Cryofilm/Cryotape Sectioning Protocol
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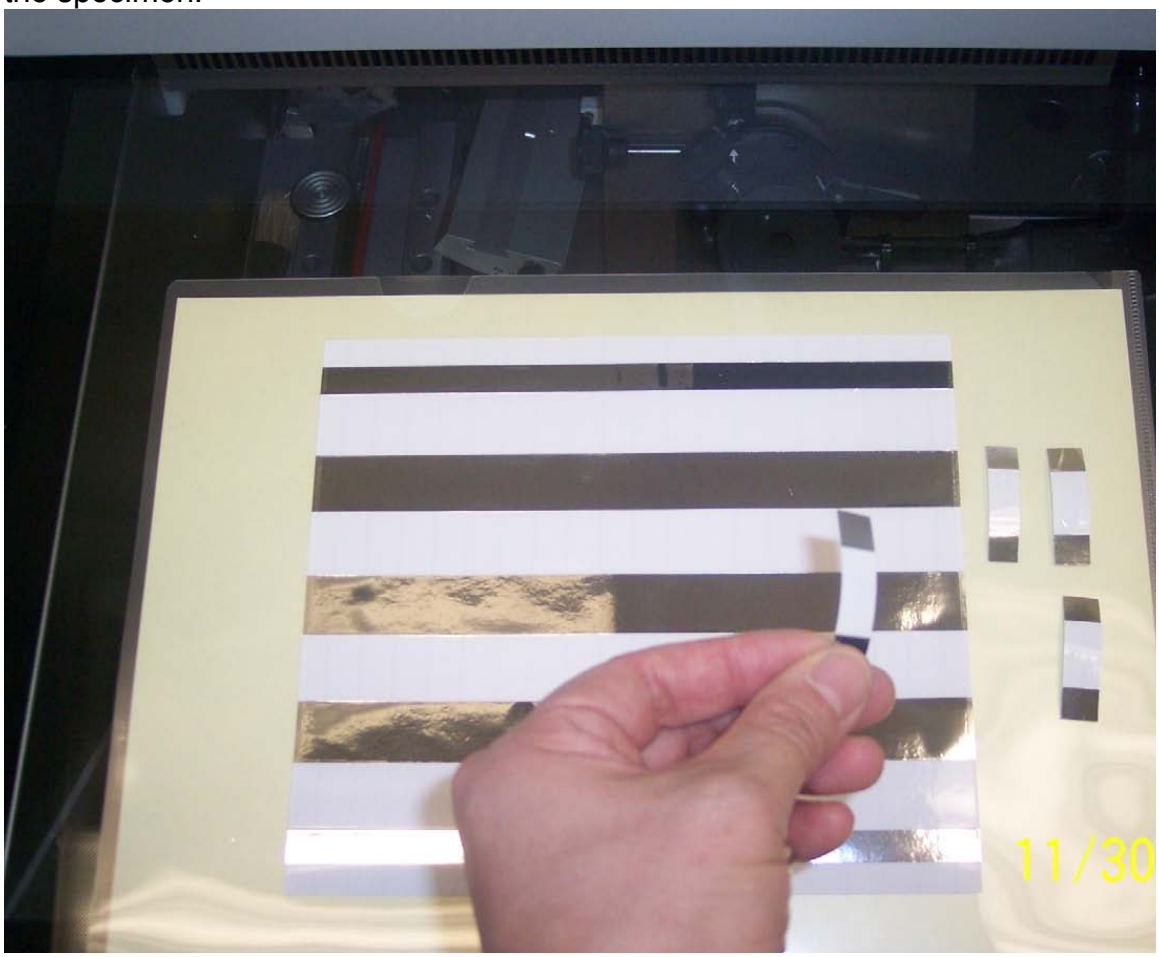
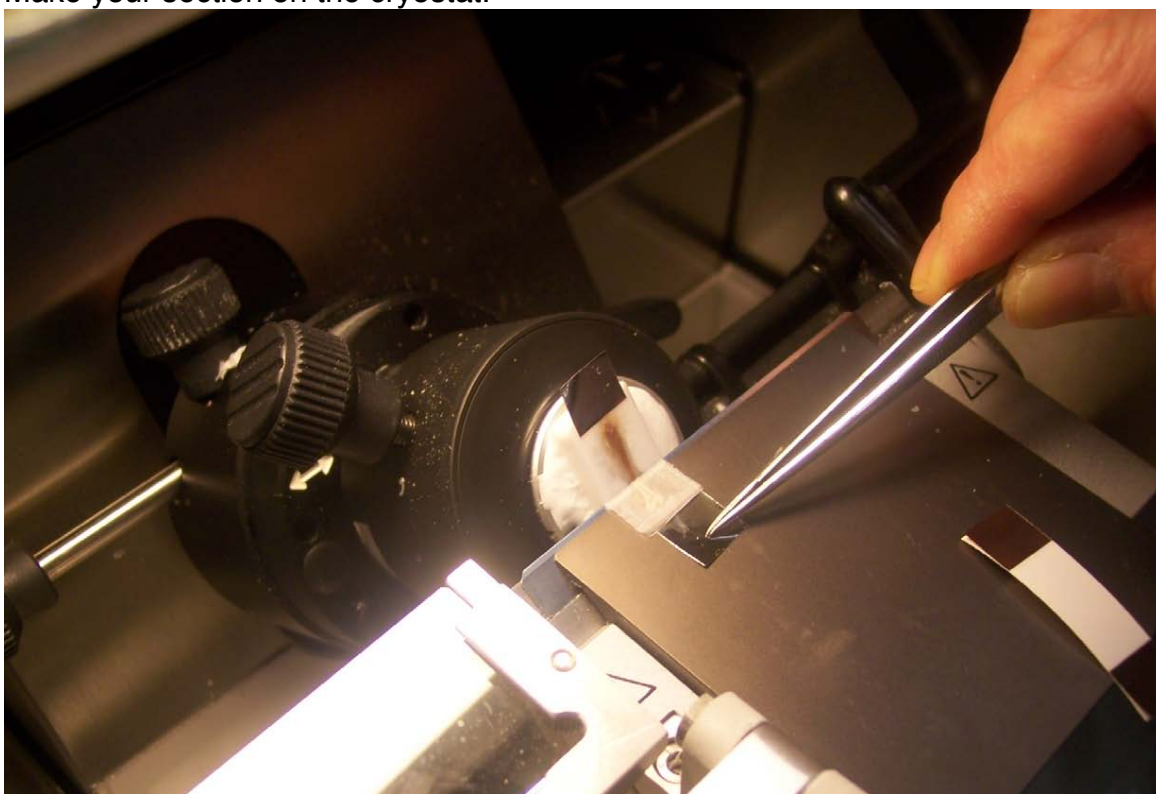
This protocol describes a novel cryosectioning technique using cryofilm, which allows for sectioning of calcified tissues while maintaining tissue morphology. The advantages of this method is that 1) thin (5μm) sections can be made in calcified specimens embedded in normal cryo-embedding media and cut with disposal cryostat blades and 2) multiple rounds of imaging can be done on each section because of the strong adhesion of the specimen to the cryofilm, allowing for removal and remounting of coverslips.

