FINAL SCRIPT: APPROVED FOR FILMING

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Title: RNA Isolation from Mouse Ocular Lens Epithelium and Fiber Cell Bulk Masses

Authors and Affiliations:
Peter N. Huynh, Michael P. Vu, Catherine Cheng

School of Optometry and Vision Science Program, Indiana University

Corresponding Authors:
Catherine Cheng (ckcheng@iu.edu)
Email Addresses for All Authors:
Peter N. Huynh (pehuynh@iu.edu)
Michael P. Vu (mpvu@iu.edu)
Catherine Cheng (ckcheng@iu.edu)

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**

   SCOPE: 2.2.1, 2.2.2, 2.2.3, and 2.3.1
   Videographer: Please film all SCOPE shots with a SCOPE kit

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: 16
Number of Shots: 35

Introduction

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

1.1. **Catherine Cheng:** Our research focuses on the lens, a specialized, transparent tissue in the anterior chamber of the eye. The lens is composed of two cell types, epithelial cells and fiber cells, that have different functions and diverse transcriptomes.

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

What are the current experimental challenges?

1.2. **Michael Vu:** There are difficulties in extracting a sufficient concentration of RNA from epithelial cells in the lens monolayer, which has impeded the study of the transcriptomes for epithelial cell vs. fiber cell.

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

What advantage does your protocol offer compared to other techniques?

1.3. **Peter Huynh:** This protocol enables the clean separation of epithelial and fiber cell compartments, allowing RNA concentration and transcriptomic analysis of epithelial cells from a single pair of mouse lenses.

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

How will your findings advance research in your field?

1.4. **Peter Huynh:** The ability to cleanly separate the major cell types of the lens allows deeper investigation of lens maintenance and dysregulation. This enables the characterization of cell-type-specific disruptions responsible for age-related lens pathologies.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.1*

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

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at Indiana University.

Protocol

2. Mouse Lens Dissection and RNA Isolation for Downstream Molecular Analysis

Demonstrator: Peter Huynh

2.1. To begin, microdissect the lens from freshly enucleated eyes of a euthanized mouse **[1].** Gently roll the lens on a clean, delicate task wipe using curved forceps to remove any remaining adherent extralenticular tissue **[2]**.

2.1.1. WIDE: Talent holding a dish with extracted lens.

2.1.2. Talent placing a dissected lens onto a task wipe and gently rolling it with curved forceps

2.2. Using fine straight forceps, shallowly pierce the lens capsule near the equator **[1]**, then gently peel the lens capsule away from the fiber bulk mass, leaving the lens epithelial cells attached to the capsule **[2]**. Remove any large fiber pieces from the capsule that may have come off during decapsulation **[3]**.

2.2.1. SCOPE: Talent piercing the lens capsule near the equator with fine straight forceps.

2.2.2. SCOPE:Talent carefully peeling the capsule from the fiber mass.
AUTHOR'S NOTE: 2.2.1-2.2.2 were shot together. Please use take 2

2.2.3. SCOPE: Talent removing excess fiber material from the capsule using forceps.

2.3. Now gently grip the capsule with fine straight forceps and swirl it in PBS to dislodge any remaining fibers **[1-TXT]**.

2.3.1. SCOPE: Shot of the capsule being swirled in a plate of phosphate-buffered saline.
TXT: Loosely attached fiber cells will dissociate from the capsule and epithelial cells

2.4. Pipette 400 microliters of cold TRIzol *(tri-Sol)* reagent into a clean 1.5-milliliter microcentrifuge tube per sample **[1]**. Deposit 2 lens capsules or 2 fiber bulk masses into each respective tubes **[2].** Tightly cap the tubes **[3].** ~~then move them to a chemical fume hood for subsequent steps **[4].**~~

2.4.1. Talent pipetting 400 µL of TRIzol reagent into separate microcentrifuge tubes.

2.4.2. Talent transferring 2 lens capsules or fiber masses into separate microcentrifuge tube containing TRIzol.

2.4.3. Talent sealing the tubes .
AUTHOR'S NOTE: 2.4.2-2.4.3 were shot together.

2.4.4. Shot of the tubes being placed in a fume hood.
NOTE: Shot deleted by authors

2.5. Gently homogenize the lens fiber bulk masses in the tubes using a clean plastic pestle then incubate **[1-TXT]**.

2.5.1. Talent using a plastic pestle to homogenize fiber masses in TRIzol inside the fume hood. **TXT: Incubation: 30 min, RT**

2.6. In the fume hood, add 200 microliters of chloroform per 400 microliters of TRIzol reagent, into each tube **[1]**. Close the tubes tightly and shake vigorously by hand for 15 seconds, keeping the thumb on the bottom of the tube and the forefinger on the cap **[2]**.

2.6.1. Talent pipetting chloroform into tubes containing TRIzol.

2.6.2. Talent shaking the tubes as described, demonstrating the correct grip.

2.7. After incubating the samples at room temperature for 10 to 15 minutes to allow phase separation **[1]**, centrifuge the samples at 14,000 *g* for 15 minutes at 4 degrees Celsius **[2]**.

