Protocol for E. coli Cultures Homogenization in the Bullet Blender®

The protocol described in this document is for the use of the Bullet Blender® 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*, aspirator, Bullet Blender®, homogenization buffer, pipettor, microcentrifuge tubes, and 0.5mm zirconium oxide beads (part number ZrOB05)

Instructions

1. Pour overnight bacterial culture into a microcentrifuge tube.
2. Centrifuge culture to yield a cell pellet (2000g for one minute).
3. Completely aspirate the supernatant liquid. Place tube on ice.
4. Inspect the volume of the pellet. It should be 300µL or less in order to get efficient homogenization.
5. Add volume of zirconium oxide beads equal to the size of the cell pellet.
6. Add 0.1mL to 0.6mL buffer (2 volumes of buffer for every volume of cells).
7. Snap the caps centrifuge tubes closed.
8. Place tubes into the Bullet Blender®.
9. Set controls for **SPEED 8** and **TIME** to **3** minutes. Press **Start**.
10. After the run, remove tubes from the instrument.
11. Visually inspect samples. If homogenization is unsatisfactory, run for another two minutes at the **SPEED 8.**
12. 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.

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