Embedding in Paraffin Manually - Bones

Fixation:

-10% Buffered Formalin (or 4% PFA) O/N to 24 hrs, RT, oscillating

-1x PBS wash, 3 x 10 minutes

Decalcification:

-10% EDTA (saturated EDTA), pH 5.5; RT, oscillating

-4 days for skulls, long bones, rib cage; slice skulls on day 3 and decal one more day

-6-7 days for spines and arms.

-Change EDTA every ~2 days

-1x PBS wash, 1 x 10 minutes

-1x PBS wash O/N, RT, oscillating

-70% EtOH at 4°C until processing; minimize time in 70% EtOH and process ASAP

Processing Day 1 (all oscillating):

-Slice rib cages in half down sternum; slice spines transversely into 2 mm sections; slice hand off arm

-80% EtOH, 2 x 30 minutes -90% EtOH, 2 x 30 minutes -95% EtOH, 1 x 30 minutes -95% EtOH, O/N 4°C

Processing Day 2:

-100% EtOH, 2 x 30 minutes, oscillating RT

-50% EtOH, 50% Histoclear, 1 x 30 minutes, oscillating RT

-100% Histoclear, 1 x 30 minutes, oscillating RT

-50% Histoclear, 50% Wax, 1 x 30 minutes, in embedder/incubator to keep warm

-100% Wax, 3 x 1 hr, in embedder/incubator to keep warm

-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; tap skulls to remove bubbles. Skulls should be cut side down, long bones aligned in the same direction, arm shoulder blade down, rib cage cut side of sternum down.

-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