2.7.1. Shot of the separated phases in the tube.

2.7.2. Talent placing tubes into a centrifuge and initiating the spin.

2.8. In the fume hood, carefully transfer the clear, top aqueous phase into a clean 1.5-milliliter microcentrifuge tube and note the volume **[1]**. Then add 200-proof ethanol to the aqueous phase in a 1 to 1 volumetric ratio **[2]**.

2.8.1. Talent extracting the top layer with a pipette and transferring it to a labeled clean tube.

2.8.2. Talent adding ethanol to the aqueous solution in an equal volume.

2.9. Now, gently mix the solution by inverting the tube several times **[1]**. Transfer the mixed solution to an RNA spin column using a pipettor **[2-TXT]**.

2.9.1. Talent mixing the tube by inversion.

2.9.2. Talent transferring the solution into a spin column. **TXT: Be careful not to contact the filter membrane**

2.10. Centrifuge the spin columns at 16,000 *g* for 30 seconds at 4 degrees Celsius **[1]**. Then discard the flowthrough **[2],** and pipette 400 microliters of RNA Prep Buffer to the spin column **[3]**.

2.10.1. Talent loading spin columns into a centrifuge and starting the run.

2.10.2. Talent discarding flowthrough.

2.10.3. Talent pipetting RNA Prep Buffer into the spin column.

2.11. After centrifuging the columns again, discard the flowthrough **[1].** Add 700 microliters of RNA Wash Buffer to the spin column **[2-TXT]**.

2.11.1. Shot of the flowthrough being discarded.

2.11.2. Talent pipetting 700 µL RNA wash buffer into the tubes. **TXT: Perform wash and centrifugation again, with 400 µL RNA wash buffer**

2.12. Once the final wash is complete and the flowthrough has been discarded, centrifuge once more at 16,000 *g* for 30 seconds at 4 degrees Celsius to ensure the spin column is dry **[1].**

2.12.1. Shot of the tubes being placed in a centrifuge.
AUTHOR'S NOTE: 2.12.1 is the same as 2.10.1. Use same file clip

2.13. Move the dried spin column to a new, clean, and labeled 1.5-milliliter microcentrifuge tube **[1]**. Then pipette 15 microliters of RNase-free water directly onto the membrane filter and incubate for 2 minutes at room temperature **[2-TXT]**.

2.13.1. Talent transferring the spin column to a fresh labeled tube.

2.13.2. Talent pipetting RNase-free water onto the membrane and setting the tube aside for incubation. **TXT: Do not contact the filter membrane**

2.14. Centrifuge the spin column one final time at 16,000 *g* for 30 seconds at 4 degrees Celsius to elute the purified RNA **[1]**. Now remove and discard the spin columns **[2]**. The purified RNA will be present in the liquid collected in the microcentrifuge tubes **[3]**.

2.14.1. Talent loading columns into the centrifuge.
AUTHOR'S NOTE: Use take 2

2.14.2. Talent discarding the used spin column.

2.14.3. Shot of final liquid RNA sample in the bottom of the tube.

2.15. ~~Tightly close the microcentrifuge tubes, ensuring a tight seal **[1].** Then~~ Incubate the RNA samples at 55 to 65 degrees Celsius for 10 minutes to promote resolubilization **[2].** Immediately place the samples on ice following the heating step **[3]**.

2.15.1. Talent sealing the tubes.
NOTE: Shot deleted by authors

2.15.2. Talent placing them in a heat block for incubation.
AUTHOR'S NOTE: Use take 2

2.15.3. Talent moving tubes from heat block to ice bucket.

2.16. Store the samples at minus 80 degrees Celsius **[1-TXT]**.

2.16.1. Talent placing tubes into an ultra-low freezer. **TXT: Alternatively, reverse transcribe into cDNA for long term storage**

Results

3. Results

3.1. Differential gene expressions were observed between the isolated epithelium and fiber cell bulk mass **[1].** The gene *Gja1 (G-J-A-One)*, also known as connexin 43, was expressed primarily in epithelial cells **[2]**. In contrast, the gene *Gja3*, which encodes connexin 46, had higher expression in fiber cells **[3]**. *Gja8* or connexin 50 was expressed in both epithelial and fiber cells **[4].**

3.1.1. LAB MEDIA: Figure 3. *Video Editor: please highlight part of the graph titled "Epithelium Enriched" and then the "Fiber enriched" part of the graph*

3.1.2. LAB MEDIA: Figure 3. *Video editor: Highlight the white bar labeled "Gja1 (Cx43)"*

3.1.3. LAB MEDIA: Figure 3. *Video editor: Highlight the grey bar labeled "Gja3 (Cx46)"*

3.1.4. LAB MEDIA: Figure 3. *Video editor: Highlight the white and grey bars labeled "Gja 8 (Cx50)"*

3.2. Expression of *Cdh1 (C-D-H-one)*, encoding E-cadherin, was high in epithelial cells **[1]** while expression of *Cdh2,* encoding N-cadherin, was detected in both epithelial cells and fiber cells **[2]**.

