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Murine Source Protocol -- University of Minnesota TMC

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Cellular Senescence Net...

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We use this protocol and it's working

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Abstract

The following protocol includes murine source information regarding mice used at the University of Minnesota TMC.

Troubleshooting

Mouse Euthanasia

1 Mice are euthanized primarily with CO2 asphyxiation and secondarily with cervical dislocation.

Adipose Tissue Collection

2 **Subcutaneous adipose:**

- Without cutting into the peritoneal cavity, make an incision longitudinal

across the cavity and down the leg. Pull back the skin to expose subcutaneous adipose tissue
attached to the inner skin wall.

- Lymph node separates the two fat pads, Chi et al described more innervation in inguinal

as compared to dorsolumbar.

- Blood vessels and sympathetic innervation can be seen feeding into dorsolumbar

adipose in lean mice. Willow et al, Chi et al and Vaughan (24480348).

Gonadal adipose:

- Pull tissue out at an angle to expose gonads (uterine tubes or epididymis).
- Starting from the base, cut upwards along gonads, to collect all adipose tissue, but avoiding

collecting non-adipose.

- Ovaries are often buried in the fat - collect this

Mesenteric adipose tissue:

- Stomach leads to duodenum, leading to small intestines.
- Remove the entire intestines+ adipose tissue and lay out flat to expose arms that stretch to intestines.
- Lymph nodes are visible from the “under” side only.
- Starting at the duodenum end of the small intestines, use a pair of scissors to make small cuts and separate adipose tissue from intestines.
- To avoid collecting lymph nodes only “arms” of adipose will be collected.

Brain Tissue Collection

3 **Brain Tissue Collection**

- Cut off head.
- Make one small cut on each side of the skull proximal to (but not cutting) the brain stem.

- Carefully peel off the fragments of the skull that covers this area until the cerebellum and brain stem are fully exposed.
- If dissecting the brain in half: take small shallow bladed scissors and cut precisely down the midline of the brain starting at the end of the cerebellum. If NOT dissecting the brain in half- continue to make shallow cuts up the sides of the skull near the brain, making sure not to cut the brain tissue. Use sharper and bigger necropsy scissors to cut/sever the ethmoid bone (i.e. the bone that lies between the two eye sockets). This will make it easier to take pieces of the skull off and will separate the olfactory bulbs from the connective tissues within the skull. Make sure to slide forceps in to disconnect the meninges from the brain to avoid ruining brain tissue.
- The brain should be able to easily disconnect from the skull where it sits . Take the brain and place it precisely in the brain matrix so that the midlines match up (must be flat and centered to get correct regions isolated).
- Now that the brain is out of the skull and in the matrix, the first cuts will be made with a surgical razor blade.
- The first cut will be made down the midline of the brain. It is recommended that the blade stays in place while making the remainder cuts (i.e. the remainder cuts will be with a surgical scalpel blade.) (size #10)
- The second cut will be between the cerebellum and midbrain. (reference image below)
- The matrix will have lines/spaces in it for blades to enter that will guide these cuts. Skip one space between the second cut made and this will be the third and final cut which will be in the midbrain region. This cut will isolate the Ventral Midbrain and Dorsal Midbrain regions.
- These are all of the cuts needed to be done in the matrix. Pull out the brain and place one half in the desired preservation method and take the other half with the cuts made and place on a flat surface to isolate the remainder regions further.
- Once half the brain (with cuts made previously) is on the flat surface, collect the Olfactory Bulbs. The olfactory bulbs are located on top of the brain.
- Between where the second and third cuts were made will be the area where the Ventral Midbrain and Dorsal Midbrain regions will be located. Separating the cerebellum and brain stem cuts will leave these two regions, the superior portion is the Dorsal Midbrain the inferior portion is the Ventral Midbrain.
- Where the second cut was made will have separated the ½ Cerebellum and Brain Stem from the midbrain. Brain stem will be white in color and inferior to the cerebellum. Make sure the two are clearly separated and no brain stem is left behind on the cerebellum.
- Take the curved, small and sharp forceps and place between the cortex and hippocampus (will be a lighter shade and banana shaped), let the forceps gently and slowly open to naturally separate the hippocampus from the cortex. If done correctly the hippocampus should be facing out towards you so you can gently pull it to collect.
- Once the hippocampus is taken out there will be a space where it once sat, the tissue that is proximal to you will be the Thalamus and it will be a triangle shape. Take all of