1. Mount sample block on the cryostat and trim to region of interest.
2. Determine the size of the tape based on size of the specimen in the block.
3. Cut the tape into pieces such that the size of each piece is just larger than the size of the specimen.

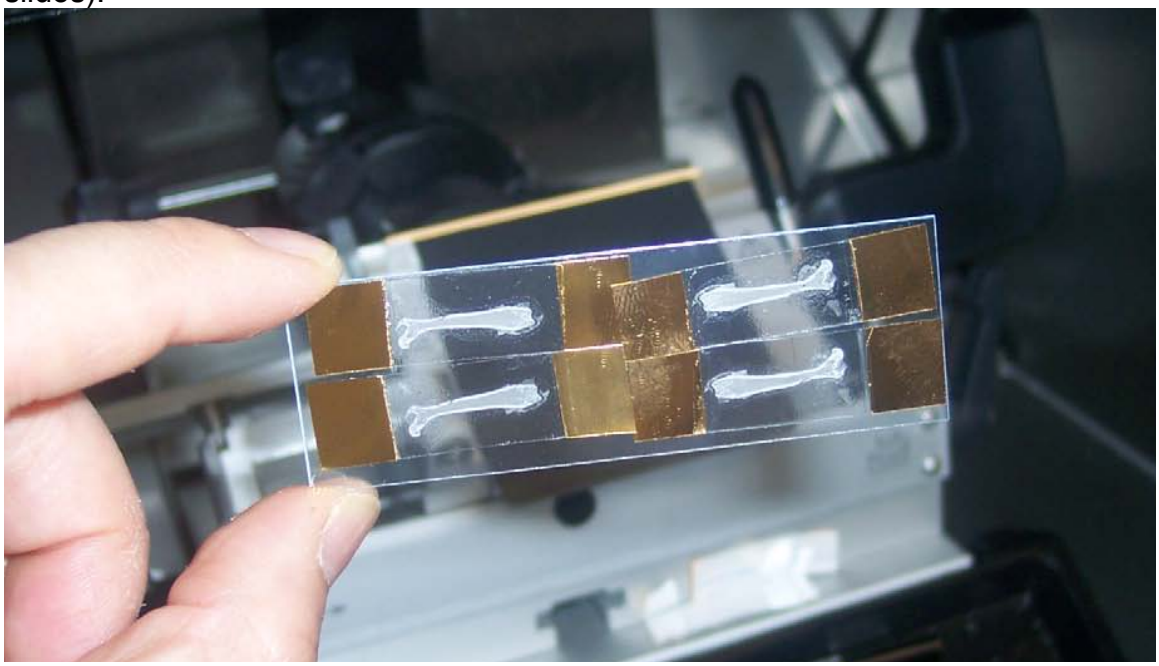


4. Peal off paper backing of a piece of tape and roll it onto your sample block such that the adhesive side is down (the tab is gold on the adhesive side and silver on the NON-adhesive side of the tape).

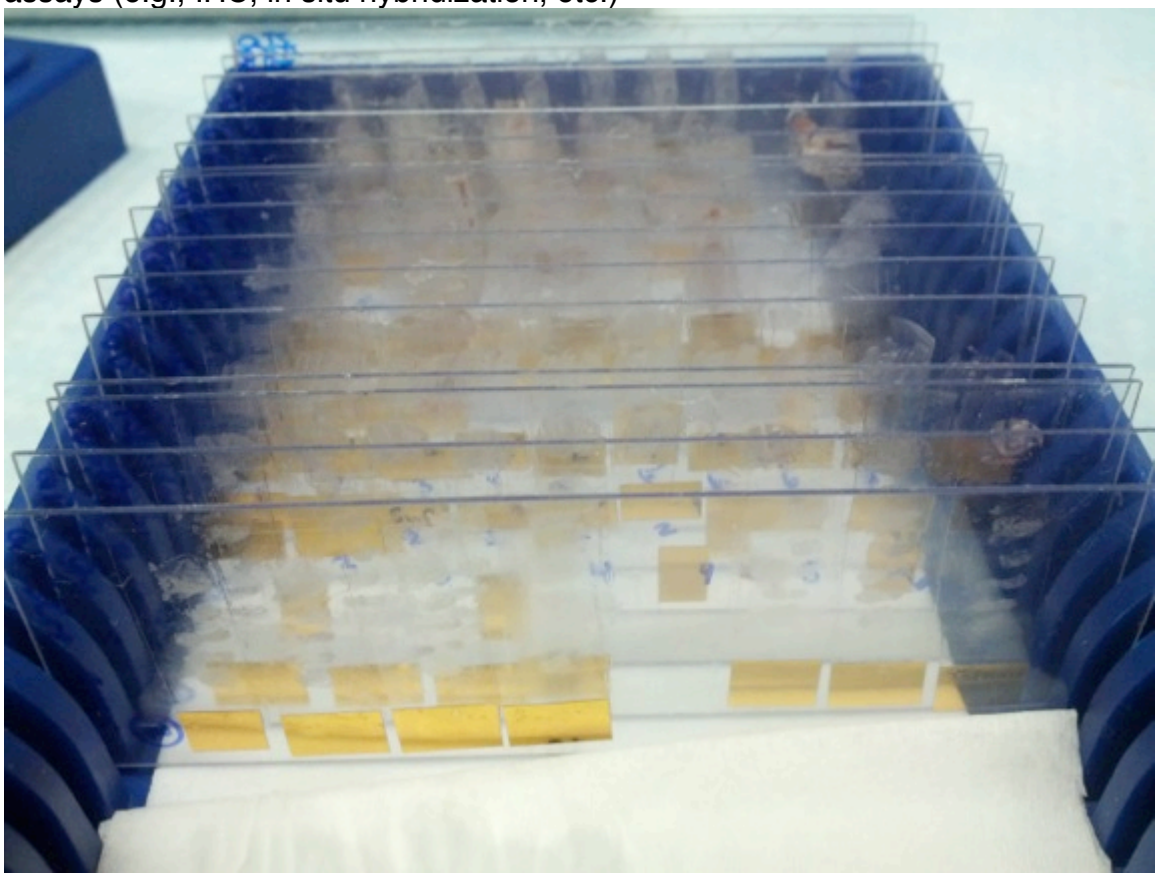
5. Make your section on the cryostat.



