Amamoto Lab, 2022

Protocol: Retina Cryosectioning

Sample Preparation

1. Place the dissected retina in 4% PFA (See Reagents) and incubate for 20 minutes at RT on an orbital shaker. If the tissue is for SABER FISH, use a fresh PFA ampule each time.
2. Wash 2x with PBS.
    a. Note: If you only want to section the electroporated region, you can go under a fluorescent dissection scope and cut out the region of interest by making a flatmount and using a razor blade.
3. Using forceps, remove the lens from the retina.
4. Incubate the fixed retina in 30% sucrose at RT on an orbital shaker until the tissue sinks (~30 minutes).
5. Prepare an ethanol-dry ice slurry in a plastic container (i.e. top of a pipette tip box), making sure that the ethanol is cold.
6. Prepare a small cryomold (Tissue Tek, cat. #25608-922) with a label.
    a. Notes: Make sure to use a VWR pen, not a Sharpie to label the cryomold. If the dorsal-ventral orientation is important, and if it's indicated by a cut on the retina, label the cryomold with the orientation.
7. Transfer the tissue into the cryomold using a transfer pipette. Use a P200 pipette to remove residual liquid.
8. Fill the cryomold with OCT/Sucrose Mix with a P1000 to the top until concave.
9. Use forceps to move the OCT/Sucrose so that the tissue is centered in the mold. NEVER touch the retina itself with the forceps as it will damage the tissue.
10. Slowly place the cryomold in the slurry so that it floats.
    a. Note: This technique takes practice. Make sure to drop the cryomold flat onto the surface so that ethanol does not get into the cryomold.
11. Wait until the OCT turns white.
12. The frozen tissue in OCT can be stored at -80C indefinitely.

Cryosectioning

13. Make sure the temperature inside the cryostat is set at -20C for both the chamber and the specimen.
14. Place the frozen tissue, brushes, chucks, and anti-roll plate in the chamber and wait at least 15 minutes.
15. If the orientation is marked on the cryomold, use a marker to label the same side on the OCT.
16. Remove the tissue/OCT block from the cryomold and place on top of chuck with ~3 mm of OCT. Wait until the OCT freezes.
17. During the wait, prepare the slides. For a typical retina, prepare 10 slides, numbered 1 through 10. Make sure to label with the Sample number, Age of animal, Date, and the Manipulation.
18. Place the chuck onto the holder and tighten.
19. Adjust the angles so that the blade cuts at the desired angle.
20. Use the trim setting (100 um) to reach the tissue, which may be slightly hard to see. Before you get to the tissue, make sure that the anti-roll plate is positioned correctly.
21. Section the tissue at 30 um.
    a. Note: If the tissue is ripping, it is likely because of the position of the anti-roll plate. The plate should line up slightly in front of the blade. Other things to note are the temperature, fixation, and blade sharpness.
22. The section should lay flat either on the metal stage or the anti-roll plate. Either is ok. Place the (+) side of the Superfrost Plus slide onto the section. You should not need to pick up the section at any time. The OCT will dissolve onto the slide.
    a. Note: Make sure to use a Superfrost Plus (or equivalent). If it's not plus charged, the sections will come off easily during subsequent steps.

23. For the next section, use the next slide (Slide #2). This is called Serial Sectioning, which allows you to have a sampling of different retinal regions on each slide.
24. If there is any tissue or OCT on the blade, brush it away using a brush, making sure to brush away from you (so that you don't cut the brush).
25. After sectioning, the slides can be stored at RT for a couple of hours. For long-term storage, place them at -80C. Make sure to keep a storage log of the sections.
26. Clean the cryostat by throwing away the bits of OCT.

Reagents

4% PFA

10 mL 16% PFA Ampule (Thermo, cat. #28908)
30 mL PBS

Store at 4°C for up to 1 month

30% Sucrose

12g Sucrose
PBS up to 40 mL (Sucrose will take up volume as well)

Store at 4°C until contaminated

OCT/Sucrose Mix (50%/15%)

20 mL OCT (Tissue Tek, cat. #25608-930)
20 mL 30% Sucrose

Store at RT