

Sectioning of whole mount hybridized embryos

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.

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. Cryostat sectioning

- 1 Fix the stained embryos overnight in 4% paraformaldehyde in PBS.
- 2 Wash several times in PBS, then equilibrate overnight with 30% sucrose in PBS.
- 3 Mount on a cryostat chuck with OCT compound and freeze on dry ice.
- 4 Cut 10–25 µm sections on a cryostat.
- 5 Mount under a coverslip with 70% glycerol in PBS.

B. Wax sections

1. Fix the stained embryos overnight in 4% paraformaldehyde in PBS. This step is especially important for wax embedding because otherwise the NBT/BCIP reaction product will dissolve in the solvents used.
2. Wash the embryos twice for 10 min with PBS, then replace solution with 75% then 100% methanol (twice) for 10 min each, and with HistoClear three times for

10 min each. Shake gently to ensure efficient equilibration. These washes will need to be longer for large tissues (e.g. > 9.5 d mouse embryos), but should not be excessively prolonged.

3. Replace the solvent with three changes of paraffin wax at 60 °C for 15 min each, with occasional shaking to mix. The length of these incubations may need to be adjusted according to the size of the tissue.
4. Transfer to an embryo dish at 60 °C, place at room temperature, orientate using a warmed needle (if necessary, observing with a dissection microscope), and let the wax set.
5. Cut sections, mount on subbed slides (Section C: *Preparation of subbed slides, in [Protocol for Preparation of slides for in situ hybridization](#)*), and dry at 37 °C overnight.
6. Dewax for 2–5 min with HistoClear.
7. While the slide is still wet, mount the sections under a coverslip using Permount (Sigma) or DPX (BDH) mounting agent.