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RNA extraction from colonial tunicates

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This protocol has been successfully used with Botrylloides diegensis and has been adapted from the following publication:

An efficient low-cost laboratory workflow for the study of blood cells and RNA extractions in marine invertebrates

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Change gloves frequently, particularly as the protocol progresses from crude extracts to more purified materials. Use sterile tubes. Perform all steps on ice and use RNAse-free water unless otherwise stated.

Liquid nitrogen
Sterile tubes and plastic pestles
Extraction buffer : 0.2M Tris-HCl pH 7.5 , 0.1M LiCl, 5mM EDTA, 1/10 of the total volume of SDS 10%
Phenol pH 4 (4 C)
Chloroform
LiCl (for 50mL: 12.6g 6M LiCl ; 6.3g 3M LiCl)
SC-EtOH: Sodium acetate + 100% Ethanol (1/3 : 2/3)
70% and 100% Ethanol
RNase-free water

1 Clean the slide from which you will take the colony of your interest. See Cleaning colonial ascidians.

2 Isolate a cleaned colony composed of approx. 20 zooids.

2m

2.1 Transfer to a tube and spin at maximum speed for **00:02:00** .

2.2 Remove the excess water and shock-freeze the tube in liquid nitrogen.

3 Add **400 µL** of extraction buffer to the frozen sample and macerate with a plastic pestle.

4 Add **100 µL** more of extraction buffer and **500 µL** of 1:1 phenol:chloroform.

5 Mix the tube by inversion a couple of times until it gets cloudy.

5m

6 Centrifuge the homogenate at 1400 g for **00:05:00** at **4 °C** .

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7 Carefully collect **400 µL** of the upper phase into a new tube.

7.1 Note: if desired this sample could be used for DNA extraction - carefully transfer **200 µL** of the interphase into a new tube (See DNA extraction from colonial tunicates).

8 Add **500 µL** of **6 Molarity (M)** LiCl to the supernatant.

9 Incubate the mixture at **-80 °C** for **01:00:00** . 1h

10 Centrifuge at 1400 g for **00:10:00** at **4 °C** . 10m

11 Discard the supernatant and resuspend the pellet in **1 mL** of **3 Molarity (M)** LiCl.

12 Shake slowly for **00:15:00** at **Room temperature** on a linear shaker. 15m

13 Centrifuge at 1400 g for **00:10:00** at **4 °C** . 10m

14 Discard the supernatant and resuspend the pellet in **1 mL** of SC-EtOH solution.

15 Incubate at **-80 °C** for **00:15:00** . 15m

16 Centrifuge at 1400 g for **00:15:00** at **4 °C** . 15m

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17 Discard the supernatant and wash the pellet with **1 mL** of **70 % volume** Ethanol.

18 Centrifuge at 1400 g for **00:05:00** at **4 °C** . 5m

19 Discard the supernatant and place the tubes up-side-down on a paper towel for **00:05:00** to **00:10:00** . 15m

20 Resuspend the pellet in RNase-free water (**20 µL** to **100 µL** depends on the amount of pellet).

21 Quantify the RNA concentration and quality using the NanoDrop, the capillary electrophoresis and/or the Bioanalyzer.

22 Store at **-80 °C** .

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