3.2.1. LAB MEDIA: Figure 3. *Video editor: Highlight the white bars labeled "Cdh1 (E-Cad)"*

3.2.2. LAB MEDIA: Figure 3. *Video editor: Highlight the grey and white bars labeled "Cdh2 (N-Cad)"*

3.3. *Pax6 (Packs-Six)* expression was strongly enriched in epithelial cells and was nearly absent in fiber cells **[1]**. In contrast, the *Crygs (CRY-G-S*) gene encoding the gammaS *(Gamma-S)*-crystallin protein, exhibited highly elevated expression in fiber cells compared to epithelial cells **[2]**.

3.3.1. LAB MEDIA: Figure 3. *Video editor: Highlight the white bar labeled "Pax6.*

3.3.2. LAB MEDIA: Figure 3. *Video editor: Highlight the grey bar labeled "Crygs"*

Pronunciation Guide:
Epithelium
Pronunciation link:
https://www.merriam-webster.com/dictionary/epithelium

IPA: /ˌɛpɪˈθiːliəm/
Phonetic Spelling: eh-puh-thee-lee-um

Transcriptome / Transcriptomes
Pronunciation link:
https://www.merriam-webster.com/dictionary/transcriptome

IPA: /ˈtrænskrɪpˌtoʊm/
Phonetic Spelling: tran-skrip-tohm

Monolayer
Pronunciation link:
https://www.merriam-webster.com/dictionary/monolayer

IPA: /ˈmɑnəˌleɪər/
Phonetic Spelling: mon-oh-lay-er

Transcriptomic
Pronunciation link:
No confirmed link found
IPA: /ˌtrænskrɪpˈtɑmɪk/
Phonetic Spelling: tran-skrip-tom-ik

Pathologies
Pronunciation link:
https://www.merriam-webster.com/dictionary/pathology

IPA: /pəˈθɑlədʒiz/
Phonetic Spelling: puh-thaa-luh-jeez

Enucleated
Pronunciation link:
https://www.merriam-webster.com/dictionary/enucleate

IPA: /iˈnuːkliˌeɪtɪd/
Phonetic Spelling: ee-noo-klee-ay-tid

Extralenticular

Pronunciation link:
No confirmed link found
IPA: /ˌɛkstrəˌlɛntɪˈkjulər/
Phonetic Spelling: ek-struh-len-tik-yoo-ler

Decapsulation
Pronunciation link:
https://www.merriam-webster.com/dictionary/decapsulation

IPA: /diˌkæpsjəˈleɪʃən/
Phonetic Spelling: dee-cap-shuh-lay-shun

Phosphate-buffered saline (PBS)
Pronunciation link:
https://www.merriam-webster.com/dictionary/phosphate

IPA: /ˈfɑsˌfeɪt ˈbʌfərd ˈseɪˌlin/
Phonetic Spelling: fahs-fayt buh-ferd say-leen

TRIzol
Pronunciation link:
https://www.howtopronounce.com/trizol

IPA: /ˈtraɪzɒl/
Phonetic Spelling: try-zol

Chloroform
Pronunciation link:
https://www.merriam-webster.com/dictionary/chloroform

IPA: /ˈklɔrəˌfɔrm/
Phonetic Spelling: klohr-uh-form

Ethanol
Pronunciation link:
https://www.merriam-webster.com/dictionary/ethanol

IPA: /ˈɛθəˌnɔl/
Phonetic Spelling: eh-thuh-nol

Centrifuge
Pronunciation link:
https://www.merriam-webster.com/dictionary/centrifuge

IPA: /ˈsɛntrəˌfjuːdʒ/
Phonetic Spelling: sen-truh-fyooj

RNase
Pronunciation link:
https://www.howtopronounce.com/rnase

IPA: /ˈɑrˌneɪz/
Phonetic Spelling: ar-nayz

Resolubilization
Pronunciation link:
No confirmed link found
IPA: /riˌsɑljəblɪˈzeɪʃən/
Phonetic Spelling: ree-saw-lyoo-buh-li-zay-shun

Connexin
Pronunciation link:
https://www.merriam-webster.com/dictionary/connexin

IPA: /kəˈnɛksɪn/
Phonetic Spelling: kuh-nek-sin

Cadherin
Pronunciation link:
https://www.merriam-webster.com/dictionary/cadherin

IPA: /kædˈhɪrɪn/
Phonetic Spelling: kad-hear-in

Pax6
Pronunciation link:
No confirmed link found
IPA: /ˈpæks sɪks/
Phonetic Spelling: packs-six

Crystallin
Pronunciation link:
https://www.merriam-webster.com/dictionary/crystallin

IPA: /ˈkrɪstəlɪn/
Phonetic Spelling: kris-tuh-lin