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Protocol for Tail Snips Homogenization in the Bullet Blender™

The protocol described in this document is for the use of the Bullet Blender™ for the homogenization of tail snips. This protocol was developed using rat tail snips; note that the time and speed settings may differ due to the variation in size and toughness of tissue from species to species. This protocol was used for RNA extraction, and uses Qiagen's RNeasy Lysis Buffer (RLT), however you may choose a buffer that is most appropriate for your downstream application (nucleic acid isolation, protein extraction, etc.).

Materials Required: tail snips, Bullet Blender™, Qiagen RLT buffer, pipettor, microcentrifuge tubes, and 0.5mm stainless steel beads (part # SSB05) or 0.5mm zirconium oxide beads (part # ZROB05).

Note: To preserve RNA integrity, this experiment was carried out in a cold room.

Instructions

1. If necessary, cut tail snips into appropriately sized pieces for analysis (< 100mg) and place into a microcentrifuge tube.
2. **OPTIONAL:** Wash tissue 3x with ~1mL PBS. Aspirate. **NOTE:** This step removes external contaminants.
3. Add beads. If using zirconium oxide, use a mass of beads equal to 2x your mass of tissue. If using stainless steel beads, use 3x your mass of tissue.
4. Add 2 volumes of RLT buffer for every mass of tissue (for example, add 100µL buffer for 50mg tissue).
5. Close the microcentrifuge tubes.
6. Place tubes into the Bullet Blender™.
7. Set controls for **SPEED 10** and **TIME 5** minutes. Press **Start**. Run again for 5 minutes.
8. After the run, remove tubes from the instrument.
9. Visually inspect samples. If homogenization is unsatisfactory, run for another five minutes at the **SPEED 10.**
10. Proceed with your downstream application.

This protocol was tested and found to generate high-integrity RNA (RIN = 8.5)

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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