Bullet Blender® 5
Homogenization Protocol for E. coli Cultures

The protocol described in this document is for the use of the Bullet Blender® 5 for the homogenization of *Escherichia coli* (or other bacterial) cultures. 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: *E. coli*, Bullet Blender® 5, homogenization buffer, pipettor, 5mL Axygen® brand tubes, and 0.5mm zirconium oxide beads (part number ZROB05)

Instructions

1. Pour bacterial culture into a 5ML tube.
2. Centrifuge culture to yield a cell pellet (1000g for two minutes).
3. Completely aspirate the supernatant liquid. Place tube on ice.
4. Inspect the volume of the pellet. It should be 0.75ML or less in order to get efficient homogenization.
5. Add a volume of beads equal to the volume of cells.
6. Add 0.2mL to 1.5mL buffer (2 volumes of buffer for every volume of sample).
7. *Tightly* screw the centrifuge tubes closed and place them into the Bullet Blender®.
8. Set controls for **SPEED 8** and **TIME 3** minutes. Press start.
9. After the run, remove the tubes from the instrument.
10. Visually inspect samples, if homogenization is unsatisfactory, run for another two minutes at **SPEED 9**.
11. 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.