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 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: tail snips, Bullet Blender®, Qiagen RLT buffer, pipettor, microcentrifuge tubes, and Navy bead lysis kit/0.9-2.0mm stainless steel bead blend (product number SSB14B) and 3.2mm stainless steel balls (product number SSB32)*.

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. a. P*rotocol step using pre-loaded tubes*
   Place the sample in Navy bead lysis kit tube.
   b. *Alternate protocol step for bulk beads*
   Place sample in microcentrifuge tube and add the beads to the tube. Use a volume of stainless steel bead blend equal to the mass of tissue in addition to 3-6 3.2mm stainless steel balls*. NOTE: 100mg ≅ 100µL.
4. Add 2 volumes of buffer for every mass of tissue (for example, add 100mL 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.

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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