

Preparation of sections for *in situ* hybridization

Qiling Xu

Division of Developmental Biology, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK.

David G. Wilkinson

Division of Developmental Neurobiology, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK.

Equipment and reagents

- ◆ For wax sectioning: microtome, graded methanol series in PBS, HistoClear (National Diagnostics), paraffin wax (melting temperature 56–58 °C, e.g. Fibrowax, BDH), incubator or wax embedding apparatus at 60 °C, bath for floating sections or slide warmer at 50 °C
- ◆ For cryostat sectioning: cryostat, OCT medium (BDH), 0.5% low melting temperature agarose, 5% sucrose in PBS, 30% sucrose in PBS
- ◆ Microscope slides (preferably with a frosted end so can be labelled with pencil)
- ◆ Glass vials
- ◆ Glass embryo dishes
- ◆ 4% paraformaldehyde in PBS (see [Fixation and pre-treatment of embryos for whole mount hybridization](#))
- ◆ TESPA (3-aminopropyltriethoxysilane, Sigma)
- ◆ Acetone

A. Embedding in paraffin wax and sectioning

- 1 Dissect the tissue or embryos and fix them in 4% paraformaldehyde in PBS, overnight at 4 °C.
- 2 Wash with PBS, twice for 10 min.
- 3 Dehydrate by taking through methanol series in PBS (25% methanol, 50% methanol, then 75% methanol), then twice in 100% methanol, for 10 min each. Large embryos or tissues should be washed for longer to ensure complete dehydration. It is convenient to carry these and the subsequent steps out in sterile glass vials.
- 4 Equilibrate with HistoClear, three times for 20 min, then with molten paraffin wax at 60 °C, three times for 20 min, occasionally agitating the vial.
- 5 Transfer the embryos to glass embryo dishes (pre-heated to 60 °C), orientate them with a warmed hypodermic needle under a dissection microscope, and allow the wax to set. Paraffin wax blocks can be stored indefinitely at 4 °C until required for use.

- 6 Trim the block, mount on the microtome (the way this is done will depend on the apparatus), and cut 6–10 μm sections as ribbons.
- 7 Float a suitable length (that will fit onto a slide) of the ribbon on a bath of distilled water at 50 °C until the creases disappear, and collect on TESPA subbed slides (see section C: *Preparation of subbed slides*). If a slide-bath is not available, can lay slides on slide warmer at 50 °C, pipette on a pool of distilled water, and then float the ribbon of sections on this until the creases have disappeared.
- 8 Drain the excess liquid, then dry the sections onto the slides at 37 °C overnight. They can be stored desiccated at 4 °C.

B. Cryostat sectioning

1. (a) For small tissues: fix in 4% paraformaldehyde in PBS overnight at 4 °C. To facilitate orientation of very small tissues, can then embed in 0.5% low melting temperature agarose, 5% sucrose in PBS. Equilibrate in 30% sucrose in PBS overnight at 4 °C.
2. (b) For large tissues: fix, then equilibrate with 30% sucrose (as above), or freeze directly in liquid nitrogen.
3. Use OCT medium to glue the tissue or agarose block onto the cryostat holder (pre-chilled to –20 to –30 °C).
4. Cut sections onto TESPA subbed slides (see section C: *Preparation of subbed slides*).
5. Air dry, and store in airtight container at –70 °C.

C. Preparation of subbed slides

1. Dip the slides in 2% TESPA in acetone for 10 sec.
2. Rinse twice with acetone, and then with distilled water.
3. Dry at 37 °C.