Submission ID #: 68676
Scriptwriter Name: Poornima G
Project Page Link: https://review.jove.com/account/file-uploader?src=20946228

Title: A Minimally Invasive Method for Generating a Syngeneic Orthotopic Mouse Model of Lung Cancer

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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? **No**

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? **Yes**

   If **Yes**, how far apart are the locations? A few feet

Current Protocol Length
Number of Steps: 20
Number of Shots: 47

Introduction

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

1.1. **Sunny Huang:** Our current research focuses on understanding lung cancer disease and how to develop new treatment modalities that are compatible with standard of care therapy including chemotherapy, radiation therapy, and immunotherapy.
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 research gap are you addressing with your protocol?

1.2. **Danielle Foster:** We could not find an orthotopic lung cancer model protocol that established criteria to specifically study early-stage lung cancer. We worked to provide clear and replicable guidelines to address that gap in this protocol.
1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.5.1*

What advantage does your protocol offer compared to other techniques?

1.3. **Danielle Foster:** This protocol is minimally invasive and is simple to perform. Additionally, with the criteria we have provided, this protocol allows for screening of precise tumor implantation and enables early tumor detection through bioluminescent imaging.
1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll:5.1.1*

How will your findings advance research in your field?

1.4. **Jennifer Petsche:** This protocol enables researchers to study early-stage lung cancer in the host tissue, allowing for preclinical advancement of novel cancer therapeutics and investigation into the tumor microenvironment.
1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.2.1*

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

1.5. **Jennifer Petsche:** We will use this technique to evaluate the mechanisms and efficacy of exciting new lung cancer treatments preclinically, and hope that it can lead to future patient care.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.1.1*

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

Ethics Title Card

This research has been approved by the University of Iowa Institutional Animal Care and Use Committee

Protocol

2. Presurgical Animal Preparation

Demonstrator: Danielle Foster

2.1. To begin, place the anesthetized mouse in a nose cone in the prone position on a warming device **[1-TXT]** and apply ophthalmic ointment to both eyes of the mouse **[2]**.

2.1.1. WIDE: Talent positioning the mouse in a prone position in a nose cone on a warming pad. **TXT: Anesthesia: 2% Isoflurane**

2.1.2. Talent gently applying ointment to each eye of the anesthetized mouse using a sterile applicator.

2.2. Once anesthesia is confirmed, use electrical clippers to remove fur from a 2 by 3-centimeter rectangular area on the left dorsal side of the mouse **[1]**. Shave from the spine medially, the table laterally, the last rib inferiorly, and the superior edge of the scapula towards the head until no fur is visible **[2]**.

2.2.1. Talent trimming a rectangular patch of fur with electrical clippers on the mouse's left dorsal area.

2.2.2. Close-up of the full area after complete shaving, with no fur visible along defined anatomical boundaries.

2.3. Place the mouse in an empty cage on a warming device **[1]**. Observe the breathing pattern until the mouse regains alertness **[2]** and then return it to its home cage until surgery **[3]**.

2.3.1. Talent placing the mouse gently into an empty cage on a warming pad.

2.3.2. Shot of the mouse becoming alert.

2.3.3. Talent transferring it into its home cage once it is awake.

3. Preparation of Cell Suspension for Injection

Demonstrator: Jennifer Petsche

3.1. On the day of cell harvesting, add fresh media containing 50 milligrams per milliliter of gentamycin to the culture plate 3 to 4 hours before collection **[1]**.

3.1.1. Talent pipetting fresh media with gentamycin into a culture flask and gently swirling to mix.

3.2. Aspirate the media from the culture plate **[1]** and wash the attached cells with 1X PBS **[2]**. Then, incubate the cells with trypsin-EDTA solution for 1 to 2 minutes at 37 degrees Celsius **[3]**.

