Dissection of Mouse Legs/Processing of Tissues

Reagents/Equipment Required:
Thick tweezers (No. 2)
Thin tweezers (No. 5)
Paraplast (Oxford Labware, order # HRI8889-501006)

Working Solutions to Prepare:
4% paraformaldehyde in PBS
for 1L EDTA solution EDTA Na2 90 g+ EDTA Na4 100g. May need heating to get into solution. -
Isopropanol/Paraplast solution - 1:1 (vol/vol) solution

Procedure:
Dissection
1. Sacrifice mice by CO_2 asphyxiation followed by cervical dislocation.
2. Spray down hindlimbs with 70% ethanol washbottle.
3. For each leg, first trim off nails with small dissecting scissors. Then make a cut in the skin around the full circumference of the ankle. Make a second cut down the inside of the leg, starting at the ankle cut and ending at the tip of the 3rd metatarsal.
4. Using thick (No. 2) tweezers, peel off skin from the ankle toward the phalanges.
5. Make another cut up the inside of the leg, starting at the original ankle cut, continuing along the tibia and femur. Peel off the skin to the level of the hip, and sever the leg at the hip.
6. Store the legs on ice in PBS.

Processing
1. Fix samples in 4% paraformaldehyde in PBS, overnight at 4ºC.
2. Wash samples in PBS at 4ºC for several hours, changing the PBS twice an hour.
3. Decalcify samples in EDTA at 4ºC, changing EDTA solution every other day:
   - 3 days for samples from mice up to 1 week old
   - 7 days for samples from mice 1-4 weeks old
   - 10 days for samples from mice > 4 weeks old
4. Following decalcification, wash samples in distilled water for 4 hours, changing the water each hour.
5. Dehydrate samples:
   - 50% ethanol, 1 hour at 4ºC
   - 70% ethanol, 1 hour at 4ºC
   - 80% ethanol, 1 hour at 4ºC
   - 90% ethanol, 1 hour at 4ºC
   - 100% isopropanol, 30 min. at 4ºC
6. Transfer samples to isopropanol/Paraplast solution for 2-3 hours at 56ºC.
7. Transfer samples to Paraplast solution; incubate overnight at 56ºC.
8. Change paraplast solution, incubate 1 additional hour at 56ºC. Embed samples.