Harvesting *Mus Musculus* Organs Through Perfusion

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1 Introduction

The perfusion technique here is used to preserve the organs of a mouse for analysis without the need for freezing, which can create artifacts. Freezing also typically utilizes a compound such as OCT, which is not compatible with some downstream applications, such as array tomography. Perfusion also removes red blood cells, which can create imaging artifacts. Preserving organs through pumping a fixative into organs through the vascular network of a mouse allows the relatively quick fixing of these organs before they have the time to decompose. The general outline to accomplish this perfusion is to anesthetize the mouse, flush the blood out with saline, and then deliver the fixative. This is best done with two people, although possible with one. Some steps are tricky, so it is recommended to practice on less critical samples first. The following is for brains, but other organs will be fixed too. Additional fixation or sucrose dehydration may or may not be necessary for the organs you intend to use.

2 Materials

2.1 Reagents

1. Avertin: 2.5% concentration diluted the day of use from a 100% stock in PBS, adjusted to pH 7.4 if needed. Mix in an airtight dark eppendorf tube until the alcohol phase dissolves and the solution is clear. Dosage is approximately 250 mg/kg, or 10 microliters per gram of mouse, however an estimation of the mouse's weight is generally acceptable. 100% stock solution (an approximately 40x stock) should be made with 10g tribromoethyl alcohol (2, 2, 2 tribromoethanol), Aldrich T4, 840-2 and 10mL tertiary amyl alcohol (2 methyl-2-butanol), Aldrich 24, 048-6. Add the tribromoethanol to the tertiary amyl alcohol and dissolve by heating and stirring over hot water in a beaker, as tertiary amyl alcohol is flammable. This can be made up to 30 days prior to the procedure and should be stored at -80 degrees Celsius with minimal light exposure.

2. PBS: Phosphate Buffered Saline, without magnesium or calcium.

3. PFA: Paraformaldehyde, 4%, 30mL per mouse plus extra for organ storage

2.2 Equipment

4. Stand with two attachments for syringes

5. Two 50ml syringe bodies (no plungers)

6. Two IV tubes with roller clamp and drip chamber

7. 23-25 gauge butterfly needle with tubing attachment compatible for the IV tubes being used

8. 1mL syringe to deliver the avertin to the mouse.

9. Dissection tools for organ harvesting

10. Falcon tubes for storage of organs

3 Methods

3.1 System Preparation

This procedure was done with a manual in vivo perfusion system from Auto-Mate Scientific, in which the setup is shown below, however it is also possible to use a peristaltic pump. Using a peristaltic pump makes the procedure take less time, however some flow control would be lost.

1. Set up stand either on a procedure table cleaned with NPD detergent or in the hood. Gravity fed solution might flow faster if elevated above the workspace, say by half a meter.

2. Attach the syringes to the vertical rod on the stand.

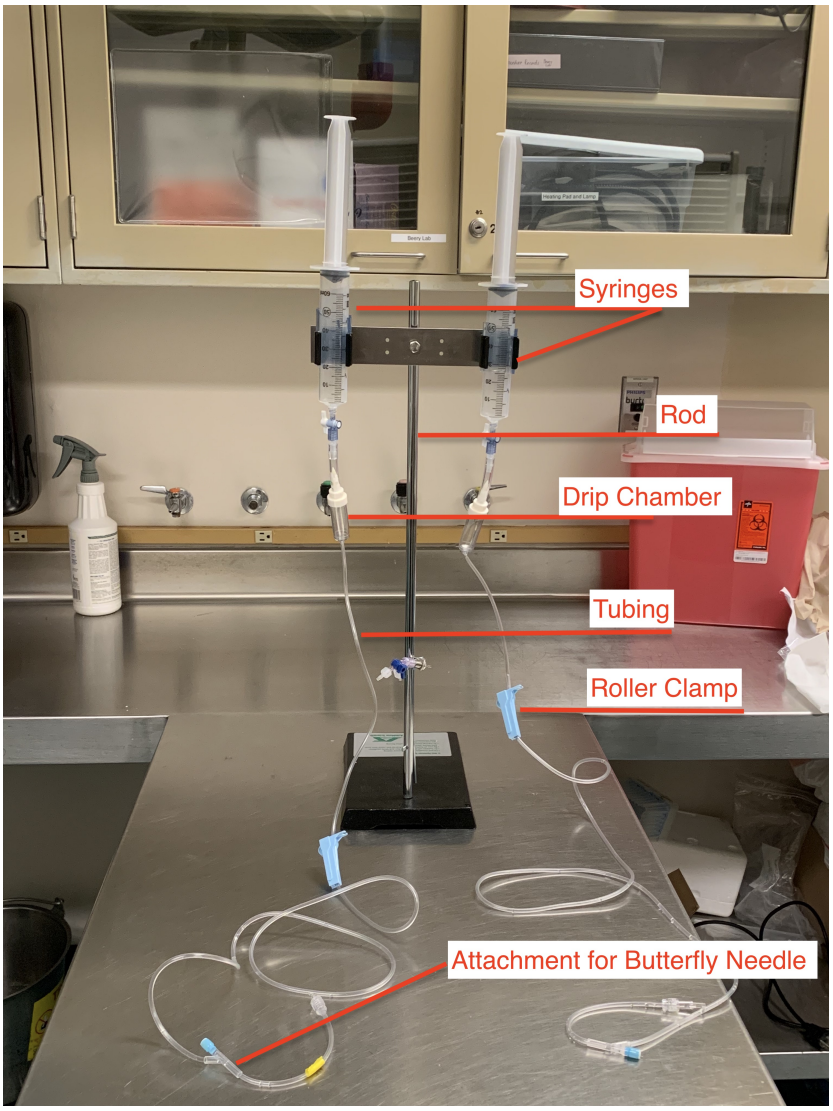
3. Set the height of the rod that the syringes are attached to maximum. The higher the syringes are with relation to the mouse, the faster the flow rate of the reagents, allowing for a quicker perfusion.

