**Ferret perfusion-fixation protocol**

**You will need:**

4% PFA  
Heparinized saline  
Plastic tissue containers  
Plastic 1gal waste container  
PE tubing  
2 pumps and controllers  
Formalin absorbing towels  
Blue pads  
PFA instruments (in metal container-keep separate)  
Isoflurane materials  
2 1ml syringes that come with needles  
Beuthanasia  
Bleach

**Preparation:**

1. Make 2L heparinized saline and 2.5L 4% PFA the morning of the perfusion.
2. Separate 500mL PFA for tissue collection.
3. Place a layer of formalin absorbing pads, followed by blue pads on the surgery table.
4. Set up surgery space for isoflurane anesthetization.
   1. Monitor HR and breathing for DLAR anesthesia monitoring forms.
5. Set up 2 vacuum pumps on white table.
6. Feed tubing through pumps. You will need enough length on one end to reach the animal on the table. The other end needs to be able to reach the waste container or the saline/PFA.
7. Test the direction of the pumps by putting the end in some water or saline. One needs to vacuum from the animal and one needs to pump toward the animal.
8. Place a waste container under the table and the flasks of saline and PFA on the table next to the pump. Make sure the waste container is stable.
9. Set up the tubing.
   1. The vacuum tube needs to go from the animal to the waste container. The animal end will be fitted with a cutoff 1ml syringe.
   2. The pump tube needs to go from the saline and PFA flasks to the animal. The animal end will be fitted with a cutoff 1ml syringe and perfusion needle.

**Perfusion-fixation:**

1. Induce animal with iso in chamber, then move to mask.
2. Monitor HR and breathing and record for DLAR.
3. Test impedances and record baseline for 10min.
4. Prep the pump tube by filling it with heparinized saline up to the needle on the animal end. Make sure there are no bubbles.
5. 2 surgeons are needed for the perfusion.
   1. One will position the pump needle in the heart.
   2. One will vacuum saline and PFA out of the animal.
6. Once everything is ready for the perfusion, administer 2ml IP beuthanasia.
7. Begin perfusion immediately after animal stops breathing.
8. Once needle is in position, pump heparinized saline at a slow pace (following surgeon’s instructions) and turn vacuum on to the highest speed.
9. At surgeon’s instructions, switch from saline to PFA:
   1. First, stop the pump while the tube is in the saline.
   2. Then quickly move the tube from the saline flask to the PFA flask.
   3. **Very briefly** turn the pump on in the BACKWARD direction to push any bubbles out of the tube. Avoid bubbles as much as possible.
   4. Switch the pump to the FORWARD direction and begin to pump PFA to the animal.
10. Stop pump and vacuum when perfusion is complete
11. Handle the full tubing carefully to avoid spills.
    1. Empty the tubing by pumping remaining liquid into waste container/leftover PFA
12. Close waste container
13. Soak all instruments and tubing in 10% bleach solution briefly, then clean with soap and water.
    1. Do not let instruments soak overnight!
14. Wipe down pumps and surgery area with 10% bleach solution.