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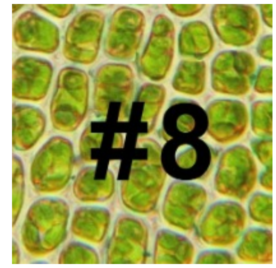
RNA Isolation from Plant Tissue Protocol 8: CTAB/Acid Phenol/Silica Membrane Method



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Peer-reviewed method

In 1 collection

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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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Abstract

Implemented by: Michael Deyholos

This protocol combines elements of standard CTAB, acid phenol, and silica-membrane protocols.
The protocol was developed to extract total RNA from a wide range of plant species and tissues, and to do so in the smallest volume possible to yield >20 µg of total RNA using as little as 50 mg of fresh tissue. Maintaining a small extraction volume also allows for many samples to be processed in parallel in microcentrifuge tubes; at least 12 samples can be processed in 3–4 hours.
The protocol has been used successfully with dozens of species, including tissues rich in polysaccharides and/or secondary metabolites. There are four organic extractions in total (three chloroform and one phenol:chloroform). For many species/tissues, the full set of chloroform extractions in the order specified is required to maximize RNA purity and to prevent phase inversion during the acid phenol extraction.

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...
276KB

Guidelines

Because the RNA is protected from RNase by denaturants throughout most of the protocol, it is not necessary to use specially treated (e.g. baked or DEPC) labware or solutions.
We have also found that **QIAshredder columns** have little positive or negative impact on quality and yields, although they may be useful for some tissues that are not easily disrupted by grinding in a mortar.

Instead of using a Qiagen silica membrane spin column (also available from other manufacturers), it is also possible to precipitate the RNA after the last organic extraction (step 22). However, the silica membrane columns provide more reliable recovery of RNA (especially in a high-throughput, service environment), and allow for the convenient removal of residual DNA through an on-column digestion. We have not found it beneficial to collect more than one RNA elution from a spin column, or to pass the eluate through the same column twice.

The binding capacity of the Qiagen column (~100 µg RNA) exceeds the yield of RNA that can be extracted from tissue in a single 2 ml microcentrifuge tube. Therefore, when yields of >20 µg total RNA/~1 g fresh weight of tissue are required, it is most efficient to aliquot the tissue sample between two tubes, process the aliquots independently through stage 24, then pool the samples into a single column.

Both the acid phenol extraction and the QIAgen silica membrane washes are biased in favour of the recovery of RNA over DNA. Indeed, there appears to be little residual DNA present even before on-column DNase I digestion. Nevertheless, it is probably worthwhile to conduct the digestion on all samples to limit the possibility that any genomic DNA molecules could be used as a sequencing template.

Materials

MATERIALS

- QIAshredder **Qiagen**
- Buffer RLT **Qiagen Catalog** #79216
- RNeasy Plant Mini Kit **Qiagen Catalog** #74904
- Buffer RW1 **Qiagen Catalog** #1053394
- Buffer RPE **Qiagen Catalog** #1018013

Reagents

CTAB extraction buffer (for 200 ml final volume):

- 40 ml Tris-HCl pH 7.5
- 10 ml 0.5 M EDTA
- 35.04 g NaCl
- 4 g CTAB
- 4 g SDS (sodium dodecyl sulfate)
- 4 g PVP (polyvinylpyrrolidone)
- 8 ml β-ME (2-mercaptoethanol)

Note

Heat to 65°C to dissolve components in solution. SDS may not completely dissolve

Other reagents:

- Saturated NaOH solution
- Chloroform:Isoamyl Alcohol (24:1)
- Phenol:Chloroform (5:1, pH 4.5).

Note

It is essential to use acid-equilibrated phenol, rather than Tris-buffered phenol.

- Qiagen's RLT, RW1, RPE, DNase digestion solutions, plus Plant Mini Kit spin columns (pink)

Troubleshooting

Safety warnings

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

Before start

Mortars should be rinsed with saturated NaOH to remove residual RNA, and then rinsed with DEPC-treated water.

1 Grind tissue to a powder in liquid nitrogen.

2 Add 400 mg – 600 mg of ground, frozen tissue to 1.4 mL of pre-heated **extraction buffer** in a 2 ml microcentrifuge tube.

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

> Note
>
> To facilitate mixing, you may have to invert the tube on the vortex, and/or heat it briefly in a 65 °C water bath.

