Frozen sectioning of 3D structures for laser-capture microdissection Entered by Kevin Janes
Janes Lab Protocols 8/29/07

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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 acini at the periphery of the Matrigel 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. Spray 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 ~3 mm of Matrigel is visible on the cutting surface
   - *Matrigel will appear as light pink against the white Neg-50; test slides can be prepared and checked under an inverted microscope to confirm that acini are on the slides*
7. Cut 8 μm sections, adjusting the anti-roll bar to prevent the section from accordioning
   - *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
   - *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