Homogenization in the Bullet Blender[®] 5 Protocol for Trachea Tissue

The protocol described in this document is for the use of the Bullet Blender[®] 5 for the homogenization of trachea tissue (from a variety of animals). 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:

trachea tissue, Bullet Blender[®] 5, homogenization buffer, pipettor, 5mL Axygen[®] brand tubes, and 3.2 mm stainless steel beads (part number ZrOB20).

Instructions

- 1. Cut trachea into appropriately sized pieces for analysis (0.1g 0.5g).
- **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:** $100 \text{ mg} \approx 100 \text{ \mu L}$.
- **4.** Add 0.2mL to 1.0mL 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 9 and TIME 4 minutes. Press start.
- **7.** After the run, remove the tubes from the instrument.
- 8. Visually inspect samples, if homogenization is unsatisfactory, run for another three 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.

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