Rapid, high-throughput homogenization of embryonic or larval zebrafish (Danio rerio)

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Method Article

Keywords: homogenization, zebrafish, embryonic zebrafish, larval zebrafish, zebrafish homogenization, homogenize zebrafish, homogenize, lyse, lysis, disruption, extraction, Danio rerio, D. rerio

Posted Date: October 1st, 2009

DOI: https://doi.org/10.1038/nprot.2009.211

Abstract

Introduction

Danio rerio are an important model system, ideal for use in many developmental, cellular, and molecular biology experiments. As use of this highly tractable model organism increases, so do the needs for more effective and efficient methods regarding their use. Homogenization is commonly used for extraction of proteins, nucleic acids, and chemical analytes from tissue or whole organisms. Here we describe a protocol for highly consistent, high-throughput homogenization of whole or dissected larval or embryonic zebrafish. Twenty-four _D. rerio_ samples may be homogenized in a total time of five minutes.

Reagents

Lysis / Extraction Buffer \(as appropriate for your downstream application)

Equipment

Bullet Blender^{TM} Zirconium Oxide Grinding Beads \(0.5 mm or 1.0 mm)

Procedure

1. Place 10-300mg of zebrafish into microcentrifuge tubes. Unhatched fish do not require prior dechorionation. 2. To each tube, add two masses of zirconium oxide grinding beads for each mass of sample. 3. Add 2 volumes of buffer for every mass of zebrafish \(for example, with 100mg of fish use 200ml of buffer). You may choose your buffer as appropriate for your downstream application. 4. Close the microcentrifuge tubes and place the tubes into the Bullet Blender^{TM} \(they do not need to be balanced). 5. Set controls for SPEED 8 and TIME 3 minutes, then start the run. 6. Remove tubes and proceed with your downstream application.

Timing

5 minutes

Critical Steps

All of the steps are critical. The homogenization will be incomplete if you miss a step.

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.

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

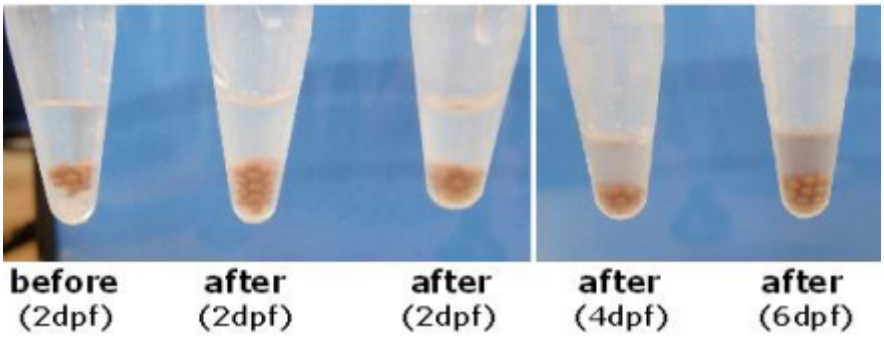

Figure 1

Homogenization of 2-6 days post-fertilization (dpf) zebrafish Before/after images of zebrafish homogenizaton in the Bullet Blender^TM^. Note that the homogenate will be darker at later ages due to increasing melanization of the zebrafish with age.