Tissue Preparation and Shearing: (Note: There will be sample loss during transfers)

In Advance: Have dry ice and a bucket of liquid nitrogen ready**.** Add 2x protease inhibitors to your buffers.

1. Quickly weigh frozen tissue.
2. Slice frozen tissue into ~ 200 mg sections
3. Place weighed frozen tissue sample into the center of labeled Kapton tissue tube (bag) with a collection tube attached and loosened 1/8 turn (note: it is best to keep the size of the tissue below 300mg per bag)
4. Keep your sample on dry ice then submerge in LN2 right before using the Covaris Hammer.
5. Turn on Covaris hammer. Note settings, setting 1 is for softer tissue, setting 6 is for tougher tissues. We always use setting 6.
6. Seat your sample in the Covaris device, select force (1-6) then activate.
7. Remove your sample quickly and submerge in LN2. Assess whether or not additional pulverization is required. Remember to keep your tissue frozen.
8. Repeat pulverization until you achieve homogenous powder. This usually requires 1 blow or activation, sometimes 2.
9. Remove tube and cap sample.
10. At this point, you can proceed with protocol or store samples at -80.
11. If you are cross-linking your tissue, you may do so directly in the collection tube provided if it is large enough, otherwise transfer tissue to a larger tube and use room temperature PBS with 2x protease inhibitors to rinse bag and tube in order to collect entire sample. We transfer frozen powder to 5ml snap cap tubes (eppendorf) prior to cross linking.