

### KOS Processing (tissue <5 mm)

#### Fixation:

- 10% Buffered Formalin (or 4% PFA) O/N to 24 hrs, RT, oscillating
- 1x PBS wash, 3 x 10 minutes
- 70% EtOH at 4°C until processing; minimize time in 70% EtOH

#### Processing (EtOH/Iso/Wax):

- Place cassettes in cassette holder in beaker holding 100% EtOH (ensure level is above cassettes); one level holds 15 cassettes.
- Run EtOH/Iso/Wax program on KOS machine (skip fixation)
- Change cassette holder to Isopropanol (kept at RT) and wax (kept in incubator to stay liquid) as dictated by program
- Prewarm plastic molds (2/skull, 1/long bone, 1/arm, 1/rib cages) and large metal molds (1/spinal cord) and label
- Fill prewarmed molds with wax, then add tissue with hot tongs
- Use hot tongs to orient tissue
- Add plastic cassette to spines and fill to top with wax
- Allow to solidify over night

#### Processing Day 3:

- Put metal molds into freezer for 5-10 minutes
- Carefully use razor to pop plastic cassette out of metal molds