Frozen Tissue Preparation Protocol

I. Purpose or Principle:

This protocol explains how to properly freeze tissue into OCT freezing medium for subsequent frozen sectioning.

II. Specimen:

Tissue morphology is best when fixed slightly before freezing. 4% PFA for a couple hours is sufficient, followed by immersion in 30% sucrose until the tissue sinks. Tissue should be sized appropriately, both thickness and "face"/footprint, for the freezing mold you intend to use.

III. Reagents (or Media), Standards, Supplies and Equipment:

- Dry ice (and appropriate PPE to handle)
- Metal block, preferably aluminum (PCR machine block works well)
- Freezing molds/trays
- 100% ethanol
- O.C.T. Freezing media
- Styrofoam container

IV. Procedure:

1. Pre-chill the metal block in a -80 freezer. You can keep this in the freezer if you have a spare block available.
2. Remove the block from the freezer and place in the center of the Styrofoam box.
3. Surround the block with dry ice, leaving the top surface open.
4. Squirt enough 100% EtOH to cover the top surface of the metal block.
5. Let cool for a couple minutes.
6. Remove tissue from 30% sucrose and trim, if needed, to fit into mold, leaving a good, outer boundary at the edges.
7. Fill the mold/tray with OCT, making sure not introduce bubbles. If bubbles are present, let sit for a few minutes to let them rise.
8. Slowly place the tissue into the OCT in the mold, being careful to not trap bubbles. Going in at an angle and gently forcing the tissue down to the bottom face of the mold works best.
9. Once tissue is in the proper orientation for sectioning (the bottom face of the mold is considered the plane of sectioning and is the first to be sectioned), place the mold/tray onto the metal block.
10. The OCT should quickly start to freeze. Once frozen, wipe off the excess ethanol from the bottom of the mold/tray and place in plastic bag for storage or transfer to the cryostat for sectioning. Excess ethanol can leach into the OCT, causing sectioning difficulty, or remove any mold labels if they are not solvent resistant.

V. Notes:

Center the tissue as much as possible in the mold to leave a good outer boundary of OCT. Tissue that is not fully surrounded by OCT at the edges is susceptible to tearing and/or rolling when sectioned.

Liquid nitrogen can be used to cool the metal block instead of dry ice, but the subsequent "fog" of the nitrogen usually makes it difficult to observe what you are doing. Liquid nitrogen containers will not usually accommodate something the size of the metal block used. **Liquid nitrogen should NEVER come into direct contact with the tissue or the OCT.** Doing so can create bubbles and/or cracks within the OCT or even the tissue, causing sometimes irreversible damage to the tissue block.

The "heat transfer" fluid, in this case ethanol but sometimes isopentane, should also never come into contact with the tissue or OCT. If this happens, it can leach into the tissue, making it too soft and sectioning impossible.

VI. Safety:

- Gloves and eye protection should be worn when working with dry ice.

VII. Author and/or Revised By:

- Toshi Kinoshita

Original Date: 1/5/22
Current Version Date: 1/5/22