

Transcardial Perfusion of Mice

**Experimental Goal:** To chemically fix the adult mouse inner ear by transcardial perfusion

**Rationale:** Transcardial perfusion with aldehyde-based fixatives is generally considered to be an effective paradigm to preserve the soft tissues of the entire inner ear. Rapid, comprehensive fixation is essential for anatomical, histological, and molecular analyses.

Procedure

1. Weigh the adult mouse to the nearest 0.1 gram.
2. Administer anesthetic. Protocols for use of sodium pentobarbital and ketamine/xylazine are provided in Methodological Considerations below.
3. Place the injected mouse in a heated cage for 5-10 minutes.
4. Assess responses to tail/toe pinches and the intactness of the ocular reflex. Proceed only after the mouse is unresponsive to the noxious stimuli and the reflex is absent.
5. Secure the mouse in the supine position (lying on the back with face upward) by gently taping the forepaws and hindpaws to a pinnable (i.e., Styrofoam) work surface inside a chemical fume hood.
6. Make an incision through the skin with surgical scissors along the thoracic midline from just beneath the xiphoid process to the clavicle. Make two additional skin incisions from the xiphoid process along the base of the ventral ribcage laterally.
7. Gently reflect the two flaps of skin rostrally and laterally making sure to expose the thoracic field completely.
8. Grasp the cartilage of the xiphoid process with blunt forceps and raise it slightly to insert pointed scissors. Cut through the thoracic musculature and ribcage between the breastbone and medial rib insertion points and extend the incision rostrally to the level of the clavicles.
9. Separate the diaphragm from the chest wall on both sides with scissor cuts.
10. Tape or pin (with 18G needles) the reflected ribcage laterally to expose the heart (and other thoracic organs).
11. Gently grasp the pericardial sac with blunt forceps and tear it fully.
12. Secure the beating heart with blunt forceps and make a 1-2mm incision in the left ventricle. Immediately insert a 24G X 25.4mm animal feeding needle (Harvard apparatus Cat. #52-4009). This is called a needle by the manufacturer (Cadence Science) but the tip is bulbous and will not damage the heart. Thread the feeding needle into the base of the aortic arch using a dissecting microscope. Clamp the needle base to the left ventricle above the incision site using a hemostat.
13. Immediately cut the right atrium with scissors and at the first sign of blood flow, begin the infusion of heparinized saline (stage 1 perfusate).
14. Continue perfusing the body until the fluid exiting the right atrium is entirely clear.
15. Switch from saline perfusate to aldehyde-based fixative (stage 2 perfusate).
16. Perfuse 20-30 ml of fixative.
17. Decapitate the mouse with large surgical scissors. Remove the skin.
18. Cut the cranium along the mid-sagittal suture. Completely hemisect the cranium with a rapid, forceful razor blade thrust.
19. Place the two halves of the mouse head into 20 mls of ice cold fixative and gently rock at 4 Celsius for 10-12 hrs.
20. Wash the fixed head in 3 changes of phosphate buffered saline, 5 mins per wash.
21. Dissect the inner ear and analyze phenotype with appropriate methods.

Methodological Considerations

**Anesthesia: Ketamine/Xylazine** The mice may be anesthetized with 120 mg/kg body weight of ketamine and 24 mg/kg body weight Xylazine in a vehicle containing 0.9% sodium chloride. The anesthetic mixture is made as follows:

<u>Component</u>	<u>Volume per mL</u>	<u>Final Concentration in Injected Anesthetic Mix</u>
100 mg/mL Ketamine	0.2 ml	20 mg/ml
20 mg/mL Xylazine	0.2 ml	4 mg/ml
0.9% saline	0.6 ml	not applicable

Example: A mouse weighing 25.0 grams receives 6 microliters of the above anesthetic mixture *per gram body weight* for a total of 0.150 mL of anesthetic mixture injected intraperitoneally.

**Anesthesia: Pentobarbital Sodium** The mice may be anesthetized with 65 mg/kg body weight of sodium pentobarbital injected intraperitoneally in vehicle containing 10% ethanol and 40% propylene glycol. The anesthetic mixture is made as follows:

<u>Component</u>	<u>Volume per mL</u>	<u>Final Concentration in Injected Anesthetic Mix</u>
50 mg/ml Nembutal (pentobarbital sodium solution, USP)	0.180 ml	9.0 mg/ml
Propylene glycol	0.400 ml	40.0%
Ethanol	0.100 ml	10.0%
0.9% saline	0.320 ml	not applicable

Example: A mouse weighing 25.0 grams receives 7.5 microliters of the above anesthetic mixture *per gram body weight* for a total of 0.188 mL of anesthetic mixture injected intraperitoneally.

