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Protocol

Histological Preparation of Embryonic and Adult Zebrafish Eyes

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INTRODUCTION

This protocol describes the histological preparation of embryonic and adult zebrafish eyes. The methods described here can be easily adapted for use on other zebrafish tissues.

RELATED INFORMATION

These methods have been employed in studies on morphological development of the zebrafish retina (Branchek and Bremiller 1984, Schmitt and Dowling 1999).

MATERIALS

Reagents

0.01 M phosphate buffered saline (PBS) (pH 7.4) (Sigma)

DPX mountant (Electron Microscopy Sciences, 13512)

Embedding resin for zebrafish eyes

Ethanol (50%, 70%, 80%, 90%, and 100%)

Fixation solution for zebrafish eyes

Histology stain

1% osmium tetroxide

Propylene oxide

Tricaine

Zebrafish embryos or adults of interest

Equipment

Coverslips, No. 1 thickness

Embedding mold

Fume hood

Heating block

Knives, glass

Microscope, light

Microtome

Needles (25-30 gauge)

Oven, preset to 60°C

Razor

Scalpel

Slides, gelatin coated or positively charged (e.g., ESCO Superfrost Plus, Erie Scientific)

Spatula, wooden

Stereomicroscope

Transfer pipettes, disposable

Tubes (1.5 mL), microcentrifuge

Tweezers

Vials (15 mL), conical

METHOD

Fixation

1. Collect and fix surgically removed adult eyes or entire embryonic or larval fish in fixation solution:
   i. To dissect and fix adult eyes, euthanize fish in tricaine. Use a scalpel and fine tweezers to remove the eyes, and puncture the corneas with a 25- to 30-gauge needle. Place the eyes in a 15-mL conical vial with 3-4 mL of fixation solution. Fix at 4°C for 2-3 d.
   If desired, place one eye in fixation solution for histology, and process the contralateral eye for immunohistochemistry in 4% PFA as described in Immunohistochemistry on Cryosections from Embryonic and Adult Zebrafish Eyes *(Uribe and Gross 2007).*

   ii. To fix larval fish, place the entire fish in a 1.5-mL microcentrifuge tube containing fixation solution. Fix overnight at 4°C or at room temperature for 4-6 h.
2. Remove the fixation solution and wash the fixed samples in 0.01 M PBS:
   i. Wash adult samples three times for 15 min per wash.

   ii. Wash larval samples three times for 5 min per wash.
3. Fix the samples in 1% osmium tetroxide at 4°C:

i. Fix adult eyes for 2-3 h.

ii. Fix larval samples for 60-90 min.

Avoid light during fixation.

4. Remove the osmium tetroxide and wash samples three times in 0.01 M PBS for 5 min per wash.

Osmium tetroxide is toxic and should be disposed of with hazardous waste.

5. Remove the PBS and proceed with the following dehydration series:

i. 50% ethanol for 5 min

ii. 70% ethanol for 10 min

iii. 80% ethanol for 15 min

iv. 90% ethanol for 20 min

v. 100% ethanol for 15 min

vi. 100% ethanol for 15 min

vii. 100% propylene oxide for 10 min

viii. 100% propylene oxide for 10 min

Care should be taken with propylene oxide as it is highly volatile; propylene oxide incubations and the subsequent steps should be performed in a hood until the samples are in embedding resin and transferred to an incubator (Step 9).

Thaw embedding resin during the second propylene oxide incubation. The resin is highly toxic until it has polymerized. If possible, devote specific pieces of equipment to resin-related work (i.e., 60°C incubator, heating/stirring block, stereomicroscope).

6. Carefully mix equal amounts of embedding resin and 100% propylene oxide in a 15-mL conical tube. Begin resin infiltration with 50% propylene oxide and 50% resin for 1 h or longer.

Samples may be left for 5-6 h.

7. Remove the 50/50 embedding resin/propylene oxide mix and add 100% embedding resin to the samples. Leave the samples overnight at room temperature in the hood with caps open, to allow for propylene oxide evaporation.

8. The next day, remove the samples and place them in fresh embedding resin in an embedding mold. Under a stereomicroscope, align the samples using a small needle:

i. Place one to five embryos at the end of a single well, dorsal side up, and align as closely as possible to each other (Fig. 1A).

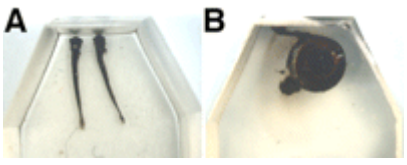

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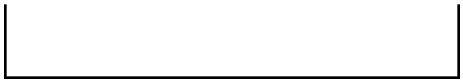
Figure 1. (*A*) Alignment of 9-dpf (days post-fertilization) embryos in a histology mold. (*B*) Alignment of an adult eye in a histology mold.



ii. Place a single adult eye, lens side up, in a well (Fig. 1B).
It is helpful to place a small piece of paper with an identifying name or code into each well before adding the resin and samples.