3.2.1. Talent aspirating media from a cell culture plate.

3.2.2. Talent pipetting in PBS to wash cells.
Added shot: 3.2.2A

3.2.3. Talent placing the flask in a 37 degrees Celsius incubator.

3.3. Add fresh media to neutralize the trypsin **[1]** , disperse the cells by gently tapping or pipetting the plate **[2]**, then collect the cell suspension into a 50-milliliter conical tube **[3]**.
NOTE: The VO has been edited to match the flipped shots

3.3.1. Talent dispersing cells in the plate by pipetting up and down.

3.3.2. Talent adding media to the flask.
Videographer's Note: Please move 3.3.2 before 3.2.1

3.3.3. Talent transferring the suspension to a conical tube.

3.4. Now, centrifuge the conical tube at 1,200 *g* for 3 minutes at room temperature **[1]**. After confirming the presence of a compact pellet at the bottom **[2]**, aspirate the supernatant **[3]**, and wash the pellet with PBS **[4-TXT]**.

3.4.1. Talent placing the conical tube in a centrifuge and closing the lid.

3.4.2. Close-up of a compact white pellet at the bottom of the conical tube.

3.4.3. Talent aspirating the supernatant without disturbing the pellet.

3.4.4. Talent pipetting PBS into the tube. **TXT: Repeat this wash and spin step 3x**

3.5. Resuspend the washed cell pellet in PBS to achieve a concentration of approximately 1 to 2 million cells per milliliter for counting **[1]**. Filter the suspension through a 40-micrometer cell strainer before proceeding **[2]**.

3.5.1. Talent swirling the tube gently to mix.

3.5.2. Talent pouring the cell suspension on a 40 micrometer cell strainer connected to a clean tube.

3.6. Next, combine 10 microliters of the cell suspension with 10 microliters of Trypan Blue **[1]**. Load the stained sample into a hemocytometer and count the cells **[2]**. Calculate the total number of cells and the cell viability **[3]**.

3.6.1. Talent mixing cell suspension with Trypan Blue in a microcentrifuge tube.

3.6.2. Talent loading the hemocytometer.

3.6.3. Talent writing on a notepad.

3.7. Centrifuge the cells again at 1,200 *g* for 3 minutes at room temperature **[1]** and aspirate the PBS, leaving the pellet **[2]**.

3.7.1. Talent placing the tube in the centrifuge and closing the lid.

3.7.2. Talent aspirating the liquid carefully, leaving only the pellet.

3.8. Resuspend the cell pellet in 0.5 milligrams per milliliter of Matrigel in PBS **[1]**. Adjust to the desired cell number in a total injection volume of 50 microliters **[2]**. Keep the cell suspension on ice to prevent Matrigel solidification **[3]**.

3.8.1. Talent pipetting Matrigel solution into the cell pellet and mixing.

3.8.2. Talent looking at the tube closely.

3.8.3. Talent placing the final tube on ice and covering it with a lid.

4. Orthotopic Mouse Lung Injection

Demonstrator: Danielle Foster

4.1. Clean the surgical site on the anesthetized animal three times using sterile gauze, alternating between betadine and 70 percent ethanol **[1-TXT]**. Place a sterilized fenestrated surgical drape with a 3 by 4 centimeter opening over the mouse **[2]** and lay out autoclaved surgical instruments on top of the drape **[3]**.

4.1.1. Talent wiping the surgical site with sterile gauze soaked in ethanol or betadine. **TXT: Anesthesia: 2% Isoflurane; Wipe in a circular motion, starting at the site of the planned incision and going outwards**

4.1.2. Talent placing a fenestrated surgical drape over the mouse with the opening aligned on the back.
Videographer's Note: Try running 4.1.2 backwards for smoother drape

placement

4.1.3. Talent placing sterilized instruments in an organized manner on the surgical drape.

4.2. Using forceps, identify the cranial edge of the scapula and the caudal edge of the thoracic rib cage **[1]**. With the skin held taut between the surgeon's thumb and forefinger **[2]**, make a superficial 5-millimeter skin incision directly below the scapula using a size 10 scalpel blade **[2]**.

4.2.1. Talent pointing to the landmarks with forceps to identify incision area.

4.2.2. Talent holding the animal's skin taut between thumb and forefinger.

4.2.3. Talent stabilizing the skin and making a clean, shallow incision using a size 10 blade.

4.3. Then, use 115-millimeter straight scissors to blunt dissect the subcutaneous fat away from the ribs and intercostal muscles without cutting through them **[1]**. To stop any light bleeding caused by capillary damage, press a sterilized cotton-tipped applicator lightly onto the bleeding area **[2]**.