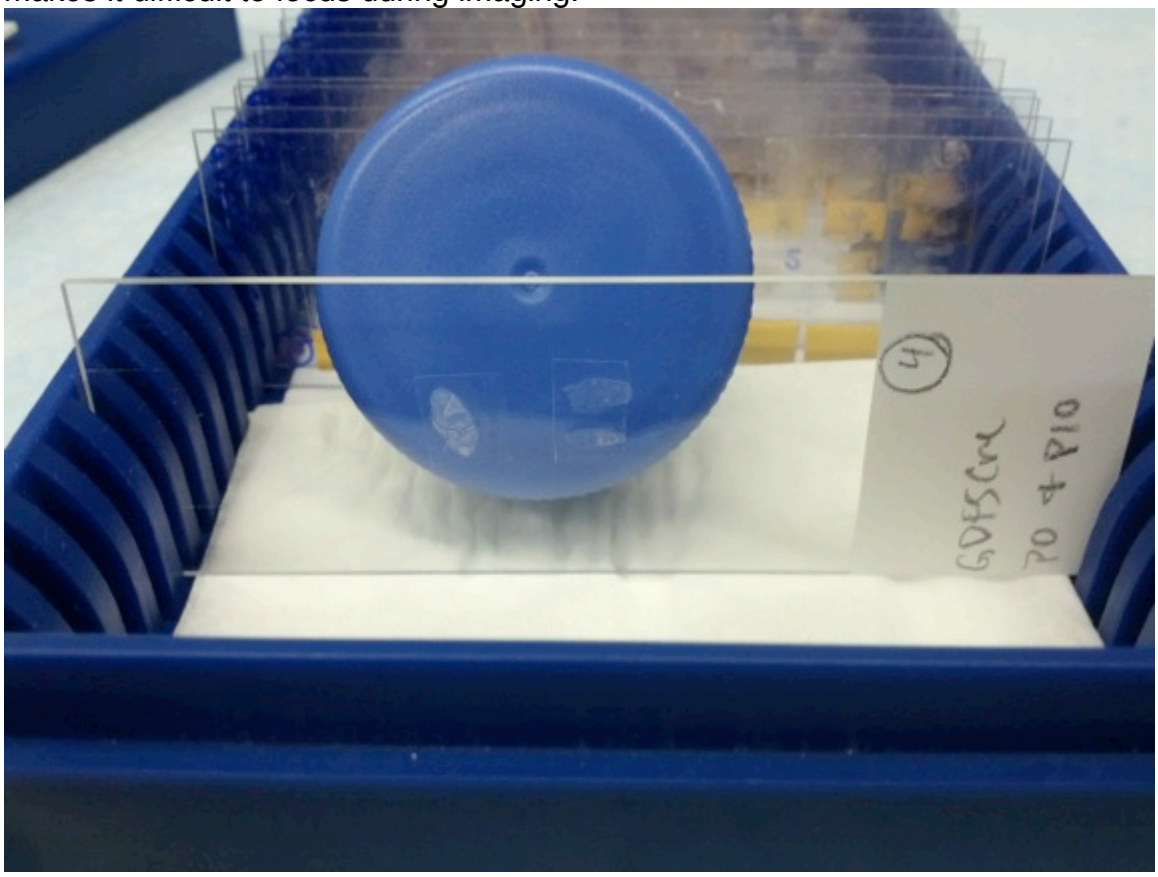
6. After making the section, take the piece of tape with the tissue adhered to it and place it tissue-side up on a slide. (We stick our sections temporarily to cheap plastic slides).



