**Harvesting of tissue from Ligation Injured Mice**

**I. Rationale:**

**Hypothesis:**

**Date of Sacrifice:**

**Ligation injury:**

**Mice:**

|  |  |  |  |
| --- | --- | --- | --- |
| **Number** | **Genotype** | **Weight** | **Re-Genotype** |
| **1.** |  |  |  |
| **2.** |  |  |  |
| **3.** |  |  |  |
| **4.** |  |  |  |
| **5.** |  |  |  |
| **6.** |  |  |  |
| **7.** |  |  |  |
| **8.** |  |  |  |
| **9.** |  |  |  |
| **10.** |  |  |  |

**II. Preparation:**

1. 1. Have plate ready with 4% PFA for tissues.
2. 5. Have an eppendorf tube ready for tail and organ pieces for each mouse for repeat genotyping.

**III. Euthanasia and Harvesting**

1. Euthanize the mouse in the CO2 chamber.
2. Weigh the mouse. Take a piece of tail for repeat genotyping.
3. Tape the mouse’s arms to 3-4 layers of paper towels and place under a light source. Swab the ventral area of the mouse with 70% ethanol.
4. Cut the skin of the mouse from the abdomen to the top of the thorax. Open the abdominal wall below the ribcage. Lift the sternum with tweezers and cut the diaphragm. Then cut away the lower part of the ribcage to partially expose the heart.
5. To begin perfusion fixation, make a small incision in the right atrium for drainage. Perfuse using a syringe with 5 cc PBS then 10 cc 4% PFA.
6. Dissect out the R common carotid by cutting at the distal end just beyond the suture. Dissect out the L common carotid at the distal end just beyond the bifurcation with the internal and external carotids. Also remove the thoracic aorta, liver, spleen, kidney, lung, heart, skeletal muscle, and colon as a control for tissue staining and for DNA genotyping.
7. Leave tissues in 4% PFA overnight with shaking at 4 deg C. Wash 2X in PBS the next day and place in 70% EtOH to prepare for processing for histology.

**Tissue for Paraffin Sections**

**Specimen Size**: No more than 3mm thick and should not touch both the top and bottom of the tissue cassette

**Volume of Fixative**: 15-20 times greater than tissue volume at room temperature

**Length in Fixative**: Small tissue: 24 hours after perfusion

 Large tissue: 48 hours after perfusion

\*There are always exceptions to the rule so if in doubt ask

**Tissue Prep**:

1. Submit tissue in cassettes labeled in **pencil** in either fixative or 70% ethanol.
2. Bring slide boxes, super frost plus slides and blades
3. Submit instruction sheets for all samples