FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 68753
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Title: Development of Mouse-Derived Organoid Lines from Fallopian Tube Epithelial Cells for High Grade Serous Ovarian Carcinoma Modeling

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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**

   NOTE: Scope shots were captured by the videographer

   SCOPE: 2.2.1-2.2.2, 2.4.2

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: 11
Number of Shots: 26

Introduction

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

Videographer's Note: Author preferred to make interview statements with a more conversational approach instead of reading directly from the written statements. I asked him to do additional takes of sections that I thought missed what was in the original statements.

1.1. **Oscar Pundel:** We are generating novel models of high grade serous ovarian carcinomas so that we may use them to better investigate the origins of the disease and treatment.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.9*

What are the most recent developments in your field of research?

1.2. **Oscar Pundel:** Like in most cancer research, immune therapies are hot, but poorly effective in ovarian cancer. We believe this is due to current models being unable to look at immune interactions.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.1.2*

What research gap are you addressing with your protocol?

1.3. **Oscar Pundel:** There is a lack of proper visual tools to help researchers do mouse work, particularly isolating samples and also injections into mice.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.8*

Videographer's Note: 1.3 clip C076_C955_0821BB_001 might potentially be used as a JOVE testimonial statement

What advantage does your protocol offer compared to other techniques?

1.4. **Oscar Pundel:** We can now investigate fully interactions between the host microenvironment and the tumor.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.1.1*

What research questions will your laboratory focus on in the future?

1.5. **Oscar Pundel:** We will use this system to investigate how the adaptive immune system shapes tumor genomic evolution, and if this phenomenon generates therapeutic vulnerabilities.

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

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

Testimonial Questions (OPTIONAL):

Videographer:

- ***Please ensure that all testimonial shots are captured in a wide-angle format, while also maintaining sufficient headspace, given that the final videos will be rendered in a 1:1 aspect ratio.***
- ***Also, kindly note that testimonial statements will be presented live by the authors, offering their spontaneous perspectives.***

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.6. **Dr. Oscar Pundel, Designation**: (authors will present their testimonial statements live)

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE? (This could include increased collaborations, citations, funding opportunities, streamlined lab procedures, reduced training time, cost savings in the lab, or improved lab productivity.)

1.7. **Dr. Oscar Pundel, Designation**: (authors will present their testimonial statements live)

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at Alexandria Center for Life Sciences and the Perlmutter Cancer Center at NYU Langone Health

Protocol

2. Intrabursal Injection and Organoid-Based Tumor Modeling in the Mouse Ovary

Demonstrator: Oscar Pundel

2.1. To begin, obtain euthanized female C57BL/6J *(C-fifty-seven-B-L-Bar-six-J)* mice **[1].** Dissect and remove the ovary and fallopian tube **[2].** Transfer them into a sterile Petri dish containing DMEM supplemented with penicillin-streptomycin at 4 degrees Celsius **[3]**.

2.1.1. WIDE: Talent placing euthanized female C57BL/6J mice on the worktable.

2.1.2. Shot of the ovary and the fallopian tube being excised.

2.1.3. Talent transferring ovary and fallopian tube into a Petri dish filled with chilled DMEM medium.

2.2. Use a dissecting microscope, fine forceps, and scissors to remove residual fat from the ovary **[1]**. Isolate the fallopian tube, including the infundibulum and part of the distal ampulla, from the ovary and uterus **[2]**.

2.2.1. SCOPE: The remaining fat is being removed from the ovary using forceps and scissors.
Videographer's Note: 2.2.1 please use take 2 clip C076_C936_0821MV_001

2.2.2. SCOPE: The fallopian tube is being isolated from ovary and uterus.
Videographer's Note: 2.2.2 please use take 2 clip C076_C937_08215I_001

2.3. Now, transfer the cleaned tissue to a tube containing the complete digestion mixture **[1]**. With a pair of fine scissors, mince the fallopian tube into pieces smaller than 0.5 millimeters **[2]**. Then incubate the tube at 37 degrees Celsius for 40 to 50 minutes before performing organoid culturing **[3]**.

