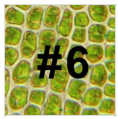

Aug 20, 2019

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

Marc T. J. Johnson[1], Eric J. Carpenter[2], Zhijian Tian[3], Richard Bruskiewich[4], Jason N. Burris[5], Charlotte T. Carrigan[6], Mark W. Chase[7], Neil D. Clarke[8], Sarah Covshoff[9], Claude W. dePamphilis[10], Patrick P. Edger[11], Falicia Goh[8], Sean Graham[12], Stephan Greiner[13], Julian M. Hibberd[9], Ingrid Jordon-Thaden[14,15], Toni M. Kutchan[16], James Leebens-Mack[6], Michael Melkonian[17], Nicholas Miles[14,15], Henrietta Myburg[18], Jordan Patterson[2], J. Chris Pires[11], Paula Ralph[10], Megan Rolf[16], Rowan F. Sage[19], Douglas Soltis[14], Pamela Soltis[15], Dennis Stevenson[20], C. Neal Stewart Jr[5], Barbara Surek[17], Christina J. M. Thomsen[1], Juan Carlos Villarreal[21], Xiaolei Wu[3], Yong Zhang[3], Michael K. Deyholos[2], Gane Ka-Shu Wong[2,3,22]

[1]Department of Biology, University of Toronto at Mississauga, Mississauga, Ontario, Canada, [2]Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada, [3]BGI-Shenzhen, Bei Shan Industrial Zone, Yantian District, Shenzhen, China, [4]International Rice Research Institute, Metro Manila, Philippines, [5]Department of Plant Sciences, University of Tennessee, Knoxville, Tennessee, United States of America, [6]Department of Plant Biology, University of Georgia, Athens, Georgia, United States of America, [7]Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey, United Kingdom, [8]Genome Institute of Singapore, Singapore, Singapore, [9]Department of Plant Sciences, University of Cambridge, Cambridge, United Kingdom, [10]Department of Biology and Intercollege Graduate Program in Plant Biology, Huck Institutes of the Life Sciences, Pennsylvania State University, University Park, Pennsylvania, United States of America, [11]Division of Biological Sciences, University of Missouri, Columbia, Missouri, United States of America, [12]Department of Botany and UBC Botanical Garden, University of British Columbia, Vancouver, British Columbia, Canada, [13]Max Planck Institute for Molecular Plant Physiology, Wissenschaftspark Golm, Am Mühlenberg 1, Potsdam-Golm, Germany, [14]Department of Biology, University of Florida, Gainesville, Florida, United States of America, [15]Florida Museum of Natural History, University of Florida, Gainesville, Florida, United States of America, [16]Donald Danforth Plant Science Center, St. Louis, Missouri, United States of America, [17]Department of Botany, Cologne Biocenter, University of Cologne, Cologne, Germany, [18]Department of Plant Biology, North Carolina State University, Raleigh, North Carolina, United States of America, [19]Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Ontario, Canada, [20]New York Botanical Garden, Bronx, New York, United States of America, [21]Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, Connecticut, United States of America, [22]Department of Medicine, University of Alberta, Edmonton, Alberta, Canada

1 *Works for me* dx.doi.org/10.17504/protocols.io.4rfgv3n

Eric Carpenter

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

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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

journal.pone.0050226.s011.PDF

MATERIALS

NAME	CATALOG #	VENDOR
RNeasy Plant Mini Kit	74904	Qiagen

MATERIALS TEXT

Reagents

- Acid phenol (pH 4.5)
- Chloroform
- Isopropyl alcohol
- 75 % ethanol (DEPC treated)
- 100 % ethanol
- 5 M NaCl
- pBIOZOL Reagent (Beijing Bai billion New Technology Co., Beijing, China)
- RNeasy Plant Mini Kit (Qiagen)
- 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 **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**.

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 **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** .

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.

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** .

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**.

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.