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Title: Cell dissociation from the tongue epithelium and mesenchyme/connective tissue of embryonic-day 12.5 and 8-week-old mice

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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**

If **Yes**, can you record movies/images using your own microscope camera?

YES **Authors: Please use your microscope camera to film all SCOPE shots and upload the videos to your project page: https://www.jove.com/account/file-uploader?src=18959763.**

If your protocol involves microscopy but you are not able to record movies/images with your microscope camera, JoVE will need to use our scope kit (through a camera port or one of the oculars). Please list the make and model of your microscope.

Olympus SZX16

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

3. Interview statements: Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

☒ Interviewees wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. Filming location: Will the filming need to take place in multiple locations? **No, multiple rooms on the same floor**

Current Protocol Length

Number of Steps: 16
Number of Shots: 38

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Mohamed Ishan:** High yield and quality of cells from complex tissues are essential to commonly used experimental analyses such as single cell RNA sequencing and primary stem cell cultures.

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

1.2. **Mohamed Ishan:** This protocol generates healthy cells at a high yield and quality from different regions and tissue compartments of the mammalian tongue, including tongue epithelium and connective tissue.

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

Ethics Title Card

1.3. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Georgia.

Protocol

2. Separation of the tongue epithelium from the mesenchyme/underlying connective tissue

2.1. To separate the epithelium from the mesenchyme of an E12.5 mouse tongue, transfer the euthanized pregnant female mouse to the surgical area **[1]** and wet the mouse abdomen with 70% ethanol to prevent fur from getting into the operating site **[2]**.

2.1.1. WIDE: Establishing shot of talent placing mouse in surgical area. NOTE: This and next shot together

2.1.2. Talent wetting the abdomen of the mouse with ethanol.

2.2. Open the abdomen using dissecting scissors to expose the uterine horns carrying the embryos **[1]**, dissect the uterine horns **[2]**, and transfer them to 15 milliliters of fresh Tyrode's solution in a 100-millimeter culture dish **[3]**.

2.2.1. Talent opening the abdomen. NOTE: This and next shot together

2.2.2. Talent dissecting the uterine horns.

2.2.3. Talent placing the uterine horns in Tyrode's solution.

2.3. Under a dissecting microscope, use mini-scissors and fine forceps to dissect the embryos from the uterine horns **[1]**. Open the mouth cavity with the fine forceps and dissect the tongue from the mandible using mini-scissors **[2]**.

2.3.1. SCOPE: 2.3.1 SCOPE please use 0s to 14s.avi. 0:00 -0:14. NOTE: Macro shot available

2.3.2. SCOPE: 2.3.2 SCOPE please use 25s to 56s.avi. 0:25 -0:56. *Video Editor: Please speed up* NOTE: Macro shot available

2.4. Wash the tongues with 15 milliliters of fresh sterile Tyrode's solution in a 100-millimeter culture dish **[1]**, then transfer the tissues to 2 milliliters of enzyme mixture of dispase and collagenase in a 35-millimeter culture dish **[2-TXT]** and incubate for 20 minutes at 37 degrees Celsius **[3]**.

2.4.1. Talent washing the tongues.

2.4.2. Talent transferring the tissues to the dispase and collagenase mixture. **TEXT: 2.5 mg/mL dispase and 1.0 mg/mL collagenase**

2.4.3. Talent putting the dish in the incubator.

2.5. Transfer tongues to 15 milliliters of fresh sterile Tyrode's solution **[1]** and gently separate the mesenchyme and the epithelium from the ventral side using fine forceps **[2]**. Wash the separated epithelia and mesenchyme twice in 15 milliliters of fresh sterile Tyrode's solution **[3]**. *Videographer: This step is important!*

2.5.1. Talent transferring the tongues to fresh Tyrode's solution.

2.5.2. SCOPE: 2.5.2 SCOPE Please use 0s to 11s.avi. 0:00 – 0:11.

2.5.3. Talent washing the epithelia and mesenchyme in Tyrode's solution.

2.6. To separate the tongue epithelium from the underlying connective tissue of an adult mouse, transfer the euthanized mouse to the surgical area **[1]** and wet the mouse head using 70% ethanol to prevent fur from getting into the oral cavity **[2]**.

2.6.1. Talent placing the mouse in the surgical area. NOTE: This and next shot together

2.6.2. Talent wetting the mouse's head with ethanol.

2.7. Use dissecting scissors to cut the corners of the mouth along the cheek to open the oral cavity **[1]**. Dissect the tongue with a mandible **[2]**, wash it, and place it in a plastic dish with a layer of plastic wrap **[3-added]**.

2.7.1. Talent cutting the corners of the mouth. NOTE: This and next shot together

2.7.2. Talent dissecting the tongue with a mandible.

2.7.3. Add shot: Talent washing the tongue with a mandible in Tyrode's solution and placing the tongue in a plastic dish with a layer of plastic wrap.

2.8. Using surgical forceps to hold the tongue, inject the enzyme mixture of dispase and collagenase in the sub-epithelial space of the tongue through the cutting edge of the posterior tongue **[1]**.

2.8.1. SCOPE: Talent injecting dispase and collagenase in the sub-epithelial space of the tongue NOTE: Didn't find scope shot, macro shot available. Shot with 2.9.1 and 2.9.2.

