**Cryosectioning Protocol**

**Required Materials:**

* Cryostat molds
  + Use a sharpie to label on the edge of mold BEFORE you attempt to embed tissue
* Aluminum foil strips (used to wrap and insulate cryoblock once made)
* Razor blade
* Forceps
* Weigh boat for heart tissue
* OCT compound
* Styrofoam container with dry ice
* Liquid nitrogen cooled Isopentane

**Procedure:**

* Gather required materials and label cryomolds with appropriate hashcodes
* Obtain the appropriate sample(s) from the cardiac biobank
  + Place the samples on the dry ice
* Remove the heart sample from the cryogenic vial and place it on the weight dish.
  + Have the corresponding cryomold ready, so that you do not mix up which sample should go in which mold.
* Carefully use the forceps and razor blade to cut a piece of tissue that will fit inside the cryomold. Keep this piece of tissue on dry ice until ready to place into mold.
  + Preferably a piece that will not touch any of the edges of the mold.
  + Smaller pieces of tissue freeze better (i.e., quicker and more evenly) which minimizes freezing artifacts
* Put unused tissue back into the vial and place the vial on the dry ice.
* Place one small dot of OCT compound in the center of the mold, then place the tissue in the mold and fill the rest of the mold with the OCT compound.
* Place the mold into the liquid nitrogen cooled isopentane for 15 secs
  + Be careful to ensure that the mold lays evenly so that the OCT compound does not fall out.
* Place molds into -80°C freezer for 30 mins.
* Move molds to cryostat at -20°C for 30 mins prior to cutting.
* Once tissue has equilibrated for 30 mins in cryostat, take one of the chucks, and apply a nickel size dot on the chuck and place the prepared sample on it, and allow that to freeze completely.
  + If the tissue has been sectioned in cryostat previously, flip the block over and cut from the side that has not been sectioned yet.
  + If both sides have been sectioned, try to determine which side was most recently cut and use the other.
  + Notes:
    - The dot will freeze quickly, so you need to have the sample already taken out of the mold, and ready to place on the platform
    - Ideally position the sample block level on the platform such that the base of the sample is parallel to the platform surface.
    - Keep the mold so that you can place the unused sample back with it later, otherwise it is hard to know the identity of the tissue.
    - It is not wise to have more than one sample outside of the molds at a time, because they will become easy to switch up.
* While you are waiting for the sample to freeze to platform note if you need a new blade. You do not need a new blade every time you use the cryostat but if you are getting tears in the tissue, it may be due to an old, chipped blade.
  + There is a lever to the left or right of the blade that can be moved to loosen the blade.
  + Use forceps/magnet to remove the old blade and add in the new blade.
  + Retighten the blade using the lever described previously
* Once completely frozen, insert the platform and adjust as necessary
* To begin, you will need to move the block toward the blade.
  + There is a “move forward” button that you can use so that you do not need to crank it all the way to the blade, but be careful, because if you go too far you can cut right into the middle of the sample and ruin it, or it will come off the block all together, and might be lost.
* Once you begin to see complete sections, you can attempt to put them on the slide. See **Troubleshooting Guide** below if you are getting poor quality sections.
  + Notes:
    - You will need to put down the glass roll protector to see if you are getting a complete section
    - You should only have one slice on the platform when trying to put the slice on the slide.
* Place the section on a pre-labeled glass microscope slide using the method below:
  + You want to hover the slide without actually touching it to the platform
    - The sample section should rise up to adhere to the slide. It may be helpful to flip the section so that the section buckles upwards or at least is in no way stuck to the cryostat.
  + DO NOT keep the slides in the cryostat, because it they get too cold the section will not adhere to the slide.
* Repeat for all of the required sections then allow slides to air dry and equilibrate to room temp for 30 mins in the fume hood.

**Troubleshooting Guide:**

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| **Problem** | **Possible Cause** | **Solution** |
| Jagged cuts/tears in sections | Blade is chipped or dull | Change out blade |
| Air pockets in OCT block | Remove block from stage and recover top of block with a thin layer of OCT. Let freeze then try again. Repeat this a couple times if it doesn’t work on the first attempt. |
| Tissue/blade is too cold | Allow time for tissue to equilibrate to cryostat temp of 20-21°C or lower cryostat temp to 20-21°C if it is colder. |
| Sections rolling up too fast | Cutting too quickly | Use slow, controlled, smooth motion to section tissue |
| Blade is “jumping” over tissue when sectioning | Tissue/blade is too cold.  Cutting too quickly; cutting too slowly. | Check temperature of cryostat. Do not forget the tissue needs to equilibrate to -20°C after storage in -80°C freezer.  If you’re cutting fast, slow down and maintain a constant, controlled speed once the blade contacts tissue. If you’re cutting slowly, speed up some but keep it steady. |