Brain Sectioning Protocol

Solutions needed

1x PBS	*400 mL*
0.1% PBS azide	*400 mL*
95% Ethanol	*as needed*
Dry ice	*2-3 lbs*
O.C.T. compound	*as needed*

Materials needed

Coffee grinder (optional)
Foam cup
Glass petri dish
Metal spatula
Microtome
Microtome blades
Paintbrush
Paper towel
Red plastic tub & lid
Spoon
Well trays

Protocol

Workspace setup

1. Bring the red plastic tub and lid to chem stores to pick up dry ice.
    a. Chem stores is open from 9-11:30am and 12:30-4pm Monday through Friday.
2. Set up the working area by putting all materials within easy reach of the microtome.
3. Fill the petri dish to about halfway with 1x PBS.
4. Label the well trays then fill to about halfway with 0.1% PBS azide (1x PBS may be used instead if the sections will be mounted in less than 24 hours).
    a. Labels should include bird I.D., tray #, solution, date, and initials.
5. Assemble the blade holder, comprised of a large bottom slab, smaller top slab, small cylindrical piece, metal rod, and two tiny springs. Slide it into place in the microtome and secure with two hand screws.
    a. Adjust the blade holder so the screws line up with the notch marks.
6. Remove a microtome blade from the blade dispenser and slide it between the two slabs of the blade holder. Line it up with the edges and use a paintbrush to push it all the way to the back. Tighten the thumbscrew to secure.
    a. DO NOT touch the front of the blade; it is extremely sharp and will cut fingers.
7. If needed, use a razorblade to cut thin slices of gelatin off of the gel-blocked brain so that the brain is level to a flat surface. Refer to the brain orientation photos in the Gel-Blocking Protocol.

Sectioning

1. Gently grind/crush dry ice with a coffee grinder or manually between paper towel.
2. Add O.C.T. compound to the mictrotome stage, starting from the middle and working outwards in a circular motion, to create a round glue blob.
3. Spoon a small amount of crushed dry ice into the well of the microtome.

4. Watch the glue and wait for it to begin to freeze. As soon as the edges begin to turn white, add more O.C.T. compound and stick the gel-block into it.
    a. Adjust the block quickly if it isn't level and (optional) add some glue to the block edges to make sure it's secure.
5. Spoon more crushed ice into the microtome well and add 95% ethanol using a squirt bottle to distribute the ice more evenly around the stage.
6. Cover the microtome well with a foam cup to speed up the gel-block freezing process. Wait until the gel-block is completely frozen. To check if it is frozen, tap it with the end of a paintbrush to see if it makes a hard sound.
7. Use the crank to adjust the stage height so that the blade is just above the gel-block and won't cut into it.
8. Move the blade forward and back to make sections.
9. As soon as the blade starts cutting into the gelatin, sweep each section off using a paintbrush and discard into the petri dish.
10. Tips for making smooth sections:
    a. Cut through the gel-block with a constant speed, smooth and slow.
    b. Dip the paintbrush into the petri dish to wet the blade with 1x PBS before making each cut.
    c. Adjust the temperature of the gel-block by adding more dry ice to the well or using a fingertip to warm the block before making a cut.
11. Begin collecting sections as necessary, adding the first section to tray 1 and the second section to tray 2. Continue back and forth between the trays to create two almost identical well trays
12. When all wells are full, restart from the top. Each well may have as many as 4 sections in it by the end of sectioning.

Cleanup

1. Let the dry ice in the microtome well evaporate. When the gel-block has warmed up enough, use a metal spatula brain to pop it completely off the stage, along with the O.C.T. compound blob.
2. Carefully remove the blade and throw in sharps disposal. Take apart all components of the blade holder and lay them on paper towel.
3. Wash all glassware and tools. Store well trays in the fridge.