, and 2, 4, 6 µl of 100 U/ml DNase I. Discard the diluted DNase I after use.