Brain Cryosectioning Protocol

Solutions needed

95% Ethanol	*as needed*
OCT compound	*as needed*
1x PBS	*as needed*

Materials needed

Cryostat
Microtome blades
Paintbrushes
Metal forceps
Kimwipes
Paper towel
SuperFrost Plus slides
SuperFrost marker OR pencil
-80°C freezer-safe slide boxes
Razor blade

Protocol

1. Set up the working area by putting all materials within easy reach of the cryostat.
2. Ensure that the chamber and operating table are set at (and maintaining) a temperature of -20°C.
3. Before sectioning, equilibrate the tissue blocks at -20°C for at least 1 HR in the cryostat.
4. Ensure all tools to be used throughout the sectioning process to manipulate sections prior to mounting (i.e.: paintbrushes, and metal forceps) are placed inside the cryostat chamber to equilibrate them at -20°C. Keep at least one fine paintbrush outside the cryostat so that it can be used to adjust mounted sections.
5. Pour 1x PBS into a glass beaker/petri dish. Place at least one fine paintbrush in the receptacle. Keep at room temperature as these will be used for adjusting mounted tissue sections.
6. Mount the tissue block onto a cryostat chuck.
    a. Wipe down both sides of the heat press using kimwipe and a small amount of 95% Ethanol
    b. Add a small (approx. pea-sized) amount of OCT to one of the cryostat tissue mounting chucks, then quickly flatten the surface using the heat press.
    c. Add another pea-sized drop of OCT and quickly place the tissue block onto the chuck and level it before the OCT freezes. Be sure to place the unmarked, flat surface of the tissue block face down. If necessary, add a bit more OCT to fill in any gaps between the chuck and the tissue block.
    d. Wait a few minutes to allow the OCT to turn white, indicating that it is frozen.
7. Attach the chuck to the specimen holder and so that the marked (dorsal) corner of the tissue block is oriented upwards (refer to *OCT-embedding-and-flash-freezing-protocol* for brain positioning). Tighten the clamping screw to secure the chuck in place.
8. Remove a microtome blade from the blade dispenser and slide it between the two slabs of the blade holder. Tighten the lever on the right to secure.
    a. DO NOT touch the front of the blade; it is extremely sharp and will cut fingers.
9. Label each slide – using a SuperFrost marker or pencil – with bird ID, experiment code, date, slide series-slide number (i.e.: slide one of series two would be written “S2-1), and your initials.
10. Keep -80°C freezer-safe slide boxes nearby, but outside cryostat chamber so that once slides are full, they can air dry at room temperature while remaining sectioning is being completed.

11. To help reduce unwanted static, a small opened bottle of 95% ethanol can be placed inside of the cryostat chamber (out of range of any moving components).
12. Use the buttons with arrows on the left control panel to adjust the chuck distance so that the blade is just in from of the tissue block but won't cut into it.
13. Section the tissue block.
    a. Use a large paintbrush to clean off the operating table surface. DO NOT use brushstrokes towards the sharp edge of the blade as this will nick the blade surface!
    b. Position the blade guard onto the operating table, over the blade.
    c. Cut one section by turning the crank on the outside of the cryostat away from you for one full turn. Tip for smooth sections: fully turn the crank using a uniform and quick motion.
    d. As soon as the blade starts cutting into the OCT, sweep unwanted sections off the operating table using a paintbrush.
14. Once the cerebellar folia start to appear in the tissue sections, mount the sections on SuperFrost Plus slides.
    a. Carefully raise the blade guard and use fine paintbrushes to manipulate the tissue section.
    b. Use the brushes to prevent the section from curling and from sticking to the guard.
    c. Flip the section over (curled side down) using metal forceps and/or small paintbrushes.
    d. Flatten the section as much as possible using the small paintbrushes.
    e. Grab a labelled, room temperature SuperFrost Plus slide. Position the slide with the mounting surface face down above the section and slowly lower the slide towards the section until the section is drawn towards the slide and adheres. DO NOT leave the slide close to the operating table surface for too long, and DO NOT touch the slide to the operating table surface. This will cause sections to freeze to the operating table surface!
    f. Remove slide from cryostat and using a small amount of 1X PBS remove any bubbles or folds in the tissue with the side of a fine paintbrush. <u>Avoid touching the tissue directly during this process as this can cause damage.</u>
    g. Carefully remove any excess 1X PBS from the slide using a kimwipe.
15. Repeat steps 13 and 14 until all desired tissue is sectioned.
    a. Cycle through one slide per series and mounting one section per slide (i.e.: if sectioning into 4 series then mount the first section on S1-1, then S2-1, then S3-1, and finally S4-1. Then start the process over until those slides are full before moving on to slide 2 for each series).
    b. Ensure sections are evenly spaced leaving ~1/2cm space between one another and from the edges. Each slide can fit up to 8 sections if the sections are small.
16. Once a slide is full, place it into one of the slide boxes to air dry at room temperature.
17. Air dry the slides at room temperature for the duration of cryosectioning and overnight at -80°C. If all the slides are not used immediately, store them at -80°C for up to 3 MONTHS.

Cleanup

1. Carefully remove the blade and throw in sharps disposal.
2. Remove to chuck from the specimen holder. Use a razor blade to carefully remove any remaining tissue block and OCT from the chuck. Run the chuck under warm water and wipe dry with a paper towel to remove any remaining OCT.
3. Brush off any remaining sections or debris from the operating table.
4. Remove the debris catching tray from below the specimen holder. Dispose of the contents in the trash.