4 Incubate the tube at 65 °C for 00:10:00 – 00:15:00 , vortexing briefly (15 seconds) twice during the incubation.

5 Spin the tube at maximum speed (> 11269 x g) for 00:03:00 in a microcentrifuge.

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

6 Pour the supernatant into a new 2 ml tube.

Solvent Extraction #1

7 Add enough 24:1 chloroform:isoamyl alcohol to fill the tube.

8 Vortex the tube for 00:00:15 or until the phases mix and appear cloudy.

9 Spin the tube at maximum speed (> 11269 x g) for 00:03:00 in a microcentrifuge.

Note

Most of the chlorophyll will be dissolved in the lower, organic phase.

10 Transfer the upper, aqueous phase to a new 2 ml tube, using a disposable pipette.

Note

Avoid transferring any of the material (usually a white precipitate) from the boundary between the phases.

Solvent Extraction #2

11 Add 24:1 chloroform:isoamyl alcohol to the tube containing the aqueous phase (this should be at least 900 µL of 24:1 chloroform:isoamyl).

12 Vortex the tube for 00:00:15 or until the phases mix and appear cloudy.

13 Spin the tube at maximum speed (> 11269 x g) for 00:03:00 in a microcentrifuge.

14 Transfer the upper, aqueous phase to a new 2 ml tube, using a disposable pipette.

Note

Avoid transferring any of the material (usually a white precipitate) from the boundary between the phases.

Solvent Extraction #3

15 Add 1 mL 5:1 phenol:chlorofom pH 4.5 to the tube containing the aqueous phase.

16 Vortex the tube for 00:00:15 or until the phases mix and appear cloudy.

17 Spin the tube at maximum speed (> 11269 x g) for 00:03:00 in a microcentrifuge.

18 Transfer the upper, aqueous phase to a new 2 ml tube, using a disposable pipette.

Note

Avoid transferring any of the material (usually a white precipitate) from the boundary between the phases.

Solvent Extraction #4

19 Add 24:1 chloroform:isoamyl alcohol to the tube containing the aqueous phase (this should be at least 900 µL of 24:1 chloroform:isoamyl).

20 Vortex the tube for 00:00:15 or until the phases mix and appear cloudy.

21 Spin the tube at maximum speed (> 11269 x g) for 00:03:00 in a microcentrifuge.

22 Transfer the upper, aqueous phase to a new 2 ml tube, using a disposable pipette.

Note

Avoid transferring any of the material (usually a white precipitate) from the boundary between the phases.

23 Estimate the volume of the aqueous phase based on the markings on the tube.

23.1 Add at least 0.5 volumes of solution RLT, and mix by briefly shaking.

24 Estimate the new total volume in the tube.

24.1 Add 0.5 volumes of 95–100 % ethanol.

24.2 Mix by briefly shaking.

25 Pour the contents of the tube into a Qiagen miniRNA spin column (pink), until the column is almost filled with liquid.

26 Cap the tube and spin for 00:00:15 at > 5000 x g .

Note

The column should be empty at the end of this spin.

27 Discard the flow-through from the collection tube.

28 Repeat the previous two steps with the same miniRNA spin column, until all of the liquid in the tube(s) has been passed through the column.

Note

The nucleic acid is now bound to the silica membrane in the spin column.

29 Apply 350 µL of solution RW1 to the spin column.

30 Cap the tube and spin for 00:00:15 at > 5000 x g .

Note

The column should be empty at the end of this spin.

31 Discard the flow-through from the collection tube.

32 Apply 80 µL of DNase digestion solution to the membrane of the spin column.

33 Incubate at room temperature for 00:15:00 .

34 Apply 350 µL of solution RW1 to the spin column.

35 Cap the tube and spin for 00:00:15 at > 500 x g .

Note

The column should be empty at the end of this spin.

36 Discard the flow-through from the collection tube.

37 Apply 500 µL of solution RPE to the spin column.

38 Cap the tube and spin for 00:00:15 at > 5000 x g .

Note

The column should be empty at the end of this spin.

39 Discard the flow-through from the collection tube.

40 Apply 500 µL of solution RPE to the spin column.

41 Cap the tube and spin for 00:00:15 at > 5000 x g .

Note

The column should be empty at the end of this spin.

42 Discard the flow-through from the collection tube.

43 Transfer the spin column to a new collection tube.

43.1 Spin at maximum speed for 00:03:00 to remove remaining liquid from the silica membrane.

44 Transfer the spin column to a new 1.5 ml conical bottom microcentrifuge tube

45 Add 44 µL of RNase-free water to the column.

46 Spin at maximum speed for 00:01:00 to elute.