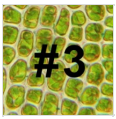

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RNA Isolation from Plant Tissue Protocol 3: CTAB-PVP Method

Beijing Genomics Institute[1]

[1]Beijing Genomics Institute

1 Works for me dx.doi.org/10.17504/protocols.io.4vygw7w

BGI

Eric Carpenter

ABSTRACT

Implemented by: Beijing Genomics Institute

A similar protocol was used by C. dePamphilis and P. Ralph.

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)

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MATERIALS TEXT

Reagents:

CTAB-PVP Buffer:

- CTAB (Hexadecyltrimethylammonium bromide; 2 % w/v)
- PVP-40 (2 % w/v)
- 100 mM Tris-HCl (pH 8.0)
- 25 mM EDTA
- 2 M NaCl (Warmed to 65 °C in a water bath to suspend in solution)
- Add β-ME to final concentration of 2 % before use

SSTE buffer:

- 1 M NaCl
- SDS (0.5 % w/v)
- 10 mM Tris-HCl (pH 8.0)
- EDTA (1 mM)

Other reagents:

- 75 % ethanol (treated with 0.1 % DEPC)
- 96–100 % ethanol
- Acid phenol (pH 4.5)
- Chloroform
- Isoamyl alcohol
- 10 M LiCl
- Glycogen (5 mg/ml)
- 3 M Sodium Acetate (pH 5.2)
- RNase free water

SAFETY WARNINGS

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

1 Grind tissue to a powder in liquid nitrogen.

2 Add **200 mg**–**500 mg** of ground, frozen tissue to **3 ml** of pre-heated extraction buffer in a 5 ml tube.

3 Vortex the tube until the tissue is mixed with the buffer.

4 Incubate the tube at **65 °C** for **00:30:00** (min), vortexing briefly (**00:00:15**) every 2–3 min during the incubation.

5 Aliquot the mixture into four 2 ml RNase free tubes, **1 ml** each tube.

6 Spin the tube at **12000 x g** for **00:10:00** in a microcentrifuge.

All of the insoluble matter should form a pellet at the bottom of the tube.

When used for algae the centrifugation was performed at **3000 x g**.

7 Pour the supernatant into a new 2 ml RNase free tube.

When used for algae, 15 ml tubes were used.

8 Add equal volume of 24:1 chloroform:isoamyl alcohol to fill the tube.

9 Vortex tubes until the phases mix and appear cloudy.

9.1 Incubate at 20 °C – 25 °C for 00:05:00 .

10 Spin the tubes at 12000 x g for 00:10:00 in a microcentrifuge.

When used for algae the centrifugation was performed at 3000 x g.

11 Transfer the upper, aqueous phase to new 2 ml RNase free tubes.

When used for algae, 15 ml tubes were used.

11.1 Repeat step 7 to 9 one more time. go to step #7

12 Transfer the upper, aqueous phase to new 2 ml RNase free tubes.

12.1 Add 1/3 volume of [M]10 Molarity (M) LiCl to each tube.

12.2 Mix and let stand at 4 °C for 06:00:00 – 08:00:00 or overnight to precipitate RNA.

13 Spin the tubes at 18000 x g for 00:20:00 in a microcentrifuge.

13.1 Decant the supernatant, taking care not to lose the pellet.

14 Add 1 ml 75 % ethanol to the pellet.

15 Spin the tube at maximum speed (> 11270 x g) for 00:05:00 in a microcentrifuge.

15.1 Decant the supernatant carefully.

16 Repeat steps 14 and 15 one more time. go to step #14

17 Open cap and air-dry the pellet.

18 Add 30 µl RNase free water to dissolve the pellet.

18.1 Then add 70 µl SSTE buffer to each tube.

19 Combine all 4 tubes into 1 tube.

20 Add equal volume of 25:24:1 acid phenol:chloroform:isoamyl alcohol to the tube.

21 Vortex the tubes until the phases mix and appear cloudy.

21.1 Then incubate at 20 °C for 00:05:00 .

22 Spin the tube at 12000 x g for 00:10:00 .

23 Transfer the upper, aqueous phase to a new 2 ml RNase free tube.

23.1 Add equal volume of 24:1 chloroform:isoamyl alcohol to the tube.

24 Vortex the tubes until the phases mix and appear cloudy.

24.1 Then incubate at 20 °C for 00:05:00 .

25 Spin the tube at 12000 x g for 00:10:00 in a microcentrifuge.

26 Transfer the upper, aqueous phase to a new 2 ml RNase free tube.

26.1 Add 2 volumes of cooled 100 % ethanol, 1/10 volumes of NaAc (pH 5.2) and **2 µl** glycogen.

26.2 Mix and incubate at **-20 °C** for **02:00:00** .

27 Spin the tube at **18000 x g** for **00:20:00** in a microcentrifuge.

27.1 Then decant the supernatant, taking care not to lose the pellet.

28 Add **1 ml** 75 % cooled ethanol to the pellet.

28.1 Leave at **20 °C** for **00:03:00** .

29 Centrifuge at **4 °C** for **00:05:00** at **12000 x g**.

29.1 Decant the liquid carefully, taking care not to lose the pellet.

29.2 Briefly centrifuge to collect the residual liquid and remove it with a pipette.

30 Wash the pellet twice with cooled 75 % DEPC-ethanol.

30.1 Open cap and air-dry the pellet.

31 Add **30 µl** RNase-free water to dissolve the pellet.

32 Treat RNA with DNase I as per supplier's protocols.