

## Standard Operating Procedure Mouse Liver Collection for DNA/RNA Extraction

### I. Purpose

This document provides the protocol for collecting liver during mouse necropsy from euthanized mice. This protocol is specifically designed for isolating different lobes of the liver and for subsequent DNA and RNA extractions.

### II. Materials

Lab supplies and reagents required for this SOP include the following:

- 1.5 mL collection tubes (e.g., microcentrifuge tubes)
- Surgical scissors
- Surgical forceps
- Razor blade
- Ethanol (70%)
- Mouse euthanasia equipment (i.e., euthanasia container, CO<sub>2</sub> tank with regulatory gauges)
- Lab bench absorptive pads
- Weigh circles
- Liquid nitrogen

### III. Procedure

#### 1) Preparation

- a. Label 6 collection tubes with mouse ID, sex, and age on each. Also include the following for the individual tubes:
  - i. 1 tube labeled for left lobe
  - ii. 1 tube labeled for medial lobe
  - iii. 1 tube labeled for right lobe
  - iv. 1 tube labeled for caudate lobe
  - v. 1 tube labeled for accessory lobes
  - vi. 1 tube labeled for tumors (this may not be required for younger mice)
- b. Clean all surgical equipment using soap and water, followed by sterilization with ethanol
- c. Place absorptive pad on lab bench in the area in which collection will take place.

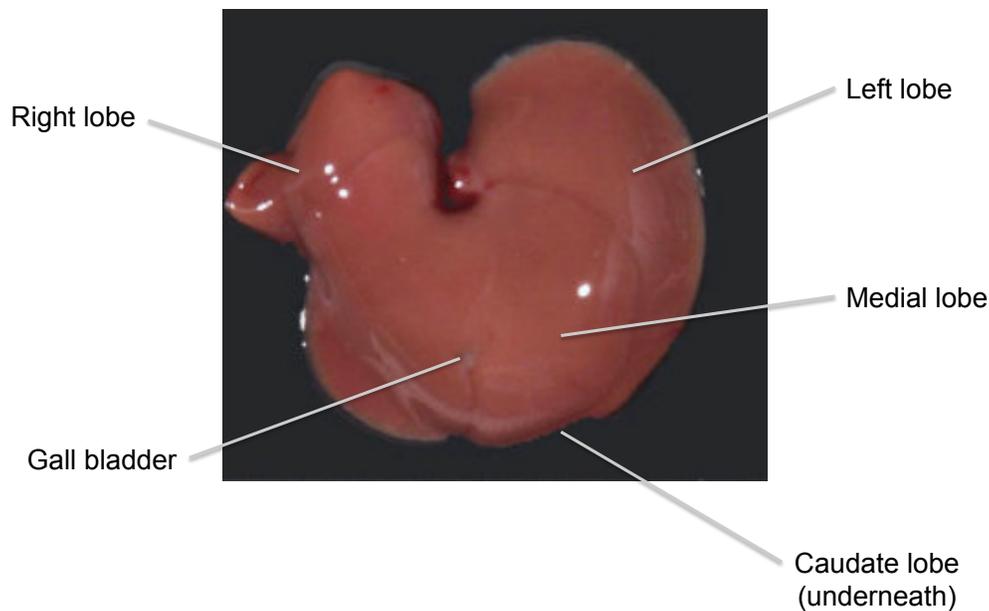
#### 2) CO<sub>2</sub> euthanasia

- a. Place mouse in euthanasia container and perform CO<sub>2</sub> euthanasia according to approved animal protocol
- b. Perform secondary euthanasia as specified in your protocol (e.g., pneumothorax)

#### 3) Open abdominal cavity

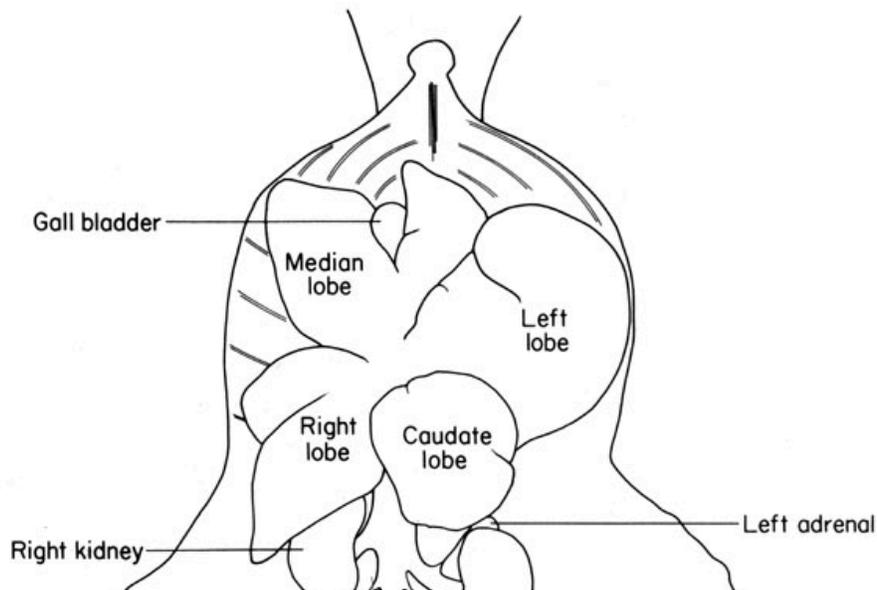
- a. Wet antral side of animal with 70% ethanol to minimize interference from hair
- b. Use forceps to lift abdominal skin, near genital region

- c. Use scissors to cut skin and abdominal cavity lining vertically towards the chest, exposing the abdominal organs. Then make a lateral cut at the chest to the left and right so that the liver is exposed and visible. Use forceps to tent the skin to avoid damaging organs as you cut.
- 4) Extract the liver
- a. Use surgical scissors to cut the liver away from the diaphragm. Work your way back to the dorsal side, cutting the liver away from other abdominal organs and from the dorsal side of the abdominal wall.
  - b. Next, use forceps and scissors to go underneath the liver and cut it away from abdominal organs until the entire liver is free.
    - i. Double check the stomach and pancreas to be sure you've freed all accessory lobes.
  - c. Place the whole liver on a weigh circle and orient it as depicted below:



**Description of lobes:**

- Medial or median lobe has the gall bladder in the center of the lobe and is shaped like a butterfly. This is the largest lobe
- The left lobe is the second largest lobe and lays relatively flat. It is located in the left portion of the mouse cavity.
- The right lobe has more of a 3-D shape and does not lay flat. It is the third largest lobe.
- The caudate lobe is also somewhat 3-D in shape and is underneath the medial and left lobes (see picture below).
- There are also often a few small accessory lobes, typically attached to the stomach.



#### 5) Sample collection

- a. To remain consistent, the left lobe will be extracted for all DNA/RNA work. Use forceps and razor blade to cut left lobe away from the other lobes.
- b. Place the left lobe in properly labeled microcentrifuge tube and immediately immerse in liquid nitrogen to flash freeze. Flash freezing with liquid nitrogen helps preserve sample quality.
- c. If there are any tumors present, attempt to isolate the tumor and place in a separate tube, then flash freeze in liquid nitrogen. Record which lobe of the liver the tumor was located in. If tumors are diffuse and it is not possible to isolate them, then collect the lobes with tumors and record this information for your records and for meta data.