2.3.1. Talent transferring dissected tissue into a digestion solution tube.

2.3.2. Shot of the fallopian tube being minced into very small pieces using fine scissors.

2.3.3. Talent placing the digestion tube into the incubator.

~~2.4. Once the organoids have grown to approximately 500 micrometers and are visually discernible **[1],** identify 4 to 5 large, well-separated organoids using a light microscope **[2].**~~

~~2.4.1. Shot of the organoid culture~~

~~2.4.2. SCOPE: Microscope view of organoids and focus on selecting separated organoids.~~

~~2.5. Inside a sterile hood, gently insert a 20-microliter pipette tip into the basement membrane matrix **[1].** Aspirate a single organoid, drawing up approximately 12.5 microliters **[2]**. Transfer the organoid into a 1.5-milliliter collection tube containing 500 microliters of mouse organoid medium **[3-TXT].**~~

~~2.5.1. Shot of 20 µL pipette tip being inserted into the BMM.~~

~~2.5.2. Talent aspirating a single organoid with a 20-microliter pipette in the sterile hood.~~

2.5.3. ~~Talent dispensing organoid into a 1.5-milliliter tube containing culture medium. **TXT: Incubate at 37 °C**~~

Videographer's Note: Steps 2.4 and 2.5 were not filmed

2.6. To develop the mouse tumor model, first expel air bubbles from the dead volume of a U-100 *(U-One-Hundred)* insulin syringe fitted a 28-gauge needle using cold PBS **[1]**. Slowly load the syringe with 40 microliters of cells from the 1.5-milliliter tube **[2-TXT]**. Keep the loaded syringes on ice until the mice are anesthetized **[3]**.

2.6.1. Talent flushing a U-100 syringe with cold phosphate-buffered saline.

2.6.2. Talent carefully loading the syringe with 40 microliters of cell suspension. **TXT: Prepare 5 injections**

2.6.3. Talent placing loaded syringes on ice.

2.7. Once ready for injection, place the syringes beside a heating lamp to allow the basement membrane matrix to warm and increase viscosity, aiding injection and engraftment **[1-TXT]**.

2.7.1. Talent positioning syringes next to a heating lamp. **TXT: Ensure the syringes are not placed directly under the lamp to prevent solidification**
Videographer's Note: Shot renamed to 2.8.1

2.8. Next, place a mouse in a pre-warmed cage under a heating lamp to prepare for surgery **[1]**. Make an incision less than 1 centimeter, along the flank above the thigh of a anesthetized mouse **[2].** Cut through the epidermis and hypodermis to reach the bursa region **[3]**.
Videographer's Note: Please move 2.8 before 2.7

2.8.1. Talent placing a mouse into a cage warmed with a heating lamp.
Videographer's Note: Shot renamed to 2.7.1

2.8.2. Talent making a small incision on the thigh of a anesthetized mouse.
Videographer's Note: Shot renamed to 2.7.2

2.8.3. Shot of the epidermis and hypodermis being cut and bursa exposed.
Videographer's Note: Shot renamed to 2.7.3

2.9. Gently pull out the ovary by locating the attached fat pad **[1]**. Then using a prepared

syringe, inject cells through the fat pad and ovary into the oviduct sac **[2]**.

2.9.1. Talent gently pulling out the ovary using forceps.

2.9.2. Talent injecting cells into the oviduct sac using a syringe.

2.10. Carefully return the ovary into the bursa **[1]**. Close the incision with surgical staples or sutures **[2]**.

2.10.1. Talent placing ovary back into bursa cavity.

2.10.2. Talent sealing the incision with either staples or thread.

2.11. Place the mouse back in the warmed cage and maintain body temperature with a heating lamp until semi-conscious, usually for more than 15 minutes **[1]**. The following day, inspect the mouse to confirm the absence of sepsis **[2]**.

2.11.1. Talent returning mouse to a heated recovery cage.

2.11.2. Talent examining mouse for signs of infection.

Results

3. Results

3.1. *Trp53 (T-R-P-Fifty-Three)* knockout fallopian tube epithelium organoids exhibited significantly larger and more numerous structures compared to wild-type organoids after 5 days of culture **[1]**, while 2-dimensional monolayers showed a denser, more uniform appearance regardless of genotype **[2]**.

