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Title: Dissection of Zebrafish Craniofacial Tissues upon Staining with Alcian Blue

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **Yes, all done**

SCOPE shots: 3.1.1, 3.1.2, 3.1.3, 3.2.1, 3.2.2, 3.3.1, 3.3.2, 3.4.1, 3.4.2, 3.5.1

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. Filming location: Will the filming need to take place in multiple locations? **No**

Current Protocol Length
Number of Steps: 15
Number of Shots: 32 (10 Scope)

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Nandini Bhargava:** We are exploring the mechanisms by which the zebrafish palate grows over time through fixed samples as well as time-lapse imaging of embryos with high spatiotemporal resolution using light-sheet microscopy.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

What advantage does your protocol offer compared to other techniques?

1.2. **Nandini Bhargava:** Our protocol is simple and easy to follow. It lays down detailed steps to carefully dissect out and separate craniofacial cartilages in a 5-day old zebrafish larva.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.3.1*

What new scientific questions have your results paved the way for?

1.3. **Nandini Bhargava:** This protocol has enabled the careful characterization of palate shape at tissue and cellular scales, ultimately allowing us to assess the effect of common teratogens on palate morphogenesis.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.2.1*

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Institutional Animal Ethics Committee at the Tata Institute of Fundamental Research, Mumbai

Protocol

2. Alcian Blue Staining of Zebrafish Larvae

Demonstrator: Nandini Bhargava

~~2.1. To begin, obtain 20 to 25 anesthetized zebrafish larvae that are 5 days old in a 2-milliliter microcentrifuge tube **[1-TXT].**~~

~~2.1.1. WIDE: Talent transferring 5-day-old anesthetized zebrafish larvae into a 2 milliliter microcentrifuge tube.~~ **NOTE**: Not filmed, VO moved to the next shot

2.2. To begin, obtain 20 to 25 anesthetized zebrafish larvae and remove the anesthetic once the larvae have stopped moving **[1-TXT]**. Add 1.5 milliliters of 4 percent paraformaldehyde to the tube **[2]** and place the sample on a rocker set to 60 revolutions per minute at room temperature for 2 hours **[3]**.

2.2.1. WIDE: Talent using a pipette to remove the anesthetic from the microcentrifuge tube. **TXT: Anesthesia: 0.05% Tricaine**

2.2.2. Talent adding 1.5 milliliters of 4 percent paraformaldehyde into the tube.

2.2.3. Talent placing the tube onto a rocker set to 60 revolutions per minute.

2.3. Using a pipette, remove as much of the fixative as possible from the tube **[1]**. Then, add 1.5 milliliters of 50 percent ethanol to the sample **[2]** and place it on a rocker at room temperature set to 60 revolutions per minute for 10 minutes **[3]**.

2.3.1. Talent using a pipette to remove the fixative from the tube.

2.3.2. Talent adding 1.5 milliliters of 50 percent ethanol to the tube.

2.3.3. Talent placing the tube on the rocker and setting the timer.

2.4. Now, using a pipette, aspirate the ethanol from the tube **[1]** and add 1.5 milliliters of Alcian blue stain solution to the sample **[2]**.

2.4.1. Talent using a pipette to remove the ethanol from the tube.

2.4.2. Talent adding 1.5 milliliters of Alcian blue stain solution to the sample.

2.5. Incubate the larvae in the stain solution for 18 to 20 hours at room temperature while rocking at 60 revolutions per minute **[1]**.

2.5.1. Talent placing the tube on the rocker and setting the timer.

2.6. Next, remove the stain solution using a pipette **[1]** and wash the larvae with 1.5 milliliters of distilled water for 1 to 2 minutes on a rocker at room temperature **[2]**.

2.6.1. Talent using a pipette to discard the stain solution from the tube.

2.6.2. Talent adding distilled water to the sample.

2.7. Then, add 1 milliliter of bleach solution to the sample **[1]** and incubate at room temperature for 20 minutes without covering the tubes **[2]**.

