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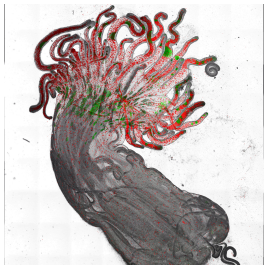
RNA extraction from adult Aiptasia



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Aiptasia Symbiodiniacea...

Kelsey Speer

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External link: http://people.oregonstate.edu/~weisv/assets/trizol_rneasyhybrid.pdf

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Protocol status: Working
We use this protocol and it's working

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Abstract

A protocol for extracting RNA from Aiptasia, modified from the Weis lab protocol by Angela Poole and Mauricio Rodriguez-Lanetty. Note that the final product has significant DNA contamination and needs to be cleaned up via DNase treatment.

Materials

MATERIALS

RNeasy Mini Kit **Qiagen Catalog #**74104

TRIzol Reagent **Thermo Fisher Scientific Catalog #**15596026

Micropestle

Safety warnings

All Trizol steps should be performed in the hood. All Trizol waste should be treated as hazardous.

Before start

Make sure to setup a clean area in the fume hood, wipe down all pipettes with RNase Away/Zap, and cool down the centrifuge.

1 Homogenize starting material in Trizol using a micropestle. Trizol volume should be ~ 1 mL per 0.1g tissue. Once the homogenate is uniform, incubate at room temperature for 5 minutes.

Safety information

All Trizol steps (1-5) should be performed in a fume hood and all Trizol waste should be treated as hazardous.

2 Centrifuge samples for 10 minutes at 12,000 x g at 4°C. This step will pellet any debris and polysaccharides. Transfer the supernatant to a new tube and discard the pellet.

3 Add chloroform to the sample (0.2 mL per 1 mL of Trizol used) and shake vigorously for 20 seconds. Incubate samples at room temperature for 2-3 minutes.

4 Centrifuge samples for 18 minutes at 10,000 x g at 4°C.

Note

Note: past this step you should be careful of RNase contamination

5 Carefully remove the top aqueous (clear) layer and transfer to a new sterile, RNase-free tube.

Note

IMPORTANT: When you take your samples out of the centrifuge, you will see three layers: aqueous (clear, top), interphase (white, middle) and organic (red, bottom). Stay away from the white middle layer - that is where the DNases and RNases are. It is better to leave a little of the aqueous layer behind than to risk contamination.

6 ***Slowly*** add an equal volume of 100% RNase-free EtOH and mix gently by pipetting or inverting tube.

7 Load sample (up to 700 µL) into an RNeasy spin column (Qiagen) and spin at room temperature for 1 min at 8,000 x g. Discard flow through.

Note

If your total volume exceeds 700 µL, repeat this step until all of the sample has passed over the column.

8 Add 700 µl of Buffer RW1 to the column and spin at room temperature for 1 min at 8,000 x g. Discard flow through.

9 Transfer spin column to a new collection tube and add 500 µl of Buffer RPE (check that ethanol has been added) and spin at room temperature for 1 min at 8,000 x g. Discard flow through.

10 Add 500 µl of Buffer RPE and spin at room temperature for 2 min at 8,000 x g. Discard flow through.

11 Spin for an additional 1 minute at 8,000 x g to get rid of remaining buffer in column.

12 Transfer the spin column to a new, RNase-free 1.5 mL collection tube and pipette 30-50 µl of RNase-free water over the center of the membrane (do not touch the membrane with your pipette tip!). Let the sample sit at room temperature for 1 minute and then spin for 1 minute at 8,000 x g to elute RNA.

13 Discard the spin column and store your RNA at -80°C until further use.