9. Bake in a 60°C oven for 2-3 d.
Baking time depends on the batch of resin. Once the resin has polymerized, the blocks can be stored indefinitely at room temperature until ready for sectioning.

Sectioning

10. Trim the block with a razor by cutting excessive plastic (polymerized resin) away from the samples. Align the specimen in the microtome block holder to provide the correct sectioning angle.

11. Using a glass knife and microtome, cut thick sections (5-10 µm) until the desired location or depth is reached. To determine location, check the sections often using a light microscope.

12. Cut semithin histological sections, 1-1.5-µm thick. Using a wooden spatula (the shaved end of an applicator tip), collect the sections and place them on a droplet of H_2O on a gelatin-coated slide.

13. Place the slide on a heating block to evaporate the H_2O droplet and allow the specimen to adhere to the slide.

14. Stain the sections with histology stain for 30 sec to 3 min.
Stain time varies with the batch of stain and batch of resin.

15. Rinse the sections with H_2O and check the samples on a microscope for the desired level of staining.

i. If the sections have not absorbed enough stain, repeat Step 14.

ii. If samples are appropriately stained, air-dry for 10-15 min.

16. Using a disposable transfer pipette, add three to four small drops of DPX mountant.
The use of disposable pipettes eliminates the risk of contaminating more expensive pipettes with the mountant.

17. Place a No. 1 coverslip onto each sample and let the DPX mountant harden overnight.

18. Image on a light microscope (Fig. 2 , Fig. 3).

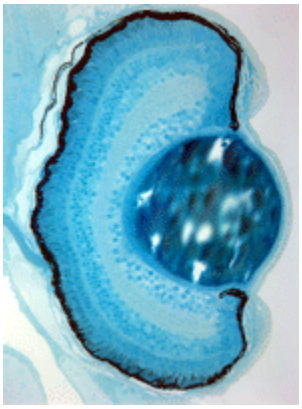


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Figure 2. Transverse histological section of 5-dpf zebrafish eye stained with histology stain (methylene blue and azure B). The dorsal side is up.

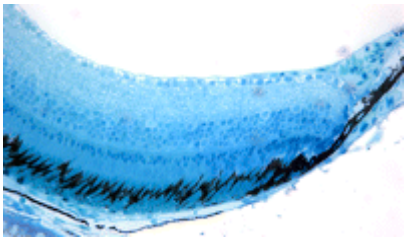

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Figure 3. Transverse histological section of an 18-mo-old zebrafish eye stained with histology stain (methylene blue and azure B). The ventral retina is shown.

ACKNOWLEDGMENTS

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REFERENCES

Branchek, T. and Bremiller, R. 1984. The development of photoreceptors in the zebrafish, *Brachydanio rerio*. I. Structure. *J. Comp. Neurol.* **224:** 107–115.[Medline]

Schmitt, E.A. and Dowling, J.E. 1999. Early retinal development in the zebrafish, *Danio rerio*: Light and electron microscopic analyses. *J. Comp. Neurol.* **404:** 515–536.[Medline]

Uribe, R.A. and Gross, J.M. 2007. Immunohistochemistry on cryosections from embryonic and adult

zebrafish eyes. *CSH Protocols* doi: 10.1101/pdb.prot4779.[Abstract/Free Full Text]

Caution

DPX

DPX is composed of Distyrene, a plasticizer, and xylene and is commercially available. Follow the manufacturer's guidelines for handling DPX.

Caution

General warning

This material contains hazardous components. Please see recipe for full details.

Caution

Osmium tetroxide (Osmic acid)

Osmium tetroxide (osmic acid) OsO_4 is highly toxic if inhaled, ingested, or absorbed through the skin. Vapors can react with corneal tissues and cause blindness. There is a possible risk of irreversible effects. Wear appropriate gloves and safety goggles and always use in a chemical fume hood. Do not breathe the vapors.

Caution

Propylene oxide

Propylene oxide is highly flammable, toxic, and may be carcinogenic. High concentrations are extremely destructive to the mucous membranes and upper respiratory tract. It may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses and use only in a chemical fume hood. Keep away from heat, sparks, and open flame.

Caution

Tricaine

See tricaine methanesulfonate.

Recipe

Embedding resin for zebrafish eyes

Reagent	Amount to add
Epon 812	25 mL
Araldite 502	20 mL
2-Dodecenylsuccinic acid anhydride (DDSA)	60 mL
DMP-30	1.1 mL

Solution must be mixed thoroughly. Store at –20°C.

Recipe

Fixation solution for zebrafish embryos

1% (w/v) paraformaldehyde (PFA)

3% (w/v) sucrose

2.5% (v/v) glutaraldehyde

0.2 M phosphate buffer (pH 7.4)

This solution can be stored at 4°C for 2 wk.

Recipe

Histology stain

1% (w/v) azure B

1% (w/v) methylene blue