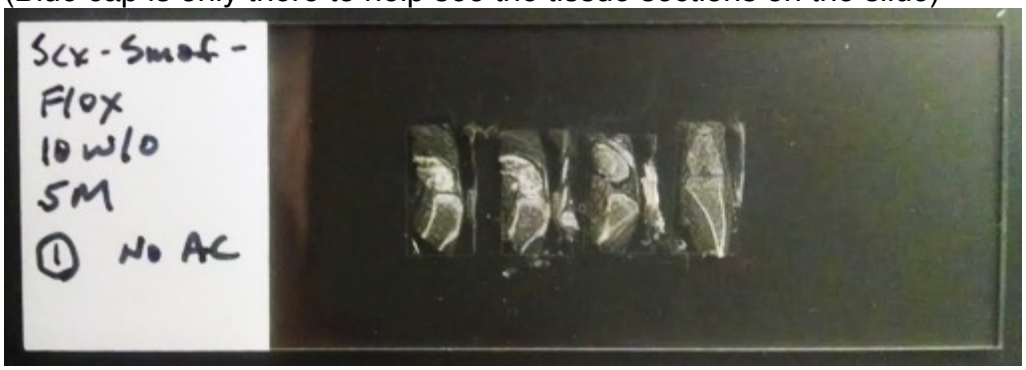
7. Store the sections at the appropriate temperature depending on your downstream assays (e.g., IHC, in situ hybridization, etc.)



8. Glue the sections down to glass microscope slides using chitosan adhesive. Place a drop of chitosan that is approximately the size of the tape section onto the glass. Then cut off the tab from the tape and place the tape tissue-side up on the glass. Drag some of the chitosan towards the edge of the glass slide and place the slide in a slide box with a kimwipe underneath it such that excess chitosan wicks down onto the kimwipe. The chitosan 1) provides a strong adhesive to the glass, 2) does not autofluoresce, and 3) when done properly makes the tape section flat against the glass. The wicking is important in getting a flat surface. If the chitosan doesn't wick down, it tends to clump underneath the tape. This will lead to an uneven surface that makes it difficult to focus during imaging.



(Blue cap is only there to help see the tissue sections on the slide)



(You can line up multiple sections if needed)

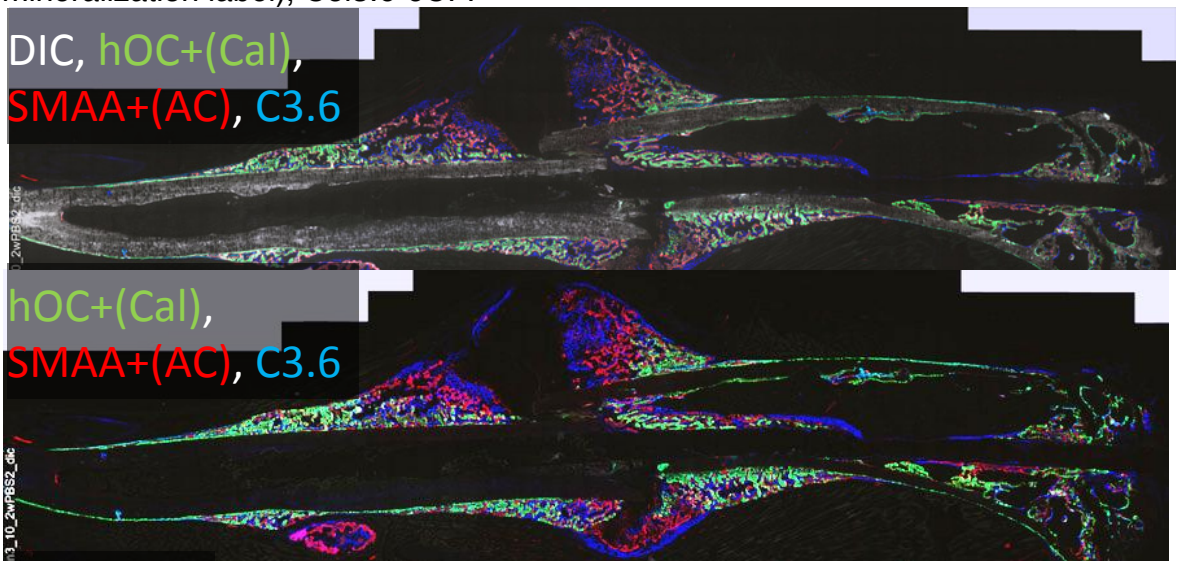
9. Allow the chitosan to dry in a slide box for 48 hours at 4°C (Prop the slide box open to improve air flow and speed up drying time).
10. After 48 hours, hydrate the slides in 1X PBS and proceed with staining, imaging, etc.
11. Once first round of imaging is complete, coverslips can be removed from slides by simply soaking them in PBS. Slides can then be re-stained and imaged again if necessary (see images on next page for an example of multiple rounds of imaging).

