Brain Removal Protocol

30% Sucrose Solution

- *Sucrose ($C_{12}H_{22}O_{11}$)* *6 g*
- *1x PBS to* *20 mL*

Solutions needed

4% PFA *20 mL*
30% sucrose solution *20 mL*

Materials needed

Absorbent bench liner
Falcon tube
Metal spatula
Metal tweezers (sharp & blunt)
Petri dish
Stereotax (optional)
Surgical microscope (optional)

Note: Only use items marked with "F" when working with fixative. Wear gloves!

Protocol

1. Set up the working area by putting all materials needed within easy reach of the surgical microscope. Turn microscope on to halfway.
   a. If removing the brain without the microscope and stereotax, jump to step 4.
2. Place the head in the stereotax, using the bird's ear holes to suspend the head, and the beak around the metal beak bar. Use a screwdriver to tighten the screws.
3. Adjust the microscrope settings.
4. Break and pull away the first two layers of skull (pia mater and arachnoid mater) with a tweezer so that only the last layer is exposed.
   a. These layers are hard and brittle compared to the soft and shiny third layer, the dura mater. The dura is best left intact so that it can be pulled away in one go at the end.
   b. The arachnoid layer is much thicker at the caudal end of the brain, work carefully here so as not to accidentally injure the delicate floculli.
5. Pull back the muscle layers at the back of the neck and cut the white spinal cord.
6. At some point the head will have to be removed from the stereotax in order to reach all sides of brain. When this happens, move the head to a petri dish to work on it.
7. Work around the sides of the skull, cutting the two optic nerves that go from the base of each eye and meet at the ventral side of the brain.
8. When most of the skull is removed and the optic nerves are cut, gently roll the brain out of the head.
9. Place the brain into a new falcon tube with 20 mL of either:
   a. 30% sucrose solution (for short-term storage, <24 hrs).
   b. 4% PFA (for long-term storage, >24 hrs).
10. Store in the fridge at 4°C.