Video Article

Plastic Embedding and Sectioning of *Xenopus laevis* Embryos

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Abstract

Protocol

Fixation

1. Transfer whole embryos or dissected explants into MEMFA or 3.7% FA+PBS in a small glass scintillation vial (Fisher cat# 03-339-25B). Incubate vial on the nutator at RT, **only** for 2 hours, and not more than that. Do not leave the embryo in FA for more than 2 hours. Longer incubation in FA will make the embryonic tissue harder and result in a poor penetration of antibodies in the detection process.
2. Aspirate most of FA and add Dent's fixative up to the top of the vial (~ 4.5ml). Gently invert the vial for a few times to wash the embryos in Dent's, exchange with another 4.5ml of fresh Dent's, and incubate it in -20°C **for at least 48 hours**. Overnight is NOT enough. This step makes the embryos more permeable for the antibody. Embryos can be kept in this condition for several weeks.

Immunofluorescence

1. Rehydrate the specimen. Incubate it in a graded series of methanol, as follows:

75% MeOH/H ₂ O	10min
50% MeOH/PBS	10min
25% MeOH/PBS	10min
PBS	10min
PBS	10min

2. Make 1.5% agarose/PBS plates with 60mm plastic dishes. Pour PBS on top of the agarose after it has become solid.
3. **Hemi-sectioning of the embryos:** Put the embryos in a 1.5% agarose/PBS plate. Grab an embryo with a pair of forceps and cut it in half through the center of the embryo using a thin razor blade. Choose the ones that you have successfully cut in half with a straight cutting plane.

4. Transfer the bisected embryos into a glass scintillation vial with PBT. Exchange with 4.5ml of 20% goat serum+PBT. Incubate at RT for 2 hours on a nutator (Blocking).
5. Prepare an appropriate dilution of the primary antibody in PBT. 0.5ml of this per samples is needed. For instance, I used 1/200 dilution of rabbit anti-C-cadherin pAb to study the subcellular localization of C-cadherin in 5mm plastic sections.
6. Aspirate the blocking solution and add 0.5ml of the diluted primary antibody. Put the vials in stand-up position on a Styrofoam tube holder. Incubate overnight at 4°C on a nutator.
7. Remove the diluted primary antibody (this diluted primary antibody can be saved and reused in the next 1-2 weeks). Wash with 4.5ml of PBT for 4 times (40-60 minutes each) at RT.
8. Exchange with 0.5ml of 1/100 dilution of Biotin-conjugated secondary antibody in PBT. Incubate overnight at 4°C on a nutator. Cover the vials with aluminum foil to protect biotin from light.
9. From this point, **care should be taken to protect the sample from light, i.e., keep the vials under some cover or aluminum foil.**
10. Remove the Biotin-secondary antibody (this can be also reused in the next 1-2 weeks). Wash with or 4.5ml of PBT for 4 times (40-60 minutes each) at RT.
11. Exchange with 0.5ml of 1/200 dilution of fluorescently-labeled (FITC, Texas-red, etc.) Streptavidin in PBT. Incubate overnight at 4°C on a nutator.

12. Remove the streptavidin (this can be reused, too). Wash with 4.5ml of PBT for 3 times (15 - 30 minutes each) at RT.
13. Fix the embryos in 4.5ml of 3.7% FA+PBS for 30 minutes at RT.
14. Wash with 4.5ml of PBS twice.
15. Dehydrate the specimen. Incubate it in a graded series of ethanol, as follows:

30% EtOH/H ₂ O	10 min
50% EtOH/H ₂ O	10 min
75% EtOH/H ₂ O	10 min
100% EtOH	10 min

16. The specimen can be stored in 100% EtOH at 4°C with a cover to protect it from light.

Infiltration

1. Make Technovit 7100 infiltration mix. I usually take 15ml of the base liquid in a 50ml conical tube, add 150mg of hardener I and mix it by rocking on a nutator for 15 minutes. This infiltration mix can be kept at 4°C for up to one month.
2. Infiltrate the specimen with the infiltration mix by exchanging with a graded series of Technovit infiltration mix/EtOH combo, as follows:
 1. 50% Technovit/EtOH - 1 hour
 2. 100% Technovit - 1 hour
 3. 100% Technovit - overnight
3. The specimen can be stored in the 100% infiltration mix for up to one month at 4°C. Put a cover on the samples to protect them from light.

Embedding

1. Make Technovit 7100 embedding mix: Take 0.75ml of the infiltration mix in a 1.5ml tube and add 50ml of hardener II. Mix it by inverting the tube several times.
2. Using a plastic transfer pipette, take one specimen infiltrated overnight into 0.5ml thin wall PCR tube. Remove most of the supernatant.
3. Add 0.25ml of the embedding mix made in step #1 of this section, "Embedding", to the specimen. Orient the bisected embryo to make the dissection plane facing to the bottom of the tube, by gently nudging with a dull-tip dissection needle (wooden-handle dissection needle).
4. Close the cap and incubate the tube in a dark place for approximately four hours to allow the plastic polymerize.
5. Make Technovit 3040 mix. Take 1 gram of the powder to a weighing dish, and add 0.5ml of the liquid. Immediately, mix powder and liquid using a bluetip. Pour on top of the hardened specimen. This yellow Technovit 3040 will prevent the entire plastic block from coming out from the tube.

Sectioning

1. Wipe Histoknife H with xylene (EtOH is often not good enough) and set it on your microtome. Angle the blade at approximately 45°. Wipe the stage area in front of the blade with xylene, too, because the cleanness of this area is critical to the quality of sectioned slices.
2. Cut the side of the tube and peel the tip off using a cardboard knife.

3. Set the specimen into the chuck holder of the microtome.
4. Attach a "press-to-seal" silicon insulator on a slide glass to make a mounting chamber for section slices. Firmly press the insulator to the slide on clean benchtop and look from the other side to make sure that the insulator is completely attached without any air-bubbles. Pour clean and warm dH₂O (~ 40°C) to the pool on the slide using a Pasteur pipette, up to the level where it can be seen that the water surface becomes flat, not concave or convex in shape.
5. Start making section slices. For immunofluorescence studies, I make 5 mm section slices. For *in situ* hybridization samples, the thickness of the slices can be adjusted in the range of ~ 3 - 8 mm, depending on the purpose and the intensities of the signals.
6. Carry the section slices from the stage in front of the blade to the mounting chamber with water, using a small paintbrush. Hold the paintbrush with a slice on top of the water pool and drop the slice down to the water by knocking it with a dull-tip dissection needle. The slice will expand into a circular shape as soon as it hits the surface of the water.
7. When most of the water surface area of the mounting chamber is filled with section slices, suck up almost half of the water using a Pasteur pipette. Incubate the slide on a slide warmer overnight to make the sample completely dry.
8. Counter-stain with 1ml of DAPI (0.1mg/ml in TE) for five minutes. Aspirate DAPI and dry the sample.
9. Remove the silicone rubber insulator from the slide. Now pictures can be taken of the sample. Store the sample at 4°C, protected from light.

References