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Title: Perfusion and Inflation of the Mouse Lung for Tumor Histology

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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. **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?

   ☒ Author interview statement opt out. Statements removed completely.

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

Current Protocol Length

Number of Steps: 13
Number of Shots: 27

Introduction

1. Introductory Statements to be read by Voiceover Talent

Introduction of Demonstrator

1.1. Demonstrating the procedure will be Mackenzie Davenport, a Graduate Student from the Edmonds laboratory.

1.1.1. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera.

Ethics Title Card

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

Protocol

2. Experimental Protocol

2.1. After euthanizing the mouse, use surgical scissors to make a 3.5 to 5-millimeter horizontal incision in the middle of the lower abdomen **[1]**. Next, insert surgical scissors into the incision and cut vertically up the center midline to just below the neck of the mouse **[2]**.

2.1.1. Talent making the incision. NOTE: This and next shot filmed together

2.1.2. Talent inserting scissors into the incision and cutting up the midline.

2.2. Pull the skin back with fingers and inspect the axillary lymph nodes **[1]**. Use surgical scissors to make a 3.5-millimeter lateral incision to open the abdominal cavity, then cut in the anterior direction up to the bottom of the thorax **[2]**. Inspect the organs in the abdominal cavity such as the liver, spleen, and kidneys **[3]**.

2.2.1. Talent pulling back the skin and inspecting the lymph nodes. NOTE: This and next shot filmed together

2.2.2. Talent opening the abdominal cavity and cutting to the bottom of the thorax.

2.2.3. Organs in the abdominal cavity.

2.3. With the flat of the surgical scissors, move the liver to expose the diaphragm. Inspect the diaphragm for tumor growth or metastases **[1]**. Then, gently snip the diaphragm on the operator's right side, allowing it to expand **[2]**. Cut the diaphragm from right to left to expose the thoracic cavity and lungs, taking care to not cut the lungs **[3]**.

2.3.1. Talent moving the liver. NOTE: This and next 2 shots filmed together

2.3.2. Talent snipping the diaphragm.

2.3.3. Talent cutting the diaphragm.

2.4. Cut up through the lateral extreme of the left rib cage to inspect the left lobe of the lungs **[1]**. Gently move the right lobes of the lung out of the way, then cut up the lateral extreme of the right rib cage and remove the rib cage **[2]**. *Videographer: This step is important!*

2.4.1. Talent cutting through the left rib cage. NOTE: This and next shot filmed together

2.4.2. Talent cutting through the right rib cage and removing the rib cage.

2.5. If fresh or frozen lung tissue is required, use hemostat forceps to clamp the bronchus of the left lobe **[1]** and resect the left lung with surgical scissors prior to perfusion **[2]**.

2.5.1. Talent clamping the bronchus. NOTE: This and next shot filmed together

2.5.2. Talent resecting the lung.

2.6. Use the forceps to lift the tissue covering the trachea and cut away any excess tissue **[1]**, then gently cut the thin tissue lining the trachea to expose the airway **[2]**. Cut through the renal artery with surgical scissors **[3]**. *Videographer: This step is important!*

2.6.1. Talent lifting tissue from the trachea and cutting it. NOTE: This and next 2 shots filmed together

2.6.2. Talent cutting the thin tissue lining the trachea.

2.6.3. Talent cutting through the renal artery.

2.7. To perfuse the lungs, use a 3-milliliter syringe with a 22-gauge needle to inject PBS with 10 units per milliliter heparin into the right ventricle of the heart **[1]**. Slowly perfuse the lungs at approximately 300 microliters per second, causing the lungs to turn white **[2-TXT]**. *Videographer: This step is difficult and important!*

2.7.1. Talent injecting PBS/heparin into the heart. NOTE: This and next shot filmed together

2.7.2. Lungs perfusing and turning white. **TEXT: Use 2.5 mL PBS** NOTE: This was filmed twice so lungs could turn white

2.8. For lung inflation, use a 3-milliliter syringe with a 22-gauge needle and hold it parallel to the trachea **[1]**. Insert the needle into the trachea and inject 10% formalin with a flow rate no greater than 200 microliters per second until the lungs have fully inflated **[2]**. *Videographer: This step is difficult and important!*

2.8.1. Talent holding the needle parallel to the trachea. NOTE: This and next shot filmed together

2.8.2. Talent inserting the needle and injecting the formalin.

2.9. Once the lungs are inflated, formalin will backflow out of the trachea. Hold the needle in place for a few more seconds and then withdraw **[1]**.

2.9.1. Formalin backflowing and talent removing the needle.

2.10. Use forceps to lift the heart, insert surgical scissors directly behind the lungs, and cut the connective tissue to resect the lungs **[1]**. Then, cut the heart to remove it from the lungs **[2]**. *Videographer: This step is important!*

2.10.1. Talent lifting the heart and cutting the connective tissue to resect the lungs. NOTE: This and next shot filmed together

2.10.2. Talent cutting the heart and removing it from the lungs.

2.11. Place the lungs in a cassette labeled with the mouse ID or study ID **[1]**, then place the cassette in 10% buffered formalin for 24 to 48 hours. Lungs can be left in fixative for

over a year if desired **[2]**. When finished, transfer the cassette containing the lungs to 70% ethanol and proceed with histology **[3]**.

2.11.1. Talent placing the lungs in the cassette. NOTE: This and next 2 shots filmed together

2.11.2. Talent placing the cassette in formalin, with the formalin container in the shot.

2.11.3. Talent transferring the cassette into ethanol, with the ethanol container in the shot.

Results

3. Results: Representative H&E Staining

3.1. This protocol allows for quick perfusion, inflation, and fixation of mouse lungs. Tumor histology in lungs which have been perfused and inflated using this technique is demonstrated here **[1]**.

3.1.1. LAB MEDIA: Figure 3.

3.2. When the perfusion step is skipped or the lungs fail to perfuse correctly **[1]**, excess blood in the lungs creates less than ideal histology and can make it challenging to fully observe lung architecture **[2]**.

3.2.1. LAB MEDIA: Figure 1.

3.2.2. LAB MEDIA: Figure 1. *Video Editor: Emphasize B.*

3.3. Inflation is an important step in this protocol **[1]**. It is more difficult to identify areas of hyperplasia in un-inflated lungs due to the compression and close proximity of the alveoli **[2]**. However, in overinflated lungs, many of the alveolar walls have been broken, which could be mistaken for emphysema **[3]**.

3.3.1. LAB MEDIA: Figure 2.

3.3.2. LAB MEDIA: Figure 2. *Video Editor: Emphasize B.*