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RNA isolation of *Pinctada fucata martensii*

Scientific Reports

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ABSTRACT

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

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

MATERIALS

⊗ TRIzol™ Reagent **Thermo Fisher Catalog #15596018**

⊗ Chloroform (Trichloromethane) **Contributed by users**

⊗ Ethanol absolute **Contributed by users**

⊗ Isopropyl alcohol **Contributed by users**

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- 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.
- 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.