Debunking Myth: “*It’s really easy to overdose a mouse with sodium pentobarbital.*” It is in fact really easy to do a careless job weighing a mouse, and then deliver an inappropriate dose of sodium pentobarbital. This will reliably generate unintended outcomes. Sodium pentobarbital is an exceptional anesthetic for mice when properly administered.

**Perfusion of Early Postnatal Mice**

The 24G animal feeding needle is oversized for early postnatal mouse heart. For postnatal mice from birth to about 7 days, fabricate a custom perfusion needle from a 27G X 12.7mm hypodermic needle. Blunt the needle tip by filing on a sharpening stone. Snap the needle from its housing and file this blunt end. Superglue or epoxy the needle into fine plastic tubing (Tygon Ultra-Soft Tubing R-1000 or any similar fine tubing that accommodates the 27G needle). Slip the opposite, open end of the tubing over a blunted, 27G X 12.7mm needle that has an intact housing (Luer Lock fitting). Superglue or Epoxy the tubing to the needle. Attach the needle to the syringe. Test that the circuit is patent by passing water through the tubing and out the blunted needle tip. Hamilton syringe cleaning wires can be used unclog debris from the perfusion needle.

**Driving Perfusate:**

Twenty milliliter plastic syringes are suitable to deliver perfusates manually. However, perfusate delivery rates tend to vary as inevitable hand fatigue sets in after the second or third mouse. A useful solution is to configure a two headed peristaltic pump for delivery of both perfusates, one pump head driving each perfusate. The change from stage 1 perfusate to stage 2 is simply a matter of turning the switch on a 3-way valve. The peristaltic pump has fine motor control for perfusate delivery, thereby enabling subtle changes in flow rate while ensuring consistency in rate delivery. We use the following peristaltic setup (contact Cole Parmer at [www.coleparmer.com](http://www.coleparmer.com) for current part numbers):

Master Flex L/S Drive 1-100 RPM peristaltic pump  
Pump Head/Easy Load II (one for each perfusate)  
Mounting Hardware F/2 Heads SS (to piggyback two heads on one pump)  
Footswitch to turn pump on and off: Cole Parmer special order item  
Stopcock, 3-way, female to male

***In Situ Correlates of Efficient Perfusion:***

The blood in the vascular system of the mouse is replaced with buffer during stage 1 perfusion. An early correlate of efficient perfusion is the blanching of the liver as blood is displaced with clear buffered saline. The liver typically begins to clear within 30 seconds or earlier during stage 1 and is a good indicator of effective systemic perfusion. The failure to detect liver clearance warrants a check of needle position in the left ventricle and flow rate of the perfusate. Another good indicator of effective perfusion is body movement. As blood is purged from the body during stage 1 perfusion, musculoskeletal contractions typically occur that look like stretching movements. It is also common to see the tail flick or sway. When fixative enters the body, subtle body movements also occur. It is important to make certain that the perfusion needle remains in the left ventricle during the entire perfusion process.

Materials

Blunt forceps  
Fine forceps: #5  
Rounded surgical scissors  
Pointed surgical scissors  
25G X 25.4mm animal feeding needle  
Two 20 ml plastic syringes  
Tape or 18G X 25.4mm needles  
Styrofoam (a 50 ml conical centrifuge tube rack)  
Single edge disposable razor blades  
Paper towels  
Safety Glasses  
Chemical fume hood

Solutions

## Heparinized phosphate buffered saline

Heparin sodium salt (from porcine intestinal mucosa): Sigma H3400

Stock solution: 75 units/microliter

Usage: 13 microliters of stock per 50 mL of saline (20 units/ml final concentration)

(A dilute solution of heparin may inhibit blood clot formation and preserve the patency of the vascular system during stage 1 perfusion).

## Calcium Free Phosphate buffered saline, pH 7.4

NaCl 137mM, KCl 2.7 mM, Na<sub>2</sub>HPO<sub>4</sub> 9.9 mM, KH<sub>2</sub>PO<sub>4</sub> 2.0 mM

## Fixatives

The choice of fixative largely depends on the downstream analysis. Paraformaldehyde in PBS (2-4%) is useful for tissues to be analyzed by immunohistochemistry or *in situ* hybridization. Glutaraldehyde/paraformaldehyde mixtures are useful for electron microscopy analyses. EM may also predicate a unique core buffer system for both stage 1 and 2 perfusions.