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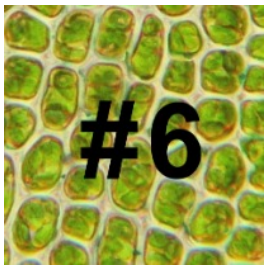
RNA Isolation from Plant Tissue Protocol 6: pBIOZOL and Qiagen RNeasy Plant Mini Kit Method



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Appendix S1 of "Evaluating Methods for Isolating Total RNA and Predicting the Success of Sequencing Phylogenetically Diverse Plant Transcriptomes" Marc T. J. Johnson et al. PLOS ONE, November 21, 2012. https://doi.org/10.1371/journal.pone.0050226

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

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) and was originally published as part of Appendix S1 of "Evaluating Methods for Isolating Total RNA and Predicting the Success of Sequencing Phylogenetically Diverse Plant Transcriptomes" Marc T. J. Johnson et al. PLOS ONE, November 21, 2012. https://doi.org/10.1371/journal.pone.0050226

Attachments

PDF

journal.pone.0050226...

283KB

Materials

MATERIALS

RNeasy Plant Mini Kit **Qiagen Catalog #**74904

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 of frozen ground tissue.

2.1 Mix by briefly vortexing or flicking the bottom of the tube until the sample is thoroughly re-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 for 00:10:00 at 12000 x g in a microcentrifuge at Room temperature .

4.1 Transfer the supernatant to a new 1.5 mL RNase-free tube.

5 Add 100 µL of [M] 5 Molarity (M) NaCl and 300 µL chloroform.

5.1 Vortex vigorously.

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

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

7.1 Add an equal volume of 5:1 acid phenol:chloroform to the tube.

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

8.1 Incubate at 20 °C for 00:05:00 .

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

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

10.1 Add to the aqueous phase equal volume of 24:1 chloroform:isoamyl alcohol.

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

10.3 Then incubate at Room temperature for 00:05:00 .

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

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

12.1 Add 1/2 volume of 100 % ethanol.

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

14 Cap the tube.

14.1 Centrifuge at 12000 x g for 00:00:15 .

Note

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

15 Discard the flow-through from the collection tube.

16 Repeat the previous two steps with the same mini RNA 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.

17 Apply 700 µL of solution RW1 to the spin column.

18 Cap the tube.

18.1 Centrifuge at 12000 x g for 00:00:15 .

Note

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

19 Discard the flow-through from the collection tube.

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

20.1 Cap the tube

20.2 Centrifuge at 12000 x g for 00:00:15 .

Note

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

21 Discard the flow-through.

22 Repeat previous two steps one time. go to step #20.2

23 Spin at maximum speed for 00:02:00 to remove remaining liquid from the silica membrane.

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

25 Add 30 µL – 50 µL of RNase-free water to the column.

25.1 Then let tube incubate at 20 °C for 00:03:00 .

26 Spin at maximum speed for 00:01:00 to collect RNA solution.