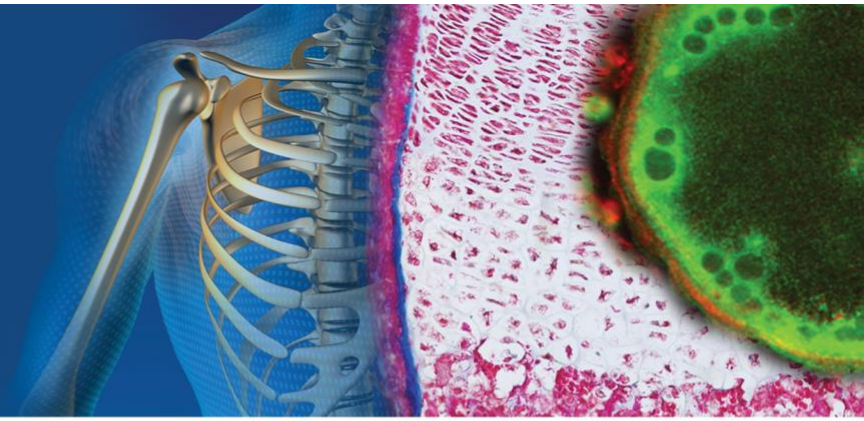
Embedding Frozen Tissue Specimens

In the HBMI Core, we snap freeze our tissue when preparing frozen blocks using a method similar to that described by Dyment, *et. al*. (Dyment, N. A., *J. Vis. Exp.* (115), e54468, doi:10.3791/54468 (2016)). Snap freezing refers to the ultra-low temperature freezing method used to prepare high-quality cryosections. Ice-crystals that form during a slow freezing process can cause distortion in tissue morphology and can lead to more difficult sectioning. If the specimen is cooled rapidly, the crystals that form are much smaller and fewer which leads to much better morphology. Dry ice (-80°C) can cool a standard sized specimen submersed in frozen embedding medium (Shandon Cryomatrix or O.C.T.) within 3 minutes, but is still not cold enough to eliminate crystal formation. The method below uses a super-cooled bath of 2-methylbutane (-150°C) that can freeze a standard specimen within 1 minute, thus, greatly reducing crystal formation.

Materials/Reagents:

- Ice bucket with lid
- Stainless steel bucket
- Dry ice
- 2-methylbutane (CAS# 78-78-4)
- Plastic staining rack with long handle
- Shandon Cryomatrix (Thermo Fisher, cat# 6769006)
- Plastic cryomolds (size dependent on tissue type)
- A marking pen to identify specimen on Cryomold



Procedure:

1. Place the stainless steel bucket inside the ice bucket and surround with dry ice. Fill the stainless steel bucket with 2-methylbutane, approximately 4cm deep, and allow to cool with the lid on for 10 minutes. *Note: this must be done in a fume hood!*

2. Create a thin layer of Cryomatrix in the bottom of a plastic cryomold and orient the tissue specimen in such a way that the cutting plane is parallel to the bottom of the mold. Place the mold on top of a flat piece of dry ice to fix the position of the specimen as the Cryomatrix begins to freeze. Once the specimen is frozen in place (approx. 30 seconds), fill the remainder of the mold with enough Cryomatrix to sufficiently cover the entire specimen. *Note: the tissue must have been fixed, decalcified (optional), and sunk into 30% sucrose/PBS for 24 hrs. prior to embedding. See protocol on Preparation of Frozen Tissue Specimens. If specimens are not to be sectioned within one month, it is recommended that they be stored in 15ml conical tubes filled with 30% sucrose/PBS at -80℃ for long term storage.*

3. Using the plastic staining rack, place the cryomold in the pre-chilled 2-methylbutane and allow to freeze completely (approx. 2-3 minutes; the Cryomatrix will become opaque when frozen).

4. Remove the cryomolds from the 2-methylbutane and shake off excess liquid. Section immediately or store at -80°C wrapped in cellophane or plastic wrap. *Note: embedded specimens can dry out over time when stored this way. It is not recommended they be stored after embedding for more than one month.*

5. When finished with embedding, allow all materials to warm to room temperature in the fume hood (or store dry ice at -80°C for future use). Return all borrowed materials to the Histology Core.