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RNA isolation of Pinctada fucata martensii

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Xiong Xinwei[1]

[1]Guangdong Ocean University

1 Works for me dx.doi.org/10.17504/protocols.io.9qgh5tw

Xiong Xinwei
Guangdong Ocean University

ABSTRACT

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

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0226367

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Xiong X, Xie B, Zheng Z, Deng Y, Jiao Y, Du X (2019) *PfmPif97-like* regulated by Pfm-miR-9b-5p participates in shell formation in *Pinctada fucata martensii*. PLoS ONE 14(12): e0226367. doi: 10.1371/journal.pone.0226367

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

NAME	CATALOG #	VENDOR
TRIzol™ Reagent	15596018	Thermo Fisher
Chloroform (Trichloromethane)	View	
Ethanol absolute	View	
Isopropyl alcohol	View	

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.

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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.

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