RNA isolation of Pinctada fucata martensii

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ABSTRACT
This protocol provides details on RNA isolation of the tissue of Pinctada fucata martensii.

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GUIDELINES

All tissue harvest and RNA isolation should be performed using RNase-free reagents and tools. The tissue should be put in liquid nitrogen quickly after harvest. And then transfer to -80°C to save until use.

MATERIALS TEXT

MATERIALS

- **TRIzol™ Reagent** Thermo Fisher Catalog #15596018
- Chloroform (Trichloromethane) Contributed by users
- Ethanol absolute Contributed by users
- Isopropyl alcohol Contributed by users

1. Take 50-100 mg of tissue to 2.0 mL tube, then add 1 mL of TRIzol™ Reagent per 50–100 mg of tissue to the tube.

2. Add a steel ball to each tube and then flacker for 3 min by TissueLayer 2. The frequency is 26 (1/S). Repeat this step one time.

3. Incubate for 5 minutes to permit complete dissociation of the nucleoproteins complex.

4. Add 200 ul Chloroform (4 °C pre-cooling), then securely cap the tube and shake for 1 min to mix it completely. Then incubate for 10 min at 4 °C.

5. Centrifuge the lysate for 15 minutes at 12,000 × g at 4 °C, then transfer the aqueous phase containing the RNA (~500 ul) to a new 1.5 mL tube.

6. Add 0.5 mL of isopropanol to per 0.5 mL aqueous phase, then mix it gently.

7. Incubate 10 min at 4 °C. Then centrifuge for 10 minutes at 12,000 × g at 4 °C.

8. Remove the supernatant as much as possible. Centrifuge for 1 minute at 12,000 × g at 4 °C to remove the supernatant completely if necessary.

9. Resuspend the pellet in 1 mL of 75% ethanol per 1 mL of TRIzol™ Reagent used for lysis.

10. Centrifuge for 5 min at 12,000 × g at 4 °C, then discard the supernatant. Repeat step 9 and step 10 for one time.

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11  Air-dry the RNA pellet for 5 min at 4 °C.

12  Resuspend the RNA pellet in 20–50 ul of RNase-free water.

13  Measuring the concentration of RNA by the instrument of SimpliNano and detecting RNA integrity using a 1% agarose gel.

14  RNA sample stored at -80 °C.