2.7.1. Talent adding 1 milliliter of bleach solution into the sample tube.

2.7.2. Wide shot showing the uncovered tube on the bench while incubating.

2.8. Using a pipette, remove the bleach solution from the sample **[1]** and treat the sample with tissue clearing solutions 1 and 2 **[2 and 3]**.

2.8.1. Talent using a pipette to remove the bleach solution.

2.8.2. Talent adding 1.5 milliliters of solution I to the tube and placing it on the rocker.

2.8.3. TEXT ON 2.8.2's background:

Solution I: 1.5 mL, rocker at 60 rpm, 40 min, RT

Remove solution I

Solution II: 1.5 mL, rocker at 60 rpm, 2 h, RT

2.9. After removing solution 2, add 1.5 to 2 milliliters of storage solution to the sample **[1]**. Due to the glycerol content, observe as the stained samples gradually sink to the bottom within 10 minutes **[2]**.

2.9.1. Talent adding 1.5 to 2 milliliters of storage solution into the sample.

2.9.2. Close-up of zebrafish larvae slowly sunken to the bottom of the container.

3. Dissection of the Craniofacial Skeleton

3.1. Place a stained larva on its lateral side in the agarose-coated petri dish **[1]**. Using forceps, gently scrape off the yolk from the body of the larva **[2]**. After yolk removal, transfer the larva to a fresh Petri dish to avoid interference during dissection **[3]**.

3.1.1. SCOPE: 3.1.1---Tranfer-of-the-sample-to-the-plate.avi– 0:00–0:06 and 0:11–0:17.

3.1.2. SCOPE: 3.1.2---yolk-removal 0:06–0:08 and 0:21–0:35.

3.1.3. SCOPE: 3.1.3---tranfer-of-sample-to-a-new-plate .

3.2. Hold the larva near the posterior of the head using one pair of forceps **[1]**. With another pair, carefully remove the eyes without damaging the surrounding craniofacial structures **[2]**.

3.2.1. SCOPE: 3.2.1---Holding-the-larva – 00:20–0:35.

3.2.2. SCOPE: 3.2.2---eye-removal 0:42–0:54 and 1:18–1:32.

3.3. While holding the larva still with one pair of forceps, make an incision at the center of the tissue dorsal to the neurocranium **[1]**, then pinch and pull off the brain and associated tissues **[2]**.

3.3.1. SCOPE: 3.3.1---Creation-of-inscision-in-the-middle-of-the-head– 0:22–0:29.

3.3.2. SCOPE: 3.3.2---removal-of-dorsal-tissues 0:26–0:30 and 01:02–1:10

3.4. Next, separate the head of the larva from the rest of the body **[1]**. The resulting head region should include the neurocranium and viscerocranium still attached at the anterior and posterior ends **[2]**.

3.4.1. SCOPE: 3.4.1---Separation-of-craniofacial-skeleton-from-the-rest-of-the-body. 00:10-00:20

3.4.2. LAB MEDIA: 3.4.2---Image-of-craniofacial-skeleton.

3.5. Carefully sever the two connecting points between the neurocranium and viscerocranium with forceps to fully separate them **[1]**.

3.5.1. SCOPE: 3.5.1---neuro-and-viscrocranium-separartion-. 0:20–0:22 and 1:37–1:44

3.6. Finally, place one or two drops of 100 percent glycerol on a clean glass slide **[1]** and transfer the dissected neurocranium or viscerocranium to the slide **[2]**. Using forceps, gently place a coverslip on top of the tissues, ensuring no air bubbles are trapped **[3]**, and seal the coverslip with nail polish before imaging **[4]**.

3.6.1. Talent dispensing glycerol onto a clean glass slide.

3.6.2. SCOPE: 3.6.2---transfer-of-neurocranium-to-glycerol 0:10–0:22

3.6.3. Talent placing the coverslip gently with forceps.

3.6.4. Talent sealing the edges with nail polish.

Results

4. Results

4.1. The dissected neurocranium displayed multiple clearly labelled structures, including the ethmoid plate, trabecula, and parachordal cartilage, enabling detailed structural analysis **[1]**.

