Preserving Organs for Histopathologic Examination

Brain

Brain morphology is best examined on perfused, fixed specimens. For the best morphology and to avoid freeze artifacts, **it is highly recommended that fixation with perfusion of the whole animal is performed**. For optimal examination of the brain, perfuse the animal with PBS (to deplete all of the blood) and then perfuse with freshly made 4% paraformaldehyde (to fix all the organs).

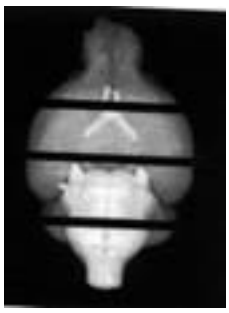
Whole Animal Perfusion

1. Set up perfusion pump via the left ventricle. Use a butterfly needle to enter the left ventricle. Use scissors to nick open the right atrium so that blood flows out as the circulation is replaced.
2. Perfuse the animal with PBS. If the perfusion is successful, the liver will blanch as the blood is replaced, followed by the kidneys.
3. Replace the buffer solution with ***freshly made*** 4% paraformaldehyde and perfuse through the body circulation for another 5-10 minutes.

If the fixed tissues are to be frozen for use in immunohistology assays after fixation, they ***must*** be immersed in 30% sucrose in PBS at 4°C ***until they sink*** in order to cryoprotect and prevent freeze artifact and loss of tissue architecture.

The **cranium** is opened by cutting around the perimeter, holding on to the foramen magnum, and it is lifted up to reveal the brain. This is removed carefully for further fixation or for freezing using the recommended protocol. **Do not forget to remove the pituitary from the sella turcica after removing the brain**. Be advised that the pituitary is small and requires special attention, so remember to confer with the Histology personnel about this.

Transverse sections provide a view of the whole brain, from the olfactory lobes, to the cerebellum, especially if different STEP sections and made and stained for viewing.



Coronal sections at specific sites, as shown in the picture to the right, allow similar areas to be examined, enabling comparisons to be made between littermate controls and from mutant animals. It is recommended that coronal sections of the entire brain be examined in order to pick up small abnormalities. Examination using special stains and immunohistochemistry will help dissect out abnormalities better.

Sagittal sections allow for easier viewing of each half of the brain, which can then be viewed from caudal to rostral aspects to detect any obvious abnormalities.

Sections of Brain may then be stained using different special stains, such as LFB (Luxol Fast Blue), which highlights myelin. Immunohistochemistry assays are also available, such as anti-GFAP (glial fibrillary acidic protein), which marks astrocytes; anti-MBP (myelin basic protein), which marks myelin; and anti-CD68, which marks macrophages (microglia).

For more information, visit The Mouse Brain Library website (www.mbl.org).

Lung

Lung morphology is best examined when all lobes are inflated. If lungs are not inflated prior to freezing, it will be very difficult to obtain good frozen sections because the tissue will collapse, which will also make accurate morphological analysis impossible. It is crucial to inflate the lungs with fixative for inhalation studies, and ***especially*** in experiments to assess **metastatic** ability of different malignancies.

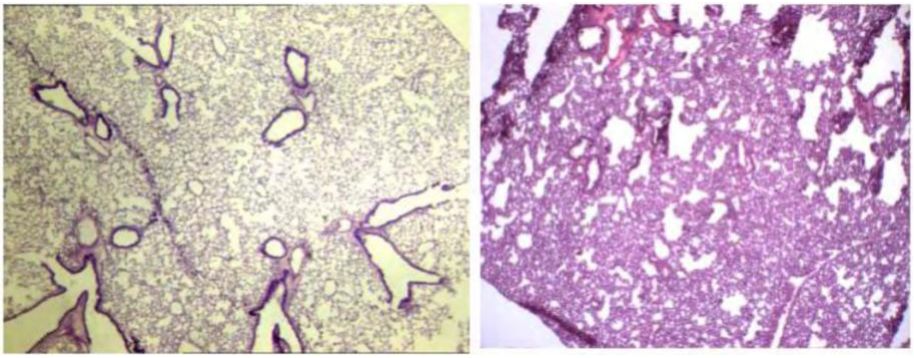


Figure 1. Left: OCT infiltrated lung prior to freezing, frozen section has good morphology. Right: non-OCT-infiltrated lung, frozen section has poor morphology.

For the best morphology, it is highly recommended to **inflate the lungs with a 1:1 mixture of OCT and PBS *prior to freezing*** in a slurry of dry ice and 2-methylbutane. All lobes should be separated from each other so that sections (and step sections) of each can be examined in order to detect small lesions.

To ensure that you will receive slides that will contain a particular abnormality that was visualized during necropsy, make sure you consult with the histotechnician when you submit the sample, so that care will be taken to embed the area of interest, such that it will be immediately visible on the stained slide. Include a part of the normal tissue that surrounds the abnormal area, so that interpretation of the stained slide will be accurate.

Heart

The heart should be trimmed away to be examined separately so that it will be possible to display all lobes of the lungs.

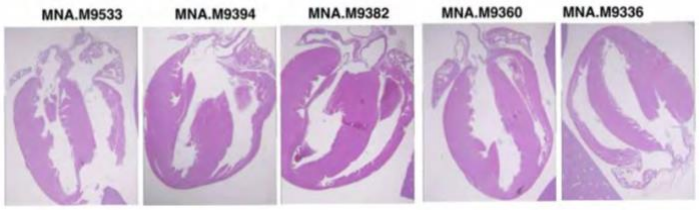


Figure 2. Multiple sections from different mouse hearts. Different orientation of the hearts can help visualize different abnormalities.

