Sectioning

Potter Group, GUDMAP Consortium

1. **Throughout this procedure be very careful not to cut yourself on the sharp blades used in the cryostat.**

2. **Wear gloves throughout to reduce RNase contamination.**

3. Use the cryostat in Prasad's lab. Place mold in the chamber for a few minutes to temperature equilibrate. Remove tinfoil. Place chuck that has been at room temp in chamber and let cool a minute, but not too much. Place OCT on chuck and let cool a minute, but not freeze, and then place tissue OCT block on chuck, and let freeze in position. Can place additional OCT around the base and spread with gloved finger to help hold in place.

4. The tissue is too brittle to section properly if the chamber is too cold. I typically use a setting for the chamber and arm of –12 to –14. We have been using –14 mostly lately, with success.

5. Use the trim setting of 40-60 to remove most of excess OCT, til see tissue. When getting close to tissue can drop back from trim to regular setting, of 9 microns for Veritas, with UV cutting, or 7 microns for old Pixcell II machine. Use a regular glass slide to check for presence of tissue in sections. When you hit a good chunk of tissue start saving on the membrane slides (see below).

6. Membrane slides are prepared as follows to allow good sticking of the sections to the slides. Dilute the Sigma poly-lysine solution one to ten, as recommended by Sigma. Dip slides and dry in vertical position.

7. Need to carve the OCT block with a razor blade, so the tissue sections are not too large. **Be very, very careful here not to cut yourself on the blade in the cryostat, which is very sharp.**

8. Collect sections on the slides. Do not want the tissue sections to be arranged so that they end up under the strut supports of the caps, as this would give cap crap. So the sections should be spaced so that the cap can rest with one tissue section centered, and with no other tissue section under the edges. So space them out, and try to get 5-10 sections per membrane slide. Try to work fairly fast,

as the RNA can go bad sitting in one section at room temp while other sections are being cut on the slide.

9. Try to get the best sections possible. Sometimes having the cut part go more slowly helps. Sometimes warming a degree or two helps. Sometimes changing the way you catch the section with the brushes as it comes off of the blade helps.

10. Place the slides with sections in box with dry ice against the slide, and then store at –80ºC.