Frozen sectioning of fresh brain samples for laser-capture microdissection Entered by Matt Sutcliffe
Janes Lab Protocols 6/13/18

1. Transport embedded samples on dry ice to the cryomicrotome
2. Place sample in the cryomicrotome box and equilibrate the box temperature to –24°C
   - *Higher box temperatures (e.g., –20°C) are acceptable but can cause distortion of the tissue during sectioning*
   - *Slide rack must be inside the cryomicrotome box to store slides after sectioning*
   - *Keep the slides at room temperature*
3. Replace microtome blade (if disposable)
4. Wipe microtome blade and anti-roll bar with a Kimwipe moistened with both ethanol and RNAse Away
   - *RNAse Away by itself will freeze to the blade*
   - *Be careful not to cut yourself when cleaning the blade*
5. Pop the embedded sample out of the cryomold, mount on the sectioning platform with Neg-50, and freeze until the sample has solidified on the sectioning platform
6. Mount the sectioning platform on the cryomicrotome and cut trim sections until the tissue is visible on the cutting surface
   - *Tissue will appear as light pink against the white Neg-50*
7. Cut 8 μm sections, adjusting the anti-roll bar to prevent the section from accordioning. Use a brush to start the section, lay down the anti-roll plate, and continue sectioning slowly and evenly through the tissue with the vacutome on sectioning mode at 100%
   - *Do not graze sections (subsequent section will be 16 μm instead of 8 μm)*
   - *Alternatively, one can omit the anti-roll bar and use the brush technique*
   - *8 μm sections are roughly one-cell thick to allow for the maximum recovery of biological material after microdissection*
8. Wick sections onto Superfrost plus slides (VWR #48311-703) and move the slide immediately to the slide rack inside the cryomicrotome box
   - *Place the slide inside the cryomicrotome box for ~10 seconds before wicking the section*
   - *The section should just barely wet onto the slide with intermittent warming from a finger (overwetting will cause fluorescence to diffuse away from the labeled cells)*
   - *Do not dry the slide at room temperature (this will ruin the RNA integrity in the tissue)*
   - *To wick a second section per slide, warm the back of the slide with your finger for a few seconds before wicking (do not warm the part of the slide that contains the existing section)*
9. Continue cutting and wicking sections until good cuts are no longer possible
   - *One embedded sample typically yields 30-60 slides with two sections per slide*
   - *Do not return a half-sectioned sample to –80°C (the exposed sample will dehydrate and become damaged)*
10. Move the slide box containing the frozen sections to dry ice and store at –80°C for up to one month