

Hybridization of mouse embryo sections for mRNA detection

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Equipment and reagents

- ◆ Sealable box for slides
- ◆ Incubator at 55 °C
- ◆ Hybridization mix: 50% deionized formamide, 0.3 M NaCl, 20 mM Tris-HCl pH 7.5, 5 mM EDTA pH 8, 10% dextran sulfate, 1 × Denhardt's solution, 0.5–1 mg/ml tRNA—a stock of 200 ml of hybridization mix should be prepared and stored at –20 °C

Method

- 1 Dilute the probe in the hybridization mix at a final concentration of $3\text{--}5 \times 10^4$ c.p.m./ μl .
- 2 Heat the hybridization mix at 80 °C for 5 min and cool to room temperature.
- 3 Apply hybridization mix to each dried slide^a and with a small piece of Parafilm gently distribute the mix all along the surface of the slides.
- 4 Place a piece of Parafilm over the slides and carefully squeeze out air bubbles.
- 5 Place the slide horizontally in a slide box containing tissue paper soaked in 50% formamide, 5 × SSC, then seal the humidified box.
- 6 Incubate at 55 °C for 16–18 h.

Notes

- a 60–70 μl of hybridization mix is sufficient to cover the entire surface of a slide. Therefore 3–4 ml of hybridization mix is sufficient for 50 slides that are fully covered with sections.