4. Attach the IV tubes to the syringes, and to one of the IV tubes attach a 23-25 gauge needle.

5. Fill both syringes with 15mL H2O to flush the lines and work out any issues with the tubing. Allow all the H2O to flow through the tubing, and the syringes to empty.

6. Once the H2O has flowed through the tubing, fill one syringe with 25 mL PFA and the other with 25 mL PBS. It is recommended to label both the end of the tubing as well as the syringe itself with the contents of the syringe for ease of reference during the procedure.

7. Let fluid flow through the lines to purge bubbles, then shut off flow at the thumb roller clamp. You might need to squeeze the drip chambers to purge bubbles once and for all.



3.2 Animal Handling

8. Draw up the avertin with a small syringe. Start with 10ul per gram of mouse.

9. Administer avertin by intraperitoneal injection in the abdominal cavity of the mouse.

10. Wait several minutes, then perform a toe pinch to check the effectiveness of the anesthetic. The mouse should not react to this toe pinch. If it has been over 5 minutes and the mouse is still reacting to the toe pinch, then dose with

more avertin. When the mouse stops reacting to the toe pinch, but while it is still breathing, bring the mouse over to the apparatus.

3.3 Surgical Procedure

11. Double check the PBS line for bubbles and flow through the butterfly needle.

12. Make a horizontal cut on the upper part of the mouse's abdomen. Hold just above and below the incision, and pull the skin open to expose the upper abdomen and lower thorax.

13. Cut through the diaphragm below the rib cage, and on each side of the rib cage. Flip the rib cage anteriorly and clamp to hold up near the head, and carefully tease/cut any connective tissue holding the heart to the ribs.

14. Insert the 23-25 gauge butterfly needle into the left ventricle of the heart. In order to prevent puncturing the septum between the left and right side of the heart, puncture from the bottom of the left ventricle and keep the tip of the needle just inside the heart. Hold the needle by the plastic tab.

15. Turn on PBS flow and cut through the right atrium to allow blood to flow out. As it comes out, it should start off deep red, and get lighter as the PBS flushes the cardiovascular system. If blood starts coming out of the mouse's face, this means the septum between the left and right side of the heart was pierced or the needle is in the wrong ventricle. The blood can be collected from the chest cavity with a syringe if the serum is needed for downstream use.

16. While the PBS is flushing the blood out, prime the PFA line in the same way as the PBS tube. Note: make sure the PBS line never runs out of reagent, and continue to monitor for bubbles.

17. When the liquid from the right atrium is coming out clear, unclip the butterfly needle from the PBS tubing and switch it to the PFA line. It is not necessary to remove the needle for this action, however make sure that there are no bubbles in the line. If bubbles get in the line, remove the needle to burp the bubbles and reinsert to the same place.

18. Keep the PFA line in for several minutes. Some markers that the fixative is properly getting pumped is if the tail or toes start twitching and straightening.

19. When the body is stiff, take the needle out, and the body should be ready for organ removal. This should require about 15-20mL of PFA.

3.4 Additional: Brain Extraction

20. To get to the brain, first use large dissection scissors to cut off the head. Cut any neck vertebra, muscles and fascia from the base of the skull.

21.Take the head of the mouse and slit the skin from the rear of the head in the caudal to rostral direction, at least to the eyes. Pull up to expose the skull.

22. Using smaller scissors, place the tips at the ear canals and with the blades parallel to the skull, cut the cranial cavity from the ventral part of the skull and jaw. Cut the rear occipital plates at the spinal cord hole to split the midline seam. Insert the pointed tip(s), to the center of the cross formed by the frontal and nasal plates (anterior of the eyes), and open the scissors to crack the whole skull open.

23. Use scissors to pry the skull plates off the brain, starting with the rear.

24. Snip the ventral nerves, and take the brain out. Store in 5mL of 4% PFA in a 15mL Falcon tube.

3.5 Cleanup

25. When completed, discard the mouse in the biohazardous waste.

26. Flush both syringes and tubes thoroughly with water to clear out any residual fixative in the lines.

27. Thoroughly spray down the apparatus and table used for the procedure with non-phosphate detergent or ethanol, taking care to clean all the blood and other biohazards from the surfaces so they continue to be safe to use.