RHTIC Guidelines For Preparing and Submitting Frozen Samples for Cryosectioning

1. Select a **proper size cryomold** and label it with permanent marker. Choose a mold larger than your samples. Having enough OCT around the tissue allows for better sample attachment to the chuck and makes sectioning easier. If your sample is sticking out of the mold, the Core staff won't be able to section it.

2. If your sample is in PBS or some other liquid, try to blot away as much of the liquid as possible before placing tissue in OCT. Equilibrate your sample to OCT at room temperature for 30 seconds in a small container (i.e. 50ml tube cap) before moving it to the molds*****.

3. Add a few drops of OCT to the mold, transfer your sample and orient it correctly in the mold******. The side of the sample facing the bottom of the cryomold is the side that will get sectioned first. Tissue should be placed in the center of the mold. If embedding several pieces of tissue in the same block, place them as close to the center as possible.

4. If needed, carefully add more OCT to completely cover the specimen. Avoid bubbles.

5. Using long forceps, place the mold on dry ice/vapors of liquid nitrogen/dry ice-isopentane slurry*** and allow OCT to freeze completely. It should take 30 sec-1 min for OCT to harden and become white.

6. **Wrap each mold in aluminum foil to prevent samples from drying**, and write the sample ID on the foil using permanent marker. Place the block in a labeled zip lock bag. Store samples at -80C.

7. Bring samples to RHTIC on dry ice together with the printed iLabs-generated order form.

***** Lungs needs to be perfused with a 1:1 mixture of OCT and PBS to remove air before freezing to ensure good section quality and correct tissue morphology.

****** When embedding cells, try to transfer the smallest amount of PBS possible with the cell pellet to prevent ice formation. Place cell pellet in the center of a mold in a small amount of OCT and allow it to slightly freeze so the pellet doesn't float. Add remaining OCT to fill the mold BEFORE it has completely frozen to prevent a fracture line. Cells can also be placed in agarose after fixing them and them frozen in OCT.

*** Some samples might require fixation before freezing in OCT. It is preferred that fresh samples are frozen in liquid nitrogen vapors. Samples that are pre-fixed should be incubated in 15% sucrose in PBS, and then in 30% sucrose in PBS until they sink in 30% sucrose. Sucrose

helps to displace water and to prevent ice crystals formation inside the sample, which results in a better tissue morphology. Pre-fixed samples can be frozen on dry ice.