Protocol: *Apis mellifera* (Honeybee) Homogenization in the Bullet Blender[®]

The protocol described in this document is for the use of the Bullet Blender[®] for the homogenization of *Apis mellifera* thoraces, although should be suitable for whole bees or other honeybee sections or tissues as well. This protocol was created for the extraction of DNA, and does specify a particular buffer, however you may modify it in any way necessary to tailor it to your needs (RNA extraction, protein purification, etc.).

Materials Required: Apis mellifera, Bullet Blender[®], homogenization buffer, pipettor, microcentrifuge tubes, and <u>1.0mm glass beads (part number GB10)</u>.

Instructions

- **1.** Wash *A. mellifera* in PBS or other buffer, as appropriate, to remove food, surface bacteria, and other contaminants.
- **2.** Isolate the thorax using a scalpel and forceps.
- **3.** Place each thorax, individually, into a microcentrifuge tube.
- **4.** Add 100mg of 1.0mm glass beads to the tube.
- 5. Add 600 μl of lysis buffer (100 mM Tris, pH 8.0,10 mM EDTA, pH 8.0, and 1% SDS).
- **6.** Close the microcentrifuge tubes.
- 7. Place tubes into the Bullet Blender[®].
- 8. Set controls for SPEED 8 and TIME 3 minutes.
- **9.** Remove tubes from the instrument.
- **10.** Visually inspect samples, if homogenization is unsatisfactory, run for another two minutes at the **SPEED 10.**
- **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.

Reference:

Bourgeois, A.L., Rinderer, T.E. <u>Genetic Characterization of Russian Honey Bee Stock</u> <u>Selected for Improved Resistance to Varroa destructor</u>. J. Econ. Entomol. 102(3):1233-1238. 2009