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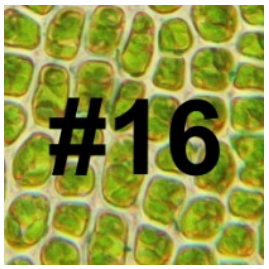
RNA Isolation from Plant Tissue Protocol 16: CTAB-Hot Acid Phenol Method for Algae



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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: 25208

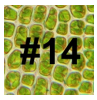
Keywords: Algae, RNA, RNA extraction, RNA isolation, RNA

Abstract

Implemented by: Falicia Goh and Neil Clarke

This RNA isolation method is a combination and modification of the hot acid phenol method (protocol 14) and that described by Asif et al[6]. This method was used for two taxa (P. cruentum and B. braunii).

Protocol



NAME

RNA Isolation from Plant Tissue Protocol 14: Ambion Trizol RNA Extraction in Microcentrifuge Tubes with Turbo DNAfree Digestion

CREATED BY

Eric Carpenter

PREVIEW

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)

[6] Asif, M.H., Dhawan, P. & Nath, P. A simple procedure for the isolation of high quality RNA from ripening banana fruit. Plant Molecular Biology Reporter 18, 109-115 (2000).

Attachments

PDF

journal.pone.0050226...

290KB

Materials

Reagents

Extraction Buffer:

- 100 mM Tris-HCl pH 8.2
- 1.4 M NaCl
- 2 % CTAB
- 20 mM EDTA pH 8.2
- 1 µl of 2-mercaptoethanol per ml of buffer just before use
- 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
- Chloroform
- Isopropanol
- 70 % ethanol (diluted in DEPC treated water H_2O)
- 3 M Sodium acetate pH 5.5
- 3 M Lithium chloride

Safety warnings

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

1 Preheat phenol and phenol:chloroform to 65 °C .

Note

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 the frozen pellet in 800 µL of preheated **extraction buffer**.

4 Incubate at 65 °C for 01:00:00 . Gently vortex every 00:15:00 .

5 Cool to Room temperature .

5.1 Add equal volume of chloroform.

5.2 Shake vigorously until 2 phases form an emulsion.

6 Collect the aqueous phase by centrifuging for 00:10:00 in micro-centrifuge at 16100 x g at Room temperature .

7 Collect aqueous phase and re-extract with an equal volume of chloroform.

7.1 Centrifuge as above.

8 Collect aqueous phase and add [M] 10 Molarity (M) LiCl to a final concentration of [M] 3 Molarity (M) .

8.1 Allow the RNA to precipitate at 4 °C overnight.

9 Recover the RNA by centrifugation at 16100 x g at 4 °C for 00:20:00 .

10 Dissolve pellet in DEPC treated water.

10.1 Extract once with hot acid phenol.

11 Extract the aqueous phase with equal volume of phenol:chloroform (5:1).

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

12.1 Spin for 00:05:00 in a micro-centrifuge at top speed.

13 Extract the aqueous phase with equal volume of chloroform.

14 Collect aqueous phase and add 1/30 volume of [M] 3 Molarity (M) sodium acetate pH 5.5 and 0.1 volume of 100 % ethanol.

14.1 Mix well and keep on ice for 00:30:00 .

14.2 Centrifuge in cold for 00:25:00 .

14.3 A white jelly-like pellet consisting mostly of polysaccharides is obtained and discarded.

15 To the clear supernatant add [M] 3 Molarity (M) sodium acetate pH 5.2 to a final concentration of [M] 0.3 Molarity (M) and 3 volumes of 100 % ethanol.

15.1 Allow the RNA to precipitate at -80 °C for 03:00:00 to overnight.

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

17 Wash the pellet with 70 % ethanol.

18 Invert tubes and air dry at room temperature.

19 Resuspend pellets in 50 µL of DEPC treated water.