## Protocol for Mouse Femur Homogenization in the Bullet Blender®

The protocol described in this document is for the use of the Bullet Blender® for the homogenization of mouse femur or other small brittle bone tissue. 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<sup>®</sup>, homogenization buffer,

microcentrifuge tubes, pipettor, and Navy bead lysis kit/0.9-2.0mm stainless steel bead blend (product number SSB14B) and 3.2mm stainless steel balls (SSB32)\*.

## Instructions

- 1. Choose appropriately sized pieces for analysis (10-100mg).
- 2. OPTIONAL: Wash tissue 3x with ~1mL PBS. Aspirate. NOTE: This step removes any external contaminants (blood etc.).
- 3. a. Protocol step for pre-loaded tubes
  Place the sample in Navy bead lysis kit tube.
  - c. Protocol step for bulk beads Place sample in microcentrifuge tube and add  $\sim 100 \mu L$  of the stainless steel blend and 6 x 3.2mm stainless steel balls.
- 4. Add 0.2 mL to 0.6mL buffer (~2 volumes of buffer for every volume of beads).
- 5. Close the centrifuge tubes.
- 6. Place tubes into the Bullet Blender.
- 7. Set controls for SPEED 10 and TIME 5 minutes. Press Start.
- 8. After the run, remove tubes from the instrument.
- 9. Visually inspect samples. If homogenization is unsatisfactory, run for another 5 minutes at the SPEED 10.
- 10. 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.



Before



After