

Synthesis of digoxigenin or fluorescein labelled RNA probe

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Equipment and reagents

- ◆ Water-bath, heater block, or incubator at 37 °C
- ◆ Agarose gel electrophoresis apparatus
- ◆ Ultraviolet light transilluminator
- ◆ Microcentrifuge
- ◆ 1% agarose gel containing 0.5 µg/ml ethidium bromide
- ◆ 5 x transcription buffer: 200 mM Tris-HCl pH 7.9, 30 mM MgCl₂, 10 mM spermidine, 50 mM NaCl
- ◆ Sterile distilled water
- ◆ Nucleotide mix: 10 mM GTP, 10 mM ATP, 10 mM CTP, 6.5 mM UTP, 3.5 mM digoxigenin-UTP (or fluorescein-UTP)
- ◆ 1 µg/µl linearized plasmid in TE buffer (10 mM Tris-HCl, 0.1 mM EDTA pH 8)
- ◆ Placental ribonuclease inhibitor at 100 U/µl
- ◆ SP6, T7, or T3 RNA polymerase at 10 U/µl
- ◆ Ribonuclease-free deoxyribonuclease I (DNase I) at 1 U/µl

Method

1 Mix these reagents in the following order at room temperature:

- sterile distilled water 9.5 µl
- 5 x transcription buffer 4 µl
- 0.1 M dithiothreitol 2 µl
- nucleotide mix 2 µl
- linearized plasmid 1 µl
- ribonuclease inhibitor 0.5 µl
- RNA polymerase 1 µl

- 2 Incubate at 37 °C for 2 h.
- 3 Remove a 1 µl aliquot and run on an agarose gel containing 0.5 µg/ml ethidium bromide. This gel must be ribonuclease-free (we use an electrophoresis apparatus designated for this purpose, i.e. not used for plasmid minipreps that contain RNase), but does not need to be denaturing. An RNA band approx. tenfold more intense than the plasmid band should be seen on a UV transilluminator, indicating that ~ 10 µg probe has been synthesized.
- 4 Add 2 µl ribonuclease-free DNase I and incubate at 37 °C for 15 min. This step is optional.
- 5 If the transcript is 1.5 kb or greater in length, reduce the average size to ~ 500 bases. Add an equal volume of 80 mM NaHCO₃, 120 mM Na₂CO₃, mix, and heat at 60 °C for a period of time (mins) = $(L-0.5)/(0.055L)$, where L is the starting length (kb) of the transcript. Check the size of the product on an agarose gel, since over-degraded probes give low signals and high backgrounds.
- 6 (a) Either adjust volume to 50 µl, load on a microspin column (e.g. Pharmacia Biotech Microspin S-400 HR column), and follow the manufacturer's instructions. The flow-through should contain probe at ~ 0.2 µg/µl.
(b) Or purify by ethanol precipitation. Add 130 µl dH₂O, 50 µl 10 M ammonium acetate, 400 µl ethanol, mix, and incubate at -20 °C for 30 min. Do not incubate at a lower temperature as this may precipitate unincorporated nucleotides. Spin in a microcentrifuge for 10 min, wash pellet with 70% ethanol, and air dry the pellet. Re-dissolve in ice-cold TE at ~ 0.2 µg/µl.
- 7 Add an equal volume of hybridization mix (recipe in [Fixation and pre-treatment of embryos for whole mount hybridization](#)) and store at -20 °C. This is stable for years. For hybridization use 1-5 µl of this stock per ml of hybridization mix.