General Frozen Sectioning Protocol

Epredia HM525 NX Cryostat

MATERIALS NEEDED:

- ✓ **Treated microscope slides *(Silane, Poly-L-lysine)* or positive charge *("Plus")* slides.**
- ✓ **Slide box** to hold slides with sections
- ✓ **OCT freezing compound** (or similar compound-provided by core user)
- ✓ **Artist paint brushes**. The lab has some available to use, but you may want to purchase your own.

Cryostat Set-Up

1. Keep all paint brushes, microtome blades and other tools in the **cryostat**. Razor blades may be kept at room temperature.
2. **Specimen holders (chucks) MUST be at room temperature before use.**
3. Place frozen blocks in cryostat for about **15 minutes** (or longer) prior to sectioning so that they equilibrate to the sectioning temperature.
4. Check that the sectioning **thickness** is set as desired and at the **temperature** is appropriate for your tissue/thickness of sections using the touchscreen. (If you need to use temperatures **lower than -24°C**, email ahead of your session so that I can set the temp in advance.)
5. Put a small amount OCT freezing compound (or similar) on the specimen holder at **ROOM TEMPERATURE.** Orient your frozen specimen as desired in the OCT and place in one of the four fast freezing stations in the cryostat. Press **"Cryobar"** to quickly cool the station.
6. **ALWAYS lock microtome handle when you are not sectioning.**
7. **The blade is VERY SHARP. Avoid touching it with your fingers.**
8. **Do NOT loosen the black lever on the left side of the blade holder.** This is to adjust the angle of the blade, which is set at **10°.** This is the best angle for the blade that is being used.

Sectioning Set-Up

1. Place specimen holder with attached specimen in the hole on the microtome and tighten knob. Adjust orientation of specimen by loosening the specimen head black clamping lever, adjusting, and retightening the lever.
2. Use the "**coarse retraction"** button on the touchscreen to move specimen all the way back. The bottom number in the center of the screen should read **28mm.** The shorter the arm is, the more stable it is.
3. Loosen blade holder with the black lever on the right side. Adjust blade holder position so that it is close to specimen, but not touching it. Tighten lever.
4. To advance the specimen to the blade, push the **"Trimming Thickness"** button on the touchscreen (usually set at 30um). This will allow you to quickly advance to your sample.
5. Unlock handle and turn it **clockwise** to advance the specimen. **ALWAYS TURN SLOWLY ON THE FIRST ROTATION!** This will make sure that you don't cut a chunk off your specimen if the blade is too close.
6. Continue sectioning on "**Trimming Thickness"** until you reach the desired area of your specimen.

7. Press the "**Fine Thickness"** button to switch back to the thickness you chose for your sections.
8. Typically, you will need to cut a few sections before you will be cutting the selected thickness.

Sectioning & Thaw Mounting Tissue

1. **Make sure there is no frost or OCT on blade.** It can be carefully cleaned with a paintbrush using **UPWARD** strokes.
2. Gently place the anti-roll plate against the blade and turn handle **clockwise** in a slow, smooth motion.
3. The section should go between the anti-roll plate and the blade. If it doesn't, adjust the position of the anti-roll plate with the knurled knob at the bottom of the anti-roll plate holder. Adjust in **small increments** (either up or down) to get the section to go between the plate and the blade.
4. Once section is on the blade, **gently** pull back the anti-roll plate. If section curls up, try leaving the anti-roll plate down for a few seconds before lifting it to pick up section. You can also use a paintbrush to gently uncurl or hold open a section.
5. **Quickly position slide (frosted slide toward blade)** close enough to allow section to melt onto it. Hold the slide steady or you will have wrinkles in the tissue.
6. If you are mounting multiple sections on one slide, start at the frosted end of the slide and work your way down, putting one section on at a time.
7. If a section is messed up, just wipe it off and add another.
8. **You will need to clean the blade with a paintbrush after each section.**
9. Fixed tissue slides may be kept **briefly** on the top of the cryostat. Unfixed tissue slides should be put into a small slide box inside the cryostat. All slides should be stored **between -20 and -80°C.**

Finishing and Cleaning

1. Lock handle and use the "**coarse retraction"** button to move specimen all the way back.
2. Remove specimen holder from microtome and set into one of the freezing stations. Add a **small amount** of OCT to the top of the block and place the metal heat sink on top. This will quickly freeze the OCT on top to protect the cut surface of the block. Remove heat sink.
3. Remove specimen holder from cryostat and put in one of the small jars on top of the cryostat. Allow to thaw slightly – about **1 min.** Pop off block from specimen holder and place in cryostat to re-cool.
4. Wrap block in Parafilm either by itself or in the embedding mold. If wrapped by itself, add a label wrapped up with the block. Store blocks at **-80°C.**
5. Using a large artist paintbrush, brush debris from around base of the microtome into the collecting bins. Remove collecting bins to empty. If there are small pieces of tissue or OCT in the cryostat, wet a Kimwipe with **95 or 100% ethanol** and wipe them up.