Bullet Blender® 5
Homogenization Protocol for Mouse Femur

The protocol described in this document is for the use of the Bullet Blender® 5 for the homogenization of mouse femur or other small brittle bone tissue. If you have difficulty with this protocol, cutting your tissue into smaller pieces will help. This protocol does not specify a particular buffer - you may choose which is most appropriate for your downstream application (nucleic acid isolation, protein extraction, etc.).

Materials Required: femur, Bullet Blender® 5, homogenization buffer, pipettor, 5mL Axygen® brand tubes, and 3.2 mm stainless steel beads (part number ZrOB20).

Instructions
1. Choose appropriately sized pieces for analysis (10-100mg).
2. **OPTIONAL:** If desired, wash the tissue 3x with 5mL PBS to remove blood and other contaminants from the tissue.
3. Place sample in 5ML tube and add beads to the tube. Use a volume of beads equal to the mass of tissue. **NOTE:** 100mg $\cong$ 100µL.
4. Add 0.025 mL to 0.3mL buffer (2 volumes of buffer for every volume of sample).
5. *Tightly* screw the centrifuge tubes closed and place them into the Bullet Blender®.
6. Set controls for **SPEED 10** and **TIME 5** minutes. Press start.
7. After the run, remove the tubes from the instrument.
8. Visually inspect samples, if homogenization is unsatisfactory, run for another five minutes at **SPEED 10**
9. Proceed with your downstream application.

SAFETY NOTE!!!
When using a centrifuge to separate your homogenate from the debris and beads, make sure your tubes are balanced.