Fixation and pre-treatment of embryos for whole mount hybridization

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Equipment and reagents

- 7 ml Bijou tubes and/or 2 ml microcentrifuge tubes (with snap-cap)
- Rocking platform
- Heater block with holder for 2 ml microcentrifuge tubes
- Phosphate-buffered saline (PBS): prepare using Dulbecco 'A' tablets (Oxoid)
- Paraformaldehyde fixative (4% paraformaldehyde in PBS) is prepared by dissolving at 65 °C and then cooling on ice.

Caution: paraformaldehyde fumes are toxic. Can be stored for months in aliquots at –20 °C.

- PBT: PBS, 0.1% Triton X-100
- Methanol
- Glutaraldehyde: 25% stock solution (Sigma)
- Proteinase K: 10 mg/ml stock solution in dH_2O. Stored in aliquots at –20 °C, and diluted just before use
- Hybridization solution for mouse, chick, or *Xenopus* embryos: 50% formamide, 5 x SSC, 2% blocking powder (Boehringer; dissolve directly in this mix), 0.1% Triton X-100, 0.1% CHAPS (Sigma), 1 mg/ml tRNA, 5 mM EDTA, 50 μg/ml heparin
- Hybridization solution for zebrafish embryos: 50% formamide, 5 x SSC pH 6 (pH of stock is adjusted with 1 M citric acid), 0.1% Triton X-100, 50 μg/ml yeast RNA, 50 μg/ml heparin
- 20 x SSC stock solution: 3 M NaCl, 0.3 M sodium citrate pH 7

Method

1. Dissect embryos free of any extra-embryonic membranes. It may be helpful to puncture any closed vesicles within which reagents may be trapped, such as the neural tube.
2. Incubate overnight in paraformaldehyde fixative at 4 °C.
3. Rinse the embryos with ice-cold PBT, twice for 5 min on rocking platform.

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4 Dehydrate the embryos by washing for 10 min at each step on a rocking platform in a graded methanol series diluted in PBT (25% methanol, 50% methanol, 75% methanol) and then twice with 100% methanol. The embryos can now be stored at –20 °C for at least several months, and are stable for at least several days at room temperature.

5 Rehydrate the embryos by washing for 10 min at each step on rocking platform in the graded methanol series in PBT (75% methanol, 50% methanol, 25% methanol) and then twice in PBT.

6 Treat the embryos with 10 μg/ml proteinase K in PBT for 5–25 min at room temperature. The length of treatment depends upon the type of embryo and the size of the embryos (see the following guide), and should be optimized for each batch of proteinase.

   Zebrafish: up to 20 somites (5 min), more than 20 somites (10min).

   Xenopus: up to stage 14 (10 min), stage 15 or older (15 min).

   Chick: stage 3-6 (5 min), stage 7-12 (10 min), stage 13-25 (20 min).

   Mouse: 7.5d (7 min), 8.5d (10 min), 9.5d (15 min), 10.5d (20 min), 11.5d (25 min).

7 Rinse the embryos for 5 min with PBT.

8 Refix the embryos with fresh 0.2% glutaraldehyde, 4% paraformaldehyde in PBT for 20 min. For zebrafish embryos, 4% paraformaldehyde in PBT is used.

9 Rinse the embryos three times for 5 min with PBT on rocking platform.

10 Remove most of the liquid, add 0.5–1 ml hybridization solution, and allow the embryos to sink.

11 Replace with fresh hybridization solution and incubate at 55–67 °C for 2 h to overnight on rocking platform. The volume used will depend upon the size and number of embryos; typically 0.5–1 ml. This can be achieved by incubating in 2 ml microtubes in a heater block. The embryos can then be stored at –20 °C for at least several weeks, but lower signals are obtained after more prolonged storage.

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