High-throughput homogenization of Drosophila melanogaster larvae using a bead mill-style homogenizer

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Method Article

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Abstract

Introduction

Drosophila melanogaster is a widely used model organism in many biological fields, due to its short life cycle, highly tractable genome, ease of handling, and many other features. Due to its widespread use, there is a great demand for more effective and efficient methods utilizing _Drosophila_. We have developed a protocol for homogenization of _Drosophila_ whereby 24 samples of up to 300mg of larvae can be easily and inexpensively homogenized using the Bullet Blender™ in under 5 minutes for extraction of proteins, nucleic acids, or other analytes. Importantly, the unique technology utilized enables samples to stay cool during homogenization.

Reagents

Lysis/extraction buffer \(you may choose a buffer as suitable for your downstream application).

Equipment

Bullet Blender™ 0.5mm glass grinding beads

Procedure

1. If you have not already, wash Drosophila larvae 3x with 1ml PBS to remove food, surface bacteria, and other contaminants. 2. Aspirate the larvae, or remove as much liquid as possible with a pipette. 3. Place 10-300mg of larvae into microcentrifuge tubes. 4. Add an equal mass of 0.5mm glass beads to the tube. 5. Add 2 volumes of buffer for every mass of larvae \(for example, with 100mg of larvae, use 200ml of buffer). 6. Close the microcentrifuge tubes and place them into the Bullet Blender™. Run for 3 minutes at speed 8. 7. Proceed with your downstream application.

Timing

5 minutes

Critical Steps

Steps 3-6 are all critical to homogenization, and homogenization will be incomplete if you miss one of these steps. It is sometimes possible to homogenize samples in the absence of buffer, however we strongly advise against it.

Troubleshooting

If your homogenization is not satisfactory, be sure you have the correct ratio of sample to beads to buffer. If you have the correct ratio and still are not achieving satisfactory homogenization, run your samples for another three minutes at speed 10. Alternatively, you may try using a denser bead, such as zirconium silicate.

Anticipated Results

The Bullet Blender™, when used appropriately and in conjunction with an appropriate cell lysis buffer, will achieve a high degree of homogenization while maintaining genomic integrity and RNA integrity. RNA integrity numbers of over 8.0 may be expected.

Figures

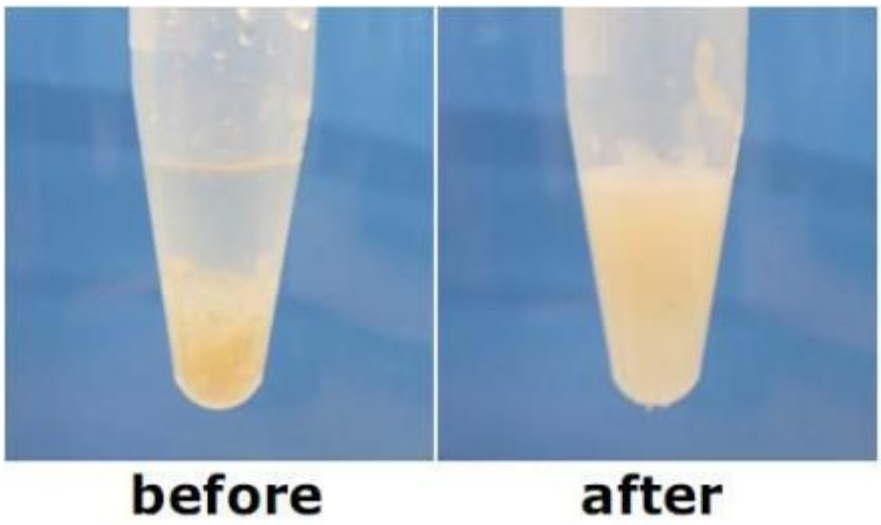

Figure 1

Homogenization of _D. Melanogaster_ larvae. Before/after images of _D. Melanogaster_ larvae homogenization in the Bullet Blender^TM^ using 0.5 mm glass beads.