3.1.1. LAB MEDIA: Figure 3 *Video editor: Highlight the lower-left panel showing large, round organoids labeled "Trp53$^{-/-}$" under the "Organoids" column.*

3.1.2. LAB MEDIA: Figure 3. *Video editor: Highlight the right two panels under "2D"*

3.2. Modified organoids produced single, visibly isolated clonal spheres that were detectable both by eye and under the microscope **[1]**.

3.2.1. LAB MEDIA: Figure 4B. *Video Editor: please sequentially show the left image and then the right. Highlight the area pointed at by the white arrow in both images*

Pronunciation Guide:

1. Organoid

- **Pronunciation link:** https://www.merriam-webster.com/medical/organoid
- **IPA:** /ˈôr-gə-ˌnȯid/
- **Phonetic spelling:** OR-guh-noid Merriam-Webster+1Merriam-Webster+4Merriam-Webster+4Merriam-Webster+4

2. Epithelial

- **Pronunciation link:** (not found—common word; presumed standard)
- **IPA:** /ˌɛp-ə-ˈθi-li-əl/
- **Phonetic spelling:** ep-uh-THEE-lee-uhl

(Note: Merriam-Webster didn't provide a separate page with pronunciation audio for this entry.)

3. Carcinoma

- **Pronunciation link:** https://www.merriam-webster.com/dictionary/carcinoma
- **IPA:** /ˌkär-sə-ˈnō-mə/
- **Phonetic spelling:** kar-suh-NOH-muh Merriam-WebsterMerriam-Webster+2Merriam-Webster+2

4. Fallopian

- **Pronunciation link:** https://www.merriam-webster.com/medical/fallopian
- **IPA:** /fə-ˈlō-pē-ən/
- **Phonetic spelling:** fuh-LOH-pee-uhn Merriam-Webster+13Merriam-Webster+13Merriam-Webster+13

5. Fallopian tube (when spoken together)

- **Pronunciation link:** https://www.merriam-webster.com/dictionary/fallopian%20tube
- **IPA: fallopian** /fə-ˈlō-pē-ən/ + **tube** /tuːb/
- **Phonetic spelling:** fuh-LOH-pee-uhn toob Merriam-Webster+3Merriam-Webster+3Merriam-Webster+3

6. Oviduct

- **Pronunciation links:**
  - Cambridge: US /ˈoʊ-vɪ-dʌkt/ Merriam-Webster+15Cambridge Dictionary+15Encyclopedia Britannica+15
  - Britannica audio confirms: "oviduct" American pronunciation Merriam-Webster+11Encyclopedia Britannica+11Myefe+11
- **IPA (American):** /ˈoʊ-vɪ-dʌkt/
- **Phonetic spelling:** OH-vih-dukt

7. Syngeneic

- **Pronunciation link:** HowToPronounce.com: phonetic guide "sĭn'jə-nē'ĭk" How To Pronounce+2How To Pronounce+2
- **IPA (approx.):** /ˌsɪn-dʒə-ˈni-ɪk/
- **Phonetic spelling:** sin-juh-NEE-ik

(Audio pronunciation is also available via YouTube tutorials like this one.) Merriam-Webster+2How To Pronounce+2

8. Mesosalpinx

- **Pronunciation link:** https://www.merriam-webster.com/medical/mesosalpinx
- **IPA:** /ˌmez-ō-ˈsal-(ˌ)piŋ(k)s/ (with variants /ˌmēz-/ or /ˌmēs-/)
- **Phonetic spelling:** mez-oh-SAL-pinks (or may-SAL-pinks) Merriam-Webster+7Merriam-Webster+7Merriam-Webster+7Merriam-Webster+1

9. Parietal peritoneum

- **Pronunciation link:** https://www.merriam-webster.com/medical/parietal%20peritoneum
- **IPA:** parietal: /pə-ˈrī-ə-təl/ + peritoneum: /ˌper-ə-ˈtē-nē-əm/
- **Phonetic spelling:** puh-RIE-uh-tuhl per-uh-TEE-nee-um Merriam-WebsterHow To Pronounce

10. Microliter *(commonly used in protocols)*

- **Pronunciation link:** (not found—standard medical English)
- **IPA:** /ˈmaɪ-krə-ˌli-tər/
- **Phonetic spelling:** MY-cruh-lee-ter