The heart may be divided into two halves and fixed in a cassette along with a section from the liver, half of a kidney, and with salivary glands – all may be fixed and processed in one block for embedding. Alternatively, the heart may be embedded separately in specific ways such that the valves may be viewed optimally, or embedded with the apex first so that sections will reveal both ventricles for evaluation of thickness, etc.

Spleen

Frozen sections of spleen are good controls for hematologic markers in immunohistochemistry. For this, the entire spleen is embedded **flat and at the bottom** of vinyl molds, in OCT, before immersion of the vinyl mold into a freezing slurry of dry ice and 2-methylbutane.

For paraffin sections, the spleen is **enclosed between two sponges** so that it will **lie flat** in the cassette ***before* it is fixed**. This procedure ensures good, flat paraffin sections without wrinkles.

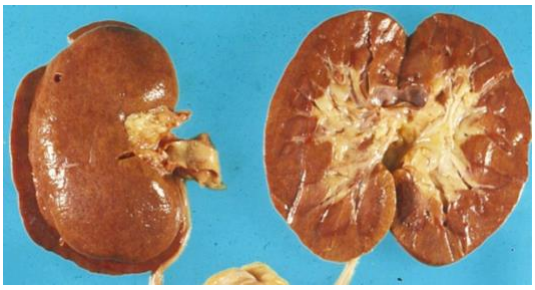
If there are obvious nodules in the spleen, or it appears abnormal, make sure the histology personnel note the position of the abnormality, so that the specific region is embedded correctly and to ensure that the eventual section that will contain the area of interest for microscopic examination and further analysis.

Pancreas

The pancreas is the fatty appearing structure that appears adherent to the hilus of the spleen. Remove as much as possible of the tan colored mass located closest to the spleen (this area usually contains a lot of the islets) and place between two sponges in an appropriately labeled embedding cassette ***before* fixing in buffered formalin**. Good, flat paraffin sections of pancreas are more difficult to obtain if not fixed in this manner.

Kidneys

Do not forget to **carefully remove the two adrenal glands before dealing with the kidneys**. The adrenals need special attention because they are so small and will need to be placed in separate cassettes, perhaps with the ovaries, which are also small. The ovaries can be isolated at the same as time as harvesting the adrenals and may not be easily visualized because they are surrounded by adipose tissue, in adjacent areas (or by following the uterine horns up from the pelvis).



The kidneys should be cut along the middle, into two parts, to reveal cortex and medulla and arcuate arteries, so that similar areas can be compared between littermate controls and in mutant animals, for microscopic observation. Embed both sides flat surface down, onto the bottom of the embedding cassettes, so that the organ is fixed while flat and thus the sections will reveal better morphologic relationships.

Remember to request that sections be made at less than 3 µm, if possible, to ensure adequate examination of **glomeruli**. Additional special stains may be requested as well as immunohistochemistry.

Intestinal Tract (Colon, Ileum)

Grasp the abdominal end of the esophagus and begin to disembowel the GI tract until the rectum is reached. **Be careful to not stretch out the mesenteric fat** so that harvesting of the mesenteric lymph nodes can be possible after fixation.

The small intestine should be sectioned into 4-5 parts before immersion fixation.

The **epithelial cells of the intestinal tract undergo autolysis readily** and thus **must be fixed as soon as possible**. This is easily accomplished by injecting fixative into the lumen. The small intestine may be cut up into smaller sections (to allow the fixative to penetrate into the mucosa) and then submitted for embedding, processing, and sectioning, as tubes laid side by side.

The colon is dissected away from the rest of the bowel.

If it is important to document the presence of either **epithelial tumors or ulcers**, the colon must be opened along the anti-mesenteric aspect and **rolled (with the mucosal end facing outwards)** so that fixative will reach all epithelium. This will make it possible to view the whole length of the colon on one section.

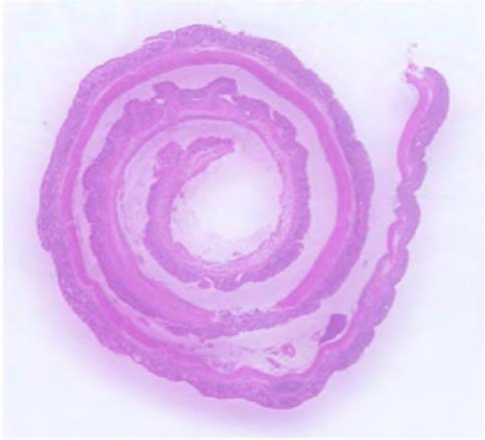


Figure 3. Histologic evaluation of colon rolls are critical when studying mouse models of inflammatory bowel disease.

Gut Roll

1. Remove and cut open colon longitudinally
2. Remove fecal contents and roll onto swab stick, making the roll compact (*note: do not try to roll after rinsing the gut in PBS – it will not stick together and will result in a poor roll*).
    a. Do not try to roll after rinsing the gut in PBS – it will not stick together and will result in a poor roll
    b. Always use the same method/orientation to roll (either anal end first or small intestine end first on the inside of the roll)
3. Fix the rolled-up colon on the stick in a 15-mL tube with fixative
4. Perfuse the animal with PBS. If the perfusion is successful, the liver will blanch as the blood is replaced, followed by the kidneys.
5. Replace the buffer solution with ***freshly made*** 4% paraformaldehyde and perfuse through the body circulation for another 5-10 minutes.