Protocol for Horseradish Root Homogenization in the Bullet Blender[®]

The protocol described in this document is for the use of the Bullet Blender[®] for the homogenization of horseradish (*Armoracia rusticana*) root. 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: Horseradish root, Bullet Blender[®], homogenization buffer, pipettor, microcentrifuge tubes, and <u>0.9-2.0mm stainless steel</u> bead blend (part no. SSB14B)

Instructions

- **1. OPTIONAL:** Wash horseradish 3x with ~1mL PBS to remove soil and other surface contaminants and debris.
- **2.** Cut horseradish into long, thin slices of 200mg or less and place each slice into a microcentrifuge tube.
- **3.** Add a a volume of beads equal to the mass of tissue. **NOTE:** $100 \text{mg} \approx 100 \mu \text{L}$.
- **4.** Close the microcentrifuge tubes and place them into the Bullet Blender[®]. **NOTE:** There should be no buffer in the tubes at this point.
- 5. Set controls for SPEED 10 and TIME 5. Press Start.
- **6.** Remove the samples from the Bullet Blender. The horseradish should be finely pulverized. If not, run for another three minutes at speed 10.
- **7.** Add 2 volumes of buffer to the tube for every mass of sample (ex. for 100 mg horseradish add 200μ L buffer).
- **8.** Close the microcentrifuge tubes and place them back into the Bullet Blender[®].
- 9. Set controls for SPEED 8 and TIME 3 minutes. Press Start.
- **10.** After the run, remove tubes from the instrument.
- **11.** Visually inspect samples. If homogenization is unsatisfactory, run for another three minutes at speed 10.
- **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.



Before



Pulverized



After