Embedding, sectioning and counterstaining of *lacZ* embryos

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Equipment and reagents

- Microtome
- Paraffin dispenser
- Paraffin section-mounting water bath (Raymond Lamb)
- PBS
- Toluene[a]
- 4% paraformaldehyde: dissolve 2 g of paraformaldehyde in 50 ml PBS; incubate at 65 °C for a few hours to aid dissolution, inverting every half hour until dissolved. The solution should be freshly made.
- DPX mounting medium (Raymond Lamb)
- Plastic moulds
- Pre-treated slides (Pre-treatment of slides for frozen and wax sections)
- Paraffin wax: pastillated Fibrowax (BDH)
- Saline (0.9% NaCl)
- Eosin B (Sigma), 0.25% in 30% ethanol (dissolve the solid and leave the solution for several days at room temperature with occasional stirring)

Method

1. Place stained, X-gal-positive embryos in 5–10 ml ice-cold paraformaldehyde and leave overnight at 4 °C.
2. Incubate with 5 ml PBS at 4 °C for 30 min, then with 5 ml PBS at 4 °C for 30 min.
3. Dehydrate the embryos by treating them with a series of 50%, 70%, 90% and 100% ethanols, each for 30 min at room temperature.
4. Clear the embryos by incubating in 10 ml of toluene at least twice for 30 min at room temperature.
5. Equilibrate the embryos in a mixture of 1:1 toluene:paraffin wax for 20 min at 60 °C.

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6 Replace the solution with pure molten wax, by incubating the embryos with three fresh changes of wax, each for 20 min at 60 °C (do not exceed these times).

7 Transfer the embryos to fresh wax in a plastic mould, orientate them as required with a warmed needle and allow the wax to set.

8 Cut ribbons of 6 μm sections on the microtome.

9 With a brush or needle lower the sections and float them on a section-mounting water bath at 50 °C.

10 Allow any creases to unfold and disappear for 2–5 min, then lift and collect sections onto pretreated slides (Pre-treatment of slides for frozen and wax sections).

11 Drain excess water onto a tissue and place the slides on a slide drier for 30 min at 37 °C.

12 Allow the slides to dry at room temperature and store them at 4 °C in a box with desiccant.

13 Remove the wax by immersing twice in toluene[a] for 10 min each at room temperature.

14 Immerse the slides for a few seconds each in a graded ethanol series: successively in 100%, 80%, 70%, 50% and 30% ethanols.

15 Wash the slides in PBS or saline for 10 min.

16 Counterstain the sections in eosin or other histochemical stains for appropriate lengths of time determined by desired intensity required for the specific counterstain. For eosin, start with 5–10 min and examine the intensity.

17 Dehydrate the sections by successive immersion through the ethanol series (30–100%); a few seconds in each is sufficient.

18 Rinse the slides in toluene for 5 sec and mount in DPX mounting medium under coverslip.

Notes

a Toluene can be replaced by xylene or Histoclear.

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