SOP: OCT embedding with liquid nitrogen (human placenta & mouse)
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Reagents:
- Tissue-Tek Optimal cutting temperature media (OCT) (Sakura 4583)
- Paraformaldehyde (PFA)
- Liquid nitrogen (LN_2)
- Cryomolds (Thermofisher, # varies with size)
- Cryochamber

Procedure:
1. Label cryomolds with pertinent information (*indelible markers do not work well on cold materials*)

2. Prepare cryo chamber, typically a hard foam, leak-proof container (**not Styrofoam**), with absorbent cloth inside (available through HVTN, commonly used for retrieving LN_2 samples)

3. Fill chamber with at least 1kg LN_2
   3.1. 2.5kg will keep chamber cold for ~1h

4. Place aluminum block in chamber and allow to equilibrate for at least 5’
   4.1. **DO NOT** fully submerge aluminum block (tissues are frozen in vapor phase of LN_2)
   4.2. If not using aluminum block, prepare plastic float (pipette box lids work well)

5. Prepare fixed or unfixed tissues as desired (preference is to embed on edge, i.e. cut-side facing down onto the bottom of the cryomold)

6. Gently dispense OCT media into cryomold, avoiding formation of bubbles in mold
   6.1. Bubbles can lead to problematic sectioning on cryostat
   6.2. Bubbles can be gently removed using a pipette

7. Carefully place cryomold onto plastic float or top of aluminum block, ensuring molds are not submerged in LN_2
   7.1. If submerged in LN_2, freezing occurs far too rapidly, causing tissues to crack when sectioned
   7.2. Allow to freeze until OCT media transitions from translucent to an opaque white

8. Transfer frozen sections into cardboard box and store at -80°C long term

NOTES: The aluminum block method is suggested to provide more even cooling than floating, better preserving tissue architecture and preventing freezing artifacts. This works well with large tissues and requires little prep time.