4.3.1. Talent using scissors to gently separate fat tissue from the ribs with a blunt dissection motion.

4.3.2. Talent applying gentle pressure with a cotton-tipped applicator to a minor bleed.

4.4. Hold the incision open using Micro-Adson forceps **[1]**. Starting at the seventh true rib, use curved medium-point forceps to count upward and locate the fourth and fifth ribs **[2]**. Mark this region as the injection site **[3]**.

4.4.1. Talent positioning Micro-Adson forceps to keep the incision open.

4.4.2. Talent methodically counting ribs from the seventh to the fourth using curved forceps.

4.4.3. Talent pointing to the identified injection site between ribs four and five.

4.5. Now, gently invert the cell suspension tube 3 to 4 times to mix **[1]**. Just before injection, draw 50 microliters of the suspension into a sterile 300 microliter 31-gauge 8-millimeter insulin syringe, making sure no bubbles are present **[2]**.

4.5.1. Talent inverting the tube with cell suspension a few times.

4.5.2. Talent drawing up 50 microliters into the insulin syringe and flicking it to remove

bubbles.

4.6. Place a 4-millimeter thick strip of sterilized metal tape flush to the syringe to act as a stopper and control injection depth **[1]**.

4.6.1. Talent attaching a pre-measured strip of metal tape to the syringe, flush with the barrel.

4.7. Inject the 50-microliter cell suspension at a 90-degree angle into the intercostal space between ribs four and five directly beneath the fourth rib **[1]**. Hold the needle in position for 2 to 3 seconds to allow the Matrigel to solidify and prevent cell leakage **[2]**.

4.7.1. Talent inserting the needle perpendicularly between ribs four and five and pressing the plunger steadily.

4.7.2. Talent holding the needle steady for a few seconds post-injection.

4.8. Slowly withdraw the needle at the same angle as inserted and discard it in a sharps container **[1]**.

4.8.1. Talent retracting the syringe at the same angle.

4.9. Finally, close the skin incision using 2 to 3 square-knotted stitches **[1]** and apply veterinary surgical adhesive over the sutures to reinforce wound closure **[2]**.

4.9.1. Talent suturing the incision with clean, square knots.

4.9.2. Talent applying surgical glue across the sutured area.

Results

5. Results

5.1. Bioluminescence imaging detected tumor-associated signals in the experimental mice as early as 1 day post injection **[1]**, and radiance visibly increased over time for both 25,000 and 50,000 cells per mouse groups **[2]**, with greater intensity observed in the 50,000-cell group **[3]**.

5.1.1. LAB MEDIA: Figure 3A*. Video editor: Highlight blue dot signal in the mouse on day "2" in the 50k panel*.

5.1.2. LAB MEDIA: Figure 3A. *Video editor: Sequentially highlight the column of images from days 2 to 20 for both 25k and 50k panels*.

5.1.3. LAB MEDIA: Figure 3A. *Video editor: highlight the 50k row*.

5.2. Total tumor flux increased exponentially in both 25,000 and 50,000 cell groups **[1]**, reaching over 10×10^8 photons per second by day 21 in the 50,000 cell group **[2]**.

5.2.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the graphs for 25k and 50k*

5.2.2. LAB MEDIA: Figure 3B. *Video editor: Highlight the 50k curve*.

5.3. Sham mice showed no luminescence or visible tumor in dissected lungs **[1]**, while experimental mice showed high luminescence and red tumor masses in lung tissue **[2]**.

5.3.1. LAB MEDIA: Figure 4A.

5.3.2. LAB MEDIA: Figure 4B.

5.4. At 21 days post injection, included mice showed tumors confined to the left lung lobe with no invasion into surrounding regions **[1]**.

