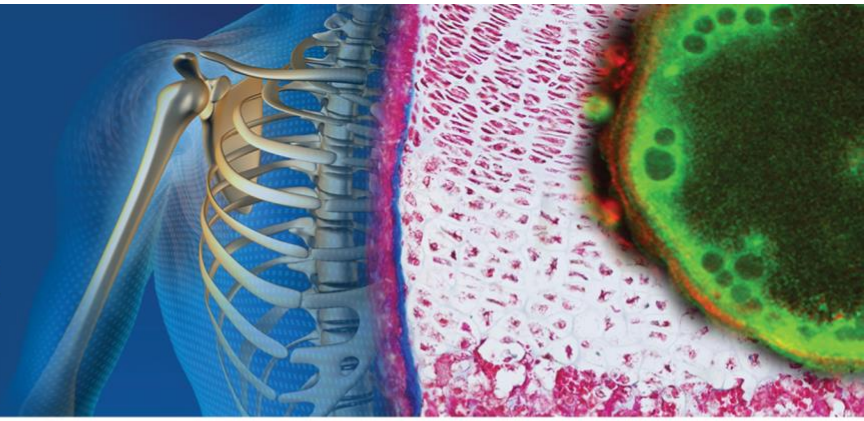

Fixation and Processing of Tissues for Frozen Specimens

1. Dissect tissue, removing all skin (with the exception of mouse embryos younger than E15.5) and as much muscle as desired. When performing gross dissection, be sure to keep your tissue hydrated in 1X PBS.

2. Fix tissues in 10% Neutral Buffered Formalin (NBF) or 4% Paraformaldehyde (PFA) at 4°C. Length of fixation time will depend on downstream applications. The following abbreviated fixation times work well for β-galactosidase assays, immunostaining, and visualization of endogenous fluorescent reporters:
   - Embryos (mouse) or soft tissue: 30 minutes
   - Postnatal limbs (mouse): 1-2 hours

   Speak to histology core members regarding fixation times of any other tissue type.

3. After fixation, rinse specimens in 1X PBS, 3 times for 5 minutes each rinse, at 4°C.

   Remove pins from all femur or tibia fractures after rinsing.

4. If desired, decalcify specimens in 14% EDTA at 4°C, changing solution every other day, using the guidelines below for murine tissues. Frozen skeletal specimens can also be sectioned undecalcified using Kawamoto's tape method (see Histology Core for details).
   - Embryos: 48 hours
   - P1-P4: 72 hours
   - Less than 3 weeks old: 5-7 days
   - Greater than 3 weeks old: 7-10 days

5. Rinse specimens in 1X PBS, 3 times for 5 minutes each rinse, at 4°C.

6. Sink specimens into 30% sucrose (in 1X PBS) overnight at 4°C.

7. Embed specimens immediately (see protocol) or store frozen in 30% sucrose/PBS at -80°C until ready to embed. *Note: transferring each specimen to individual 15ml conical tubes and freezing upright works best.*