2.9. Inject 1 milliliter of enzyme mixture evenly to the whole tongue for tissue collection **[1]**, then inject 0.5 milliliters of enzyme mixture locally to the anterior tongue for tissue collection from the tongue tip **[2]** or to the posterior tongue for circumvallate papilla tissue collection **[3]**. *Videographer: This step is important!*

2.9.1. SCOPE: 2.9.1 Scope whole tongue injection.avi. *Video Editor: Please speed up*

2.9.2. SCOPE: 2.9.2 Scope anterior tongue injection.avi. *Video Editor: Please speed up*

2.9.3. SCOPE: 2.9.2 Scope posterior tongue injection.avi. *Video Editor: Please speed up*

2.10. Wrap the tongue with plastic wrap **[1]** and incubate it for 30 minutes at 37 degrees Celsius **[2]**. Use mini-scissors to dissect the circumvallate papilla **[3a]** and tongue tip **[3b]**, then use a spatula and fine forceps to transfer the tissue to 15 milliliters of fresh sterile Tyrode's solution **[4]**.

2.10.1. Talent wrapping the tongue in plastic.

2.10.2. Talent placing the tongue in the incubator.

2.10.3. A. SCOPE: 2.10.3 Scope circumvallate dissection please use 2s to 20s.avi. 0:15 – 0:20. NOTE: Macro shot available

B. SCOPE: 2.10.3 Scope tongue tip dissection please use 0s to 21s.avi. 0:15 – 0:21.

2.10.4. Talent transferring the tissue to Tyrode's solution.

2.11. Separate the epithelium from the underlying connective tissue in the enzyme-digested sub-epithelial space using mini-scissors **[1]** and trim the tissues to a proper size according to the requirement of downstream experiments **[2]**.

2.11.1. SCOPE: 2.11.1 Scope separating the epithelium for tongue tip please use 3s to 7s.avi NOTE: Macro shot available

2.11.2. SCOPE: 2.11.2 Scope trim the epithelium and mesenchyme of tongue tip.avi. Talent trimming the tissue. NOTE: Macro shot available

2.12. Wash the separated epithelium and underlying connective tissue twice in 15 milliliters of fresh sterile Tyrode's solution in a 100-millimeter culture dish **[1]**.

2.12.1. Talent washing the epithelium and connective tissue in Tyrode's solution.

3. Cell dissociation

3.1. Use a spatula and fine forceps to transfer the tissues to 3 milliliters of 0.25% trypsin-EDTA in a new 35-millimeter culture dish **[1]**. Incubate the dish for 30 minutes at 37 degrees Celsius **[2]**, gently agitating tissues every 5 minutes with 1-milliliter pipette tips **[3]**.

3.1.1. Talent transferring tissue to a culture dish with trypsin-EDTA.

3.1.2. Talent putting the dish in the incubator.

3.1.3. Talent agitating the dish.

3.2. Add 500 microliters of 5% FBS in DMEM-F12 to stop the reaction **[1]** and transfer the medium to a 5-milliliter low binding centrifuge tube **[2]**.

3.2.1. Talent adding FBS in DMEM-F12 to the dish. NOTE: This and next shot together

3.2.2. Talent transferring the medium to a centrifuge tube.

3.3. Centrifuge the cell suspension at 200 x *g* for 8 minutes at room temperature **[1]** and remove the supernatant **[2]**. Gently re-suspend cells in 3 milliliters of DMEM-F12 containing 10% FBS and 1% BSA **[3]** and filter the cells with a 70-micrometer cell strainer, followed by a 35-micrometer cell strainer **[4]**. *Videographer: This step is difficult and important!*

3.3.1. Talent putting the tube in the centrifuge and closing the lid.

3.3.2. Talent removing the supernatant. NOTE: Use the first run before the pellet dislodges

3.3.3. Talent resuspending the cells.

3.3.4. Talent filtering the cells through strainers.

3.4. Centrifuge the cell suspension at 200 x *g* for 8 minutes at room temperature **[1]**, then remove most of the medium, leaving 50 to 300 microliters as the final volume to re-suspend the cells **[2]**. *Videographer: This step is difficult and important!*

3.4.1. Talent putting the cells in the centrifuge.

3.4.2. Talent removing medium from the tube.

Results

4. Results: Effect of cell dissociation on total cell number and viability

4.1. In the embryonic mouse tongue, a gap in the sub-epithelial space is visible after proper enzyme digestion. In the adult mouse tongue, a successful enzyme injection is indicated by the swelling in the injected areas, which suggests that the enzyme can be held by the tongue **[1]**.

4.1.1. LAB MEDIA: Figure 1 B2.

4.2. After pooling E12.5 tongues, epithelial sheets, and thin layers of mesenchyme, manual cell counting **[1]** demonstrated that the protocol yielded 63,917 cells in total with a viability of 95.2% from the epithelial sheets **[2]**, and 294,333 cells in total with a viability of 96.3% from the mesenchyme **[3]**.

4.2.1. LAB MEDIA: Figure 2.

4.2.2. LAB MEDIA: Figure 1 A3 and Figure 2 A1.

4.2.3. LAB MEDIA: Figure 1 A4 and Figure 2 A2.

4.3. When 10 adult tongues at 8 weeks of age were used, the protocol yielded 187,333 cells with a viability of 95.4% from epithelial sheets of tongue tip **[1]**, 544,000 cells with a viability of 96.3% from epithelial sheets of circumvallate papillae **[2]**, and 150,500 cells with a viability of 93% from connective tissues **[3]**.

4.3.1. LAB MEDIA: Figure 1 B3.

4.3.2. LAB MEDIA: Figure 1 B5 and Figure 2 B1.

4.3.3. LAB MEDIA: Figure 1 B6 and Figure 2 B2.

Conclusion

5. Conclusion Interview Statements

5.1. **Mohamed Ishan:** Following this protocol, the dissociated cells can be used for single cell RNA-seq and 3-D taste organoid culture to define unrecognized taste progenitor cells under lingual epithelium.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.