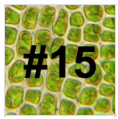

Aug 20, 2019

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

1 Works for me dx.doi.org/10.17504/protocols.io.4u3gwyn

Scott C. Edmunds
GigaScience/BGI Hong Kong/Bauhinia Genome

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).

journal.pone.0050226.s011.PDF

MATERIALS TEXT

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

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**.