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Total RNA extraction protocol for marine ostracods (bioluminescent and non-bioluminescent ostracods)

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Bioluminescent Ostracods

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We use this protocol and it's working

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Abstract

Marine ostracods are tiny crustaceans, just 1-2 millimeters in size, with important ecological roles in marine ecosystems. However, extracting RNA from single individuals and specific tissues (upper lip, gut, and compound eye) can be challenging for these tiny marine crustaceans. Here, I optimized an RNA extraction protocol using Agengcourt AMPure XP magnetic beads to extract total RNA from individuals and various tissue types for both bioluminescent and non-bioluminescent ostracods. This protocol is optimized for tissue extraction and works effectively with a single or a few individuals, ensuring high-quality RNA yield from minimal sample input.

Image Attribution

Bioluminescent ostracod - Photeros annecohennae
Image taken by Elliot Lowndes

Prep

1h

1 Clean PCR-clean workstation and lab area.

2 Remove the RNA AMPure XP magnetic beads from the fridge and allow the beads to reach room temperature for 30 minutes. 30m

3 (Optional) Remove the Proteinase K aliquot (stock 20 mg/ml) from the freezer and allow the aliquot to reach room temperature. 30m

4 Remove RNA samples from the -80°C freezer and let them thaw on ice. This process should be quick, taking about 5 minutes to defrost.

5 Prepare fresh 80% ethanol.

6 Prepare SUPERase RNase Inhibitor with RNase- and DNase-free, filtered water for Step 20.

7 (Optional) For an extra homogenization step, prepare the sterile ceramic beads by dispensing a small amount (a few microliters) into tubes for each sample.

Lysing Step

8 Spin the RNA samples for a few seconds to collect the sample and liquid at the bottom of the tube.

9 (Optional) Add Proteinase K to each sample to achieve a final concentration of 2 mg/mL. Pipette up and down at least 3 times, then let the mixture sit at room temperature between 10 - 30 minutes.

10 (Optional) Dispense the ceramic beads (a few microliters) into each sample and vortex for 30 sec to 1 minute (max speed).

10.1 (Optional) Spin the samples briefly and leave them at room temperature for a few minutes while preparing for the next step. Label new tubes for Step 10.2.

10.2 (Optional) Use a 20 µL pipette to carefully remove all the liquid from the ceramic beads and transfer it into a new labeled tube for each sample from Step 10.1. Make sure to remove all liquid from the ceramic beads. Be sure to measure the exact amount of liquid removed. After transferring, spin the tubes briefly to collect any remaining liquid at the bottom. Discard the tubes with ceramic beads.

Add RNA AMPure XP Magnetic Beads

11 Measure the volume of each sample and multiply the volume by 1.8 to determine the final amount of RNA AMPure XP magnetic beads needed for each sample. If the AMPure XP magnetic beads have been sitting out for a long time, ensure you vortex them at maximum speed for 2 minutes.

12 Thoroughly mix each sample by slowly pipetting the entire volume up and down 10-15x.

13 Incubate the samples with RNA AMPure XP magnetic beads for 30 minutes to ensure full binding to the RNA beads.

RNA Extraction

14 Place the sample tubes on a magnetic stand for 5 minutes, or until the supernatant is clear.

15 Carefully remove the clear supernatant from all samples without disturbing the beads, and discard the supernatant.

NOTE: Typically, when extracting RNA from ostracod tissue types (compound eye, upper lip, and gut), the supernatant is clear. However, when extracting RNA from the whole organism, remnants of the carapace may remain due to incomplete homogenization. If interested in the carapace, further homogenization is recommended to collect all remaining carapace material.

16 Add 200 µL of 80% ethanol to each tube and let it sit for 2 minutes.

17 Remove the ethanol and repeat Steps 16 and 17.

18 (Pay close attention to this step) Let the magnetic beads dry (with the tube cap open), but be careful not to let the beads crack! The drying time should be no more than 8 minutes. The beads should appear matte in texture once ready to elute.

19 Prepare the elution water (RNase- and DNase-free, filtered) and the SUPERase RNase Inhibitor.

20 Add the elution water over the bead pellet and carefully pipette up and down at least 20x until the beads are fully resuspended in the elution water.

21 Incubate samples for 30 minutes at room temperature.

22 Place the samples back on the magnetic stand.

23 Let the beads bind to the magnet for 5 to 10 minutes, or until the elution water is clear.

24 Collect the elute carefully and transfer it into a new tube, then place the samples on ice immediately to preserve RNA integrity.