Jan 28, 2022

RNA extraction from colonial tunicates

DOI
dx.doi.org/10.17504/protocols.io.b33nqqme

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DOI: https://dx.doi.org/10.17504/protocols.io.b33nqqme

Protocol Citation: Marta Wawrzyniak, Simon Blanchoud 2022. RNA extraction from colonial tunicates. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.b33nqqme**

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

Created: January 20, 2022

Last Modified: January 28, 2022

Protocol Integer ID: 57166

Keywords: ascidians, colonial tunicates, RNA extraction, rna extraction from colonial tunicate, rna extractions in marine, rna extraction, botrylloides diegensi, rna, extraction, colonial tunicate, blood cell

Abstract

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

Guidelines

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.

Materials

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

Troubleshooting

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.

2.1 Transfer to a tube and spin at maximum speed for 00:02:00 . 2m

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.

6 Centrifuge the homogenate at 1400 g for 00:05:00 at 4 °C . 5m

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 [M] 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 [M] 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

17 Discard the supernatant and wash the pellet with 1 mL of [M] 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 .