

Perfusion of Mice

For use in histologic methods and microscopy, adapted from [USF Training Document](#)

Procedure

1. Set up perfusion apparatus using a perfusion pump and connect plastic small gauge tubes to a butterfly needle (30 gauge). Make sure all flow is occurring as expected, with saline or phosphate buffered saline (PBS).
2. Place mouse under deep anesthesia following approved institutional protocols.
3. Dissect open skin of the chest (V shape), and using scissors already primed for bone cutting, cut on either side of the chest, through the ribs to expose the heart and lungs.
4. Make a cut into the right atrium, which may be removed.
5. Insert the butterfly needle into the left ventricle, and then stabilize.
6. Allow 5-20 mL of saline to enter the left ventricle, and watch to see the liver become bloodless, followed by the kidneys.
7. Change the perfusate to an appropriate fixative let this perfuse the animal for about 15 min, until the body becomes stiff.
8. Dissect away the required organs.
9. Perfuse the lungs again via the trachea until all lobes are filled with fixative. Separate out each lobe and places in clearly labeled cassettes for processing into paraffin blocks.
10. All organs may now be processed into paraffin blocks for sectioning at room temperature, using the microtome.

For Frozen Sections

1. After fixation place in 15% sucrose in PBS for 24 h.
2. Replace sucrose with 20% (or use 15% again) until tissue sinks for ~24 h.
3. Remove tissue from sucrose, blot off any excess and place in mold. This is an important step to avoid a "shell" of sucrose which coats the tissue and slows the freezing process.
4. NOTE: Sometimes 30% makes the tissue stickier and that 20% will give good results.
5. Transfer tissues to OCT (Optimum cutting temperature compound) chamber and surround with OCT.
6. Place mold in Styrofoam container that has dry ice/2-methyl butane slurry, and allow the block to freeze – until the OCT becomes white and is solid.
7. Remove and place on dry ice for an additional 15-30 min.
8. Store in labeled bags at -80°C until required and process for frozen sections using the Cryostat.