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Iris scissors

Small forceps

0.9% sodium chloride (20-200 ml species, size and technique dependent) Freshly prepared paraformaldehyde (PFA) or 10% buffered formalin (60-200 ml species dependent) 70% alcohol Gauze Anesthetic Chemical fume hood or down draft table Test tube rack Instrument or dissection pan Personal Protective Equipment (PPE); gloves, goggles, lab coat, mask Perfusion pump

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Carefully separate the liver from the diaphragm.

Grasp the xyphoid process and lift up, exposing the diaphragm muscle. Using a combination of blunt dissection and scissor-assisted dissection techniques, open the thoracic cavity by cutting the diaphragm from one lateral aspect to the other lateral aspect while avoiding cutting any visceral organs.

With scissors, cut along one lateral side of the ribs, carefully displacing the lungs, through the rib cage up to the collarbone. Make a similar cut on the contralateral side.

Lifting the sternum away, carefully trim any tissue connecting it to the heart. Clamp the tip of the sternum with the hemostat and place the hemostat over the head. When done properly, the thymus lifts away from the heart along with the sternum, providing a clear view of the major vessels. In mice, the ribcage may be completely removed. This is not recommended with larger rodents due to the larger vasculature.

With forceps grasp the heart gently and lift it slightly out of the chest. Make a small incision to the posterior end (apex) of the left ventricle using iris scissors.

Pass the butterfly needle through the cut ventricle into the ascending aorta. The tip may be visible through the wall of the aorta, and should not reach the aortic arch where the brachial and carotid arteries diverge.

Use a hemostat to clamp the butterfly and heart, this secures the needle and

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Gage, G. J., Kipke, D. R., Shain, W. Whole Animal Perfusion Fixation for Rodents. *J. Vis. Exp.* (65), e3564, doi:10.3791/3564 (2012). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3476408/