Materials Needed

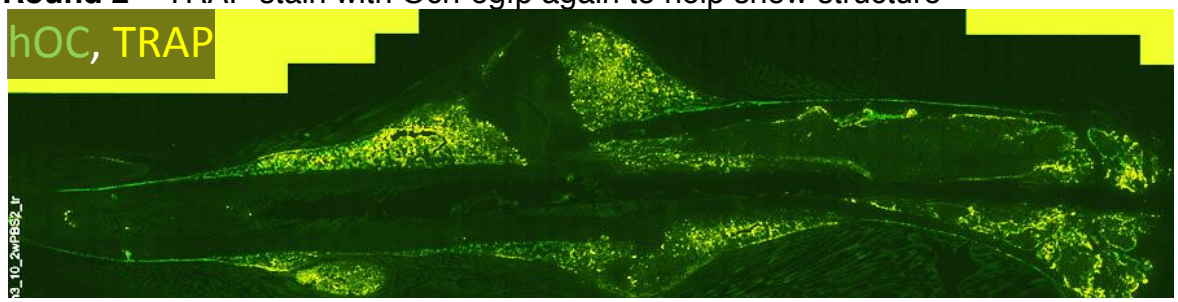
1. Cryofilm Type2C (Section Lab Co. Ltd, Hiroshima, Japan), which can be purchased from the UConn Health Center Molecular Core. Contact Trushna Desai at tdesai@uchc.edu.
2. Hand roller to roll cryofilm onto frozen block (see step 4). Catalog #62800-46 EMS Diasum.
3. Plastic slides to store dry sections from cryostat (see step 6). We prefer Disposable Rinzl Plastic Micro Slides Catalog #71890. It's cheaper than using glass microscope slides to store the sections.
4. Chitosan for gluing sections to glass slides (see step 8). Prepare chitosan (C3646, Sigma-Aldrich) by dissolving 1.5g of chitosan powder in 100ml of 0.25% acetic acid. Stir overnight at room temperature or until the powder has dissolved.
5. Glass microscope slides (see step 8). We prefer Gold Seal Rite-On Micro Slides Catalog #3051 but any glass slide will do.

Example: Tibial Fracture with 4 Rounds of Imaging

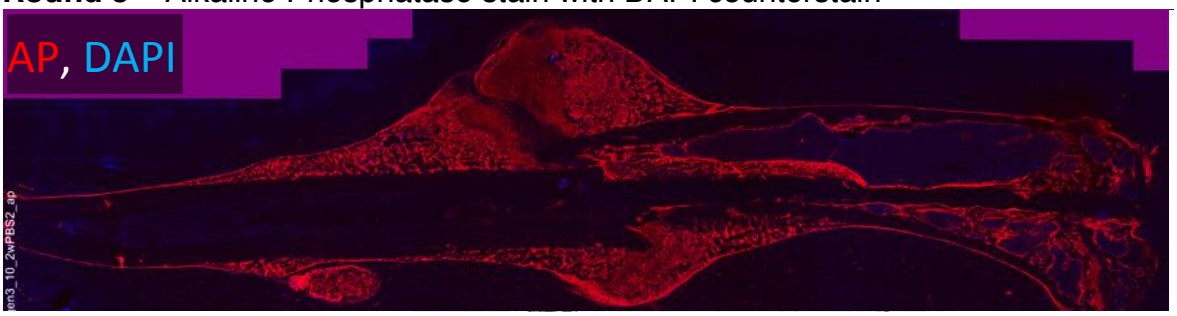
Round 1 – DIC, Ocn-eGFP (Osteocalcin reporter), Calcein (mineralization label), SMAA-mcherry (Alpha Smooth Muscle Actin reporter), Alizarine Complexone (AC; mineralization label), Col3.6-eCFP



Round 2 – TRAP stain with Ocn-egfp again to help show structure



Round 3 – Alkaline Phosphatase stain with DAPI counterstain



Round 4 – Safranin-O stain