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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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RNA Isolation from Plant Tissue Protocol 4: CTAB-PVP-TRIZOL Method

In 1 collection

GigaScience Press

BGI

Eric Carpenter

ABSTRACT

Implemented by: Beijing Genomics Institute

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>)

ATTACHMENTS

[journal.pone.0050226.s011.PDF](#)

MATERIALS

MATERIALS

TRIZOL Reagent Thermo Fisher Scientific Catalog #15596026

SAFETY WARNINGS

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

Keywords: RNA, RNA isolation, RNA extraction, plant tissue

- 1 Grind tissue to a powder in liquid nitrogen.
- 2 Add 200 mg – 500 mg of ground tissue to 3.0 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 , 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 in each tube.
- 6 Spin the tube at 12000 x g for 00:10:00 in a centrifuge.

Note

All of the insoluble matter should form a pellet at the bottom of the tube.
- 7 Pour the supernatant into a new 2 ml tube.
- 8 Add an 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 for 00:05:00 .

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

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

11.1 Repeat steps 8 to 10 one more time. [go to step #8](#)

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

12.1 Add 1/3 volume of 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 tubes at 18000 x g for 00:20:00 in a centrifuge and decant the supernatant, taking care not to lose the pellet.
- 14** Add 1 mL 75 % cooled ethanol to the pellet.
- 15** Spin the tube at maximum speed for 00:05:00 in a centrifuge.
- 15.1** Decant the supernatant carefully.
- 15.2** Repeat steps 14 and 15 one more time. go to step #14
- 16** Open cap and air-dry the pellet.
- 17** Add 30 µL RNase free water to dissolve the pellet.
- 17.1** Then add 300 µL TRIzol reagent and equal volume of chloroform to TRIzol reagent (Invitrogen).

17.2 Vortex vigorously and store at 20 °C for 00:05:00 .

18 Centrifuge at > 12000 x g for 00:10:00 .

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

19.1 Add 2 volumes of cooled 100% ethanol, 1/10 volume of NaAc and 2 µL of glycogen.

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

20 Spin tubes at > 12000 x g for 00:20:00 at 4 °C in a centrifuge.

21 Decant the supernatant taking care not to lose the pellet.

21.1 Add 1 mL 75 % ethanol to the pellet.

21.2 Let tube stand at 20 °C for 00:03:00 .

22 Centrifuge at 4 °C for 00:05:00 at > 12000 x g .

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

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

23 Repeat steps 21 and 22 one more time. go to step #21

24 Open cap and air dry the pellet.

25 Add 10 µL – 30 µL RNase free water to dissolve the pellet.