that piece as it is all the thalamus.
- What will be left is the cortex, within the cortex is the Striatum. Medial of the cortex will be the striatum and it will visually look striated; use the same forceps as before to cut around the striatum portion to successfully collect all of it.
- The tissue left after all of the regions are isolated will be the Cortex.
- To collect the spinal cord you will need an 18 gauge syringe filled with cold 1x PBS, the mouse's body, and sharp necropsy scissors. Start by cutting superior to the hips and exposing the spinal column. Once the spinal column is visible ,there will be a white small hole where the spinal cord lies within the column, place the syringe tip in the hole and apply pressure till the spinal cord is expelled from the other end of the column (where the head was once attached).
- All regions have been collected. Tools and work space can be cleaned up and mouse carcasses can be properly disposed of.

Liver Tissue Collection

4 **Liver Tissue Collection:**
- Use scissors and forceps to cut into the abdomen of the study mouse starting just above the genital area.
- Cut the skin and the cavity lining towards the chest stopping just before the diaphragm and rib cage.
- Locate the liver below the diaphragm.
- Using the scissors collect the left lobe for collection and any additional lobes if more is needed.
- The left lobe is best for OCT preservation as it is relatively flat.
- The center lobe of the liver contains the gallbladder witch is avoided for collection.

FFPE

5 Collect tissue and place in collection cup filled with 10% Neutral Buffered Formalin (NBF).

OCT

6 PREPARE YOUR WORKSPACE ON A BENCH
Things you will need:
- Styrofoam box
- Metal block
- Liquid nitrogen
- Weigh boat filled with 1X PBS
- Kim Wipes
- Cutting board

- Tools: forceps, scissors, razor blade
- OCT compound bottles (standing upside down)
- Cryo molds
- Pre- printed labels taped into small plastic storage bags

PROCEDURE

- Locate tissues in the cold room that are for “Fixed Frozen” and “Fixed Frozen SAβ-gal”
- The tissues are in histology cups and should be submerged in 30% Sucrose for at least 24 hours.
- Collect styrofoam box, metal block, liquid nitrogen and place metal block inside of the styrofoam box and pour liquid nitrogen over the block until the liquid nitrogen no longer evaporates and the block is visually frozen.
- If collecting fresh and freezing down: place tissues on cutting board and cut tissue so there is a flat cut side down.
- Next, take a weigh boat filled with 1X PBS and clean off any blood, fur or any none tissue substances off the tissue.
- Next, fill the cryomold with the O.C.T compound until the inner level is filled. Remove all air bubbles.
- Once the inner level is filled with O.C.T, place tissue in the inner sunken level of the standard tissue cryomold until the tissue is somewhat covered and cut side facing down.
- Make sure all air bubbles have been removed or are not near or on top of the tissue.
- Once tissue is placed and all air bubbles are removed, fill the rest of the cryomold with the O.C.T compound until all of the tissue is covered and all of the levels of space are covered with O.C.T.
- Place on metal block and allow to freeze. (Remove any bubbles before placing on metal block)
- Let the cryomold and O.C.T fully freeze over and ensure tissue is no longer visible.
- Lastly, take cryomold off the metal block and place in respectable plastic labeled bag and store at -80.

Fresh

7 Notify tissue lead of available tissue for pick up. Tissue is placed in a lab cold room kept at 4 degrees, on ice, or at room temperature depending on request.