5.4.1. LAB MEDIA: Figure 4D. *Video editor: Highlight the re pointed by the arrow*

Pronunciation Guide:

1. **Intercostal**
   Pronunciation link: https://www.merriam-webster.com/dictionary/intercostal Merriam-Webster
   IPA: /ˌɪn·tərˈkɒs·təl/
   Phonetic Spelling: in-ter-KOS-tuhl
2. **Luciferase**
   Pronunciation link: https://www.merriam-webster.com/dictionary/luciferase Merriam-Webster
   IPA: /ˌlu·səˈfeər·eɪs/ (or /luːˈsɪf·ər·eɪs/)
   Phonetic Spelling: loo-suh-FAIR-ays
3. **Luciferin**
   Pronunciation link: https://www.merriam-webster.com/dictionary/luciferin Merriam-Webster
   IPA: /ˌlu·səˈfɪər·ɪn/
   Phonetic Spelling: loo-suh-FEER-in
4. **Bioluminescence**
   Pronunciation link: (Merriam-Webster has "bioluminescent" but implicitly the root) https://www.merriam-webster.com/dictionary/bioluminescent Merriam-Webster
   IPA: /ˌbaɪ·oʊ·luːˈmɪn·ə·səns/
   Phonetic Spelling: bye-oh-loo-MIN-uh-səns
5. **Microenvironment**
   Pronunciation link: https://www.merriam-webster.com/dictionary/microenvironment (if present)
   IPA: /ˌmaɪ·kroʊ·ɪnˈvaɪ·rən·mənt/
   Phonetic Spelling: MY-kro-in-VY-ruhn-mənt
6. **Matrigel**
   Pronunciation link: https://www.howtopronounce.com/matrigel
   IPA: /ˈmætrəˌdʒɛl/
   Phonetic Spelling: MAT-r uh-jel
7. **Intervention**
   Pronunciation link: Merriam-Webster (standard word)
   IPA: /ˌɪntərˈvɛnʃən/
   Phonetic Spelling: in-ter-VEN-shun
8. **Subcutaneous**
   Pronunciation link: Merriam-Webster "subcutaneous"
   IPA: /ˌsʌb·kjuˈteɪ·ni·əs/
   Phonetic Spelling: sub-kyoo-TAY-nee-us
9. **Scapula**

Pronunciation link: Merriam-Webster "scapula"
IPA: /ˈskæpjə·lə/
Phonetic Spelling: SKAP-yə-luh

10. **Thoracic**
Pronunciation link: Merriam-Webster "thoracic"
IPA: /θɔːˈræs·ɪk/ or /θəˈræsɪk/
Phonetic Spelling: thor-RASS-ik

11. **Aspirate**
Pronunciation link: Merriam-Webster "aspirate"
IPA: /ˈæs·pəˌreɪt/
Phonetic Spelling: AS-puh-rate

12. **Conical**
Pronunciation link: Merriam-Webster "conical"
IPA: /ˈkɒn·ɪ·kəl/
Phonetic Spelling: KON-i-kul

13. **Pellet**
Pronunciation link: Merriam-Webster "pellet"
IPA: /ˈpɛl·ət/
Phonetic Spelling: PEL-it

14. **Sterile / Sterilized**
Pronunciation link: Merriam-Webster "sterile"
IPA: /ˈstɛr·əl/
Phonetic Spelling: STAIR-uhl

15. **Fenestrated**
Pronunciation link: (Less common; "fenestrated" may appear in dictionary)
IPA: /ˈfɛn·əˌstreɪ·tɪd/
Phonetic Spelling: FEN-uh-STRAY-tʊd

16. **Dissection**
Pronunciation link: Merriam-Webster "dissection"
IPA: /dɪˈsɛk·ʃən/
Phonetic Spelling: dih-SEK-shun

17. **Capillary**
Pronunciation link: Merriam-Webster "capillary"
IPA: /ˈkæp·əˌlɛr·i/
Phonetic Spelling: CAP-uh-ler-ee

18. **Micro-Adson forceps**

- ***Micro*** **— standard: /ˈmaɪkroʊ/ (my-kroh)**
- ***Adson*** **— proper name; likely /ˈædsən/ (AD-sən)**

- ***Forceps*** **— /ˈfɔr·sɛps/ (FOR-seps)**
  Phonetic Spelling: MY-kroh AD-sən FOR-seps

19. **Photon / Photons**
    Pronunciation link: Merriam-Webster “photon”
    IPA: /ˈfoʊ·tɒn/
    Phonetic Spelling: FOH-ton
20. **Radiance**
    Pronunciation link: Merriam-Webster “radiance”
    IPA: /ˈreɪ·di·əns/
    Phonetic Spelling: RAY-dee-uhns