

Hybridization of sections, washing, and detection of probe

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Equipment and reagents

- ◆ Slide racks and containers
- ◆ Incubator at 55–65 °C
- ◆ 50% formamide, 2 x SSC
- ◆ 25% formamide, 2 x SSC
- ◆ 2 x SSC
- ◆ 0.2 x SSC
- ◆ PBT
- ◆ AP-conjugated anti-DIG antibody (can be pre-absorbed)
- ◆ NTMT, BCIP stock solution, NBT stock solution (see [Hybridization, washing, and detection of probe \(method 1\)](#))

Method

- 1 Apply the hybridization mix containing probe ([Synthesis of digoxigenin or fluorescein labelled RNA probe](#)) to the slide adjacent to the sections (~ 5 $\mu\text{l}/\text{cm}^2$ of coverslip is sufficient) and gently lower a clean coverslip so that the mix is spread over the sections without trapping air bubbles.
- 2 Place the slides horizontally in a box containing tissue paper soaked in 50% formamide, 5 x SSC, seal the box, and incubate overnight at 55–65 °C.
- 3 Place the slides in a slide rack and immerse in pre-warmed 25% formamide, 2 x SSC at 55–65 °C until the coverslips fall off.
- 4 Wash with 25% formamide, 2 x SSC for 30 min at 55–65 °C.
- 5 Optional: rinse twice with 2 x SSC and treat with 20 $\mu\text{g}/\text{ml}$ RNase in PBS at 37 °C for 30 min. This step will decrease the signal so should only be carried out if required to reduce background or to prove specificity.
- 6 Wash with 2 x SSC, then twice in 0.2 x SSC (30 min each step) at 55–65 °C.
- 7 Wash with PBT, three times for 10 min, at room temperature.

- 8 Quickly drain each slide and place horizontally in a sandwich box containing moist tissue paper. Take care that the sections do not become dry, and quickly overlay them with 5% sheep serum in PBT. Seal the box, and incubate for 30 min.
- 9 If desired, the antibody can be pre-absorbed as described in [Pre-absorption of antibody](#).
- 10 Remove the 5% serum from the sections, replace with 1/2000 diluted AP-conjugated anti-DIG antibody in 5% sheep serum, PBT and incubate in a moist box at room temperature for 1–3 h, or at 4 °C overnight.
- 11 Wash with PBT twice for 5 min and then three times for 15 min.
- 12 Wash with NTMT, three times for 5 min.
- 13 Incubate in the dark with NTMT containing 4.5 μ l NBT, 3.5 μ l BCIP per ml. Occasionally monitor under dissecting microscope, and when sufficient signal has developed, stop the colour reaction by washing with PBT.
- 14 Fix the signal by immersing the slides in 4% paraformaldehyde in PBS for 30 min, then mount under a coverslip using 70% glycerol. Alternatively, for permanent mounting, fix for several hours or overnight, dehydrate quickly through a graded methanol series followed by HistoClear, then mount under a coverslip using Permount (Sigma) or DPX (BDH) mounting agent.