Perfusions: Rebecca General Notes

1. **Supplies**
	1. Isoflurane (under hood; flammable cabinet)
	2. Jar + kimwipes (anesthesia)
	3. Scissors
		1. Large (decap)
		2. Sharp, pointy ones (incision)
	4. Tweezers
		1. Regular flat ones (holding heart)
		2. Grippy ones; have the interlocking sides (for chest opening; brain extraction).
	5. Hemostats (holding skin flap)
	6. Spatula/scoop (for brain extraction)
	7. Pump + tubes; one end of tube in PBS/PFA, other attaches to needle
	8. Needle tips (for pump); attach to end of tube
	9. Conical tubes (PBS/PFA) + holder
	10. Diaper pads (2) + tape
	11. Ice bucket + ice (for PBS/PFA bottles)
	12. Casserole dish
		1. Styrofoam square + 4 needles (holding arms/legs)
	13. Body bag (drawer)
2. **General**
	1. Prep jar + kimwipe + iso
	2. Place mouse inside; wait until they stop breathing
	3. Remove from jar; close it
	4. Pin mouse down on styrofoam board w/ 4 large needles; arms and legs
	5. Hold middle of chest (near sternum) with grippy tweezers
	6. Cut open chest with sharp scissors; initial slice in middle of chest; cut up towards armpits in both directions carefully.
		1. You will see the ribcage; cut the diaphragm gently with the tip of scissors to expose chest cavity.
	7. Use hemostats to clip onto the sternum (white flap) and hold back the flap over head.
		1. 
	8. Use large flat tweezers to hold the heart gently while inserting a large gauge needle into the lower middle/right side of the heart (left ventricle); see below image

 

* + 1. Don’t push too much of the needle inside the heart; can use tape on the needle tip if that helps!



* 1. Use sharp scissors to cut into the right atrium; see “X” above in image.
	2. Begin running the cold PBS via pump; liver should begin to clear over time
	3. STOP pump; move over to PFA tube; START pump
		1. Usually ~25mL
	4. Body will begin to stiffen after a few minutes; the tail/legs may begin to move during fixation.
	5. STOP pump; remove from PFA; START pump to make a little bubble in the line (avoid mixing PBS and PFA)
		1. Run pump with fresh PBS before starting the next perfusion.
	6. **Brain extraction**
		1. Use large scissors to decapitate
		2. Make a midline incision with sharp scissors from neck to eyes; pull skin back in both directions to expose the skull.
		3. Use sharp scissors to remove excess muscle on back of skull
		4. Cut along the backside of the skull from the brainstem up towards the middle of the skull--make sure to apply upward pressure so you don’t cut into the brain; slowly.
		5. Make a cut between the eyes to release front of skull bone
		6. Turn the skull horizontally; use grippy tweezers to pull off the two sides of the skull; again, with outward pressure to avoid damaging tissue.
		7. Once the brain is exposed, use the spatula to gently move the brain into your conical tube filled with PFA; label tube. ****