4.1.1. LAB MEDIA: Figure 2D. *Video editor: Highlight the region labelled Ethmoid, Trabecula and Parachordal cartilage*

4.2. The viscerocranium included Meckel's cartilage **[1]** and additional elements such as the palatoquadrate and basibranchial cartilages **[2]**.

4.2.1. LAB MEDIA: Figure 2E. *Video editor: Highlight the region labelled Meckel's cartilage*

4.2.2. LAB MEDIA: Figure 2E. *Video editor: Highlight the region labelled Palatoquadrate and Basibranchial*

4.3. Alcian blue staining at 5 days post-fertilization enabled clear visualization of the ethmoid plate **[1]**.

4.3.1. LAB MEDIA: Figure 3B.

4.4. The width and length of the ethmoid plate were consistently measurable using image analysis **[1]**, and the length was significantly greater than the width in quantified data from 15 samples **[2]**.

4.4.1. LAB MEDIA: Figure 3B. *Video editor: Zoom in on the Red dashed lines for length and width*

4.4.2. LAB MEDIA: Figure 3C. *Video editor: Highlight the box plot showing a visibly taller box for "Length"*

4.5. The ethmoid plate area was extracted from image data **[2]** and confirmed through quantification, with clear boundary markings **[2]**.

4.5.1. LAB MEDIA: Figure 3D.

4.5.2. LAB MEDIA: Figure 3E.

4.6. Distinct cellular arrangements were observed in the ethmoid plate, with cuboidal cells

in the medial region **[1]** and columnar cells in the lateral parts **[2]**.

4.6.1. LAB MEDIA: Figure 3F. *Video editor: Zoom in on the central area of the ethmoid plate labeled "Mep"*

4.6.2. LAB MEDIA: Figure 3F. *Video editor: Highlight the 2 outer lateral regions of the ethmoid plate labeled "Lep"*

4.7. DAPI staining allowed visualization of individual cell nuclei within the ethmoid plate **[1]**.

4.7.1. LAB MEDIA: Figure 3G.

- **Paraformaldehyde**

Pronunciation link: https://www.merriam-webster.com/dictionary/paraformaldehyde
IPA: /ˌpærəfɔrˈmældəˌhaɪd/
Phonetic Spelling: par-uh-for-MAL-duh-hyd

- **Neurocranium**

Pronunciation link: https://www.howtopronounce.com/neurocranium
IPA: /ˌnʊroʊˈkreɪniəm/
Phonetic Spelling: noo-roh-KRAY-nee-um

- **Viscerocranium**

Pronunciation link: https://www.howtopronounce.com/viscerocranium
IPA: /ˌvɪsərəʊˈkreɪniəm/
Phonetic Spelling: vis-er-oh-KRAY-nee-um

- **Ethmoid**

Pronunciation link: https://www.merriam-webster.com/dictionary/ethmoid
IPA: /ˈɛθˌmɔɪd/
Phonetic Spelling: ETH-moyd

- **Trabecula**

Pronunciation link: https://www.merriam-webster.com/dictionary/trabecula
IPA: /trəˈbɛkjələ/
Phonetic Spelling: truh-BEK-yuh-luh

- **Parachordal**

Pronunciation link: https://www.howtopronounce.com/parachordal
IPA: /ˌpærəˈkɔrdəl/
Phonetic Spelling: par-uh-KOR-dəl

- **Palatoquadrate**

Pronunciation link: No confirmed link found
IPA: /ˌpælətəˈkwɑːdreɪt/
Phonetic Spelling: pal-uh-toh-KWAH-drayt

- **Basibranchial**

Pronunciation link: No confirmed link found
IPA: /ˌbæsɪˈbræŋkiəl/
Phonetic Spelling: bas-ih-BRANG-kee-uhl

- **Meckel's**

Pronunciation link: https://www.merriam-webster.com/dictionary/Meckel's (for "Meckel")
IPA: /ˈmɛkəlz/
Phonetic Spelling: MEK-əlz

- **Glycerol**

Pronunciation link: https://www.merriam-webster.com/dictionary/glycerol
IPA: /ˈglɪsərɒl/
Phonetic Spelling: GLIS-er-ol