Crayfish Homogenization Using the Bullet Blender

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Materials

- <u>Bullet Blender</u>[®] for 5 mL tubes
- Homogenization Buffer
- <u>FoamBlocker</u> (Optional)
- Lysis Kit or Lysis Beads
 - GREEN or NAVY Lysis Kit (from <u>PrecisionPak</u>[™] or purchased separately)
 - 3.2 mm Stainless Steel Beads + 3.5 mm UFO Beads in Eppendorf or GATOR tubes
- Sample up to 1000 mg

Table 1. Proper sample, bead and buffer volume ratios for 5 mL tubes.

Lysis Kit and Bead Choices	Sample Volume	Bead Volume	Buffer Volume
GREEN	Up to 300 mg	Pre-filled	0.5 - 1.2 mL
NAVY	300 - 1000 mg	Pre-filled	1.2 - 2.5 mL
3.2 mm Stainless Steel Beads + 3.5 mm UFO Beads	Up to 1000 mg	500 - 1000 μL	1.2 - 2.5 mL

Procedure

- 1. Use the pre-filled bead lysis kit tubes OR prepare a tube with the recommended volume of bead choices from the table above.
- 2. Add the appropriate volume of buffer according to the table above
- 3. Prepare the sample by cutting it into small thin pieces and then transfer it into the buffer-filled tubes.
- 4. (Optional) To avoid excess foaming, add FoamBlocker up to 1-2% of the total volume of the homogenization buffer.
- 5. Close the tubes tightly and place into the Bullet Blender sample chamber. If using the Gold or Gold⁺ models, pre-cool the chamber before adding sample tubes.
- 6. Set the controls to speed 16, time 4 minutes then press Start. Note: Using single-size beads instead of pre-filled lysis kits may require additional time.
- 7. After the run, remove the tubes from the instrument and visually inspect the samples. If homogenization is incomplete, homogenize for an additional 30 seconds, or repeat the homogenization step with a higher speed.
- 8. Using a pipette, transfer the homogenized samples into new tubes.
- 9. Proceed with downstream application.

Notes

This protocol does not specify a particular buffer – choose a buffer that is most appropriate for the downstream application or use the lysis buffer provided in a <u>PrecisionPak™</u>, a simplified workflow solution which also includes a bead lysis kit, supplemental reagents for high quality nucleic acids isolations, and an optimized protocol for specific samples.

The provided homogenization conditions serve as a general guideline. Homogenization times, speeds, or beads may need to be optimized based on sample characteristics and desired outcomes.





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