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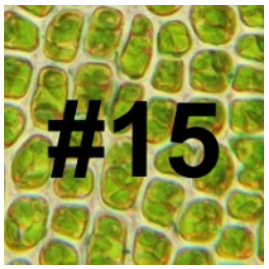
RNA Isolation from Plant Tissue Protocol 15: Hot Acid Phenol Method for Algae



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Protocol status: Working
These protocols were used for RNA extraction from plant tissues in order to support the One Thousand Plants initiative's work to produce RNA-Seq transcriptomes from a diverse collection of plant samples.

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Last Modified: August 22, 2019

Protocol Integer ID: 25211

Abstract

Implemented by: Falicia Goh and Neil Clarke

This RNA isolation method is modified from that described by Köhrer and Domdey[5].

This protocol is part of a collection of eighteen protocols used to isolate total RNA from plant tissue. (RNA Isolation from Plant Tissue Collection: https://www.protocols.io/view/rna-isolation-from-plant-tissue-439gyr6)

[5] Kohrer, K. & Domdey, H. Preparation of high molecular weight RNA. Methods in Enzymology 194, 398-405 (1991).

Attachments

PDF

journal.pone.0050226...

291KB

Materials

Reagents

Extraction Buffer:

- 1 % SDS (v/v, starting from 10 % SDS stock solution)
- 51 mM sodium acetate pH 5.5
- 10 mM EDTA
- DEPC treated water

Note

The final reaction buffer was filter purified using Nalgene 0.22 µM filter.

Other reagents:

- Acid phenol (pH 4.3)
- Phenol:chloroform (5:1) acid equilibrated to pH 4.7 from Sigma
- Isopropanol
- 70 % ethanol (diluted in DEPC treated water H_2O)
- 3 M Sodium acetate pH 5.5

Safety warnings

Please see SDS (Safety Data Sheet) for hazards and safety warnings.

1 Preheat phenol and phenol:chloroform to 65 °C. Heated phenol should not be re-used.

2 Collect algae cells via centrifugation for 00:10:00 at 16100 x g at Room temperature.

2.1 Flash freeze pellets with liquid nitrogen and keep at -80 °C until extractions are carried out.

3 Re-suspend frozen pellet in 800 µL of preheated **extraction buffer**.

4 Immediately add 800 µL of hot acid phenol.

4.1 Vortex the tubes for 00:00:15.

5 Incubate at 65 °C for 00:10:00. Vortex every 1 min for 00:00:10.

6 Centrifuge at 16100 x g at 4 °C for 00:05:00.

7 The aqueous phase was transferred to fresh 1.5 ml micro-centrifuge tube.

8 Repeat steps 5 - 7. Repeat 3x (depending on the amount of cells used). go to step #5

9 Extract with equal volume of phenol:chloroform (5:1).

9.1 Vortex for 00:01:00 at Room temperature .

9.2 Spin for 00:05:00 in a microcentrifuge at top speed.

9.3 Repeat step 9 three times. go to step #9

10 Transfer aqueous phase to a new 1.5 ml microfuge tube. Volume should be ~ 700 µL .

11 Add 1/10 volume of [M] 3 Molarity (M) sodium acetate, pH 5.5, and 1 volume of isopropanol.

11.1 Hold at 4 °C for 00:30:00 or more.

12 Spin in micro-centrifuge at 4 °C at top speed for 00:20:00 .

13 Remove the supernatant without dislodging the pellet.

14 Wash the pellet with 70 % ethanol.

15 Invert and air dry tubes at room temperature.

15.1 The pellet was re-suspended in 50 µL DEPC treated H_2O.

15.2 The RNAs were stored at -20 °C .