EMBEDDING & SECTIONING TISSUE

FIXATION

1) When possible cardiac perfuse with 4% PFA (~7.4 pH, 310mOsm) in PBS, heparinized saline may be used prior to PFA to increase perfusion efficiency
2) Post fix samples for 2-4 h in 4% PFA
3) Remove excess PFA with PBS rinse
4) Begin sucrose infiltration for cyroprotection (see below)

FROZENS:

OTC MEDIUM

1) Tissue ready for embedding should be fixed (usually in 4%PFA) and in stored in PBS
2) Sucrose infiltration (CRYOPROTECTION)
   10% Sucrose (15 min or until sample drops to bottom of vial)
   2:1 10% Sucrose:30% Sucrose (15 min or until sample drops to bottom of vial)
   1:1 10% Sucrose:30% Sucrose (15 min or until sample drops to bottom of vial)
   1:2 10% Sucrose:30% Sucrose (15 min or until sample drops to bottom of vial)
   30% Sucrose (15 min or until sample drops to bottom of vial)
   30% Sucrose (15 min or until sample drops to bottom of vial)
   30% Sucrose (15 min or until sample drops to bottom of vial)
3) Partially fill dry ice container with dry ice and add methanol to create a cool bath, let sit
4) Label Tissue Tek wells with each animal number and fill with OTC (TissueTek) freezing compound
5) Remove excess sucrose from tissue by blotting on Kimwipes and place tissue in center of well filled with OTC
6) Orient tissue into the bottom of the well and freeze by floating on methanol bath
   CAUTION: do not get methanol on the OTC, it will not freeze correctly
7) Place frozen tissue blocks in –20 freezer after they are frozen
8) Ready to be sliced after they are frozen completely
9) Note: do not store slides in the cryostat over night, they will dry out and be no good. It is also a good idea to place all tissue into plastic bags in the –20 frost free freezer to reduce drying out during storage.
10) Slice sections at 8 to 10μm on the cryostat. Cold sections will readily transfer to room temperature super frost plus or subbed slides
11) Let new slides dry (at least 2h) at room temperature in hood
12) Dunk slides in cold acetone (1-3 min) and let dry for at least 1h
13) Label slides as normal

WHOLE TISSUE

1) Whole pieces of tissue that have been cyroprotected (see above) can be frozen by placing on an aluminum "boat" floating on top a dry ice/methanol solution (see below).
   * note large pieces of tissue (brains) often don't need to be embedded in OTC

2) Slice sections at 8 to 10µm on the cryostat. Cold sections will readily transfer to room temperature super frost plus or subbed slides
3) Let new slides dry (at least 2h) at room temperature
4) If necessary, dunk slides in cold acetone (1-3 min) and let dry for at least 1h (helps the section adhere)
5) Label slides

PARAFFIN SECTIONS (Protocol from Brauer Lab)

1. After fixation, rinse tissue with PBS until fixative is completely removed.
2. Dehydrate tissue using EtOH series.*
   - 50% EtOH for 10 min.
   - 70% EtOH for 10 min.
   - 80% EtOH for 10 min.
   - 95% EtOH for 10 min.
   - 100% EtOH for 10 min.
   - 100% EtOH for 10 min.
   - 100% EtOH for 10 min.
3. Exchange EtOH with Citrisolve
   - 2:1 EtOH:Citrisolve for 10-15 min
   - 1:1 EtOH:Citrisolve for 10-15 min
   - 1:2 EtOH:Citrisolve for 10-15 min
   - 100% Citrisolve for 10-15 min
   - 100% Citrisolve for 10-15 min
   - 100% Citrisolve for 10-15 min
4. Exchange Citrisolve with paraffin. The following steps are done in a vacuum oven set for 54-58°C. We use Paraplast X-tra or Paraplast Plus (the Plus has DMSO added to facilitate infiltration-- both from Fisher). Do not let paraffin exceed 60°C for prolong periods of time because this will degrade the paraffin polymers and make it more brittle.
   - 2:1 Citrisolve:Paraffin for 30 min
   - 1:1 Citrisolve:Paraffin for 30 min
   - 1:2 Citrisolve:Paraffin for 30 min
   - 100% Paraffin for 1-2 hr
   - 100% Paraffin for 1-2 hr or overnight
5. Embed in fresh new paraffin and orient tissue as desired before it hardens (vertical for embryos).