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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 7: pBIOZOL-LiCl 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.s01](#)
 1.PDF

MATERIALS

Reagents

- Acid phenol (pH 4.5)
- Chloroform
- Isopropyl alcohol
- 75 % ethanol (DEPC treated)
- 100 % ethanol
- 2 M NaAc (pH 4.2)
- 3 M NaAc (pH 5.2)
- 5 M NaCl
- 10 M LiCl
- pBIOZOL Reagent (Beijing Bai billion New Technology Co., Beijing, China)
- RNase-free water

SSTE Buffer:

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

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

SAFETY WARNINGS

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

- 1 Grind tissue to a powder in liquid nitrogen.
- 2 Add 1.3 mL of cold (4 °C) pBIOZOL reagent for up to 100 mg gram of frozen, ground tissue.
 - 2.1 Mix by briefly vortexing or flicking the bottom of the tube until the sample is thoroughly suspended.
- 3 Incubate the tube for 00:05:00 at Room temperature .

Note

Lay the tube down horizontally to maximize surface area during RNA extraction.

- 4 Centrifuge at 12000 x g for 00:02:00 .
- 5 Transfer the supernatant to a new 1.5 ml RNase-free tube.

6 Add 50 µL of 2 Molarity (M) NaAc (pH 4.2) to the extract.

6.1 Tap tube to mix.

6.2 Then add 100 µL 5 Molarity (M) NaCl and 300 µL chloroform.

6.3 Vortex vigorously.

7 Centrifuge the mixture at 4 °C for 00:10:00 at 12000 x g to separate the phases.

7.1 Transfer the top aqueous phase (about 400 µL – 500 µL) to a new 1.5 ml RNase-free tube.

8 Add to the aqueous phase 1/3 volume of 10 Molarity (M) LiCl.

8.1 Mix and let stand at 4 °C overnight.

9 Centrifuge the mixture at 4 °C for 00:20:00 at > 12000 x g .

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

10.1 Add 1 mL of 75 % ethanol to the pellet.

10.2 Stand the tube at Room temperature for 00:03:00 .

Note

Pellet may be difficult to see.

11 Centrifuge at 4 °C for 00:03:00 at > 12000 x g .

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

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

12 Repeat the previous two steps.

13 Add 50 µL RNase-free water to dissolve the RNA pellet.

13.1 Pipette the water up and down over the pellet to dissolve the RNA.

Note

If you extract more than 100 mg plant tissues, combine different extractions to one tube.

14 Add [SSTE buffer](#) to RNA to a total volume of 600 µL .

14.1 Then add equal volume of 25:24:1 acid phenol:chloroform:isoamyl alcohol to the tube.

15 Vortex the tube until the phases mix and appears cloudy.

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

16 Centrifuge at 12000 x g for 00:10:00 in a microcentrifuge.

17 Transfer the top, aqueous phase to a new 1.5 ml RNase-free tube.

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

18 Vortex the tube until the phases mix and appear cloudy.

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

19 Centrifuge at 12000 x g for 00:10:00 .

20 Transfer the top aqueous phase to a new 1.5 ml RNase-free tube.

20.1 Add to the aqueous phase 2 volumes of 100 % ethanol, 1/10 volume of 3 Molarity (M) NaAc (pH 5.2) and 2 µL 5 mg/ml glycogen.

20.2 Invert tube to mix.

20.3 Store at -20 °C for 02:00:00 .

21 Centrifuge at 4 °C for 00:20:00 at > 12000 x g .

21.1 Decant the supernatant carefully to avoid losing the pellet.

22 Add 1 mL of 75 % ethanol to the pellet.

22.1 Incubate at 20 °C for 00:03:00 .

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

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

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

24 Repeat step 18 and 19.

25 Open cap and air-dry the pellet no more than 00:05:00 .

26 Add 30 μL RNase-free water to dissolve the pellet.

27 Before library construction, treat RNA with DNase I.