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RNA Isolation from Plant Tissue Protocol 12: Hot Acid Phenol Method for Angiosperms

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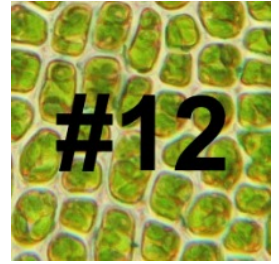
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Eric Carpenter



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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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Protocol Integer ID: 25111

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

Abstract

Implemented by: Sarah Covshoff, Rowan Sage and Julian Hibberd

This RNA isolation method is a multi-component method involving an initial extraction by hot acid phenol and then a purification and DNase treatment using the RNeasy Mini Kit by Qiagen.

The method described below is a modification of a method described by van Tunen et al.³.

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>)

³ van Tunen, A.J. et al. Cloning of the two chalcone flavanone isomerase genes from *Petunia hybrida*: coordinate, light-regulated and differential expression of flavonoid genes. The EMBO Journal 7, 1257-1263 (1988).

Attachments



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

287KB



Materials

MATERIALS

⊗ RNase-Free DNase Set **Qiagen Catalog #79254**

⊗ RNeasy® Mini Kit **Qiagen Catalog #74104**

Reagents

Extraction Buffer:

- 100 mM Tris pH 9.0
- 1 % SDS (v/v, starting from 10% SDS stock solution)
- 100 mM LiCl
- 10 mM EDTA
- RNase-free water

Note










All components were filter purified and then the final reaction buffer was also filter purified using a Millipore Stericup.

Other reagents:

- Acid phenol (pH 4.3)
- Chloroform:isoamyl (24:1)
- 70 % ethanol (diluted in RNase free H₂O)
- 4 M LiCl




Safety warnings

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

















- 1 Add  3 mL of saturated acid phenol (pH 4.3) and  3 mL of **RNA extraction buffer** to a 15 ml snap cap tube. Warm tube to  65 °C in a fume hood using a heat block.
 - 1.1 Add  3 mL of saturated acid phenol (pH 4.3) and  3 mL of **RNA extraction buffer** to a 15 ml snap cap tube.
 - 1.2 Warm tube to  65 °C in a fume hood using a heat block.
- 2 Homogenize tissue in liquid nitrogen using a mortar and pestle.
- 3 Transfer up to  1.0 g of ground tissue to a tube containing the phenol-buffer mixture and close the snap cap tube completely (second stop). Immediately mix tube by hand. Vortex until the phases mix and appear cloudy. Keep at  Room temperature or  65 °C depending on whether SDS is precipitating.

Note







Use a spatula and funnel chilled in liquid nitrogen to transfer the powdered tissue to the tube.

- 3.1 Transfer up to  1.0 g of ground tissue to a tube containing the phenol-buffer mixture and close the snap cap tube completely (second stop).
- 3.2 Immediately mix tube by hand.
- 3.3 Vortex until the phases mix and appear cloudy.
- 3.4 Keep at  Room temperature or  65 °C depending on whether SDS is precipitating.






- 4 Centrifuge at  2500 x g for  00:10:00 .
- 5 Transfer aqueous phase to a new 15 ml tube.
- 6 Add  3 mL of chloroform:isoamyl (24:1 ratio). Close the snap cap tube completely (second stop) and mix contents immediately by hand. Then vortex until the phases mix and appear cloudy.
- 6.1 Add  3 mL of chloroform:isoamyl (24:1 ratio).
- 6.2 Close the snap cap tube completely (second stop) and mix contents immediately by hand.
- 6.3 Then vortex until the phases mix and appear cloudy.
- 7 Centrifuge at  2500 x g for  00:10:00 .
- 8 Transfer aqueous phase to a new 15 ml tube.
- 9 Precipitate nucleic acids by gently adding  3 mL of isopropanol to the tube followed by gentle inversion to mix the phases. Precipitate for  01:00:00 at  4 °C .
- 9.1 Precipitate nucleic acids by gently adding  3 mL of isopropanol to the tube followed by gentle inversion to mix the phases.  01:00:00 at  4 °C .
- 9.2 Precipitate for  01:00:00 at  4 °C .
- 10 Centrifuge at  2500 x g for  00:10:00 .



- 11 Remove the supernatant without dislodging the pellet.
- 12 Rinse the pellet with  1 mL of chilled 70 % ethanol made with RNase-free H₂O.
- 13 Transfer pellet and ethanol to a new 1.5 ml tube and invert to wash.
- 14 Centrifuge at  16000 x g in a microcentrifuge for  00:02:00 and remove the supernatant.
- 15 Spin dry the RNA pellet by successive removal of any remaining liquid following  00:01:00 centrifugations at maximum speed in a microcentrifuge. Repeat this process until no liquid is seen when the tube is flicked.
Allow the pellet to air dry for  00:01:00 to  00:05:00 , depending on the size of the pellet.

Note


Avoid over-drying since this will negatively affect re-suspension in water.

- 15.1 Spin dry the RNA pellet by successive removal of any remaining liquid following  00:01:00 centrifugations at maximum speed in a microcentrifuge.
- 15.2 Repeat this process until no liquid is seen when the tube is flicked.
- 15.3 Allow the pellet to air dry for  00:01:00 to  00:05:00 , depending on the size of the pellet.

Note

Avoid over-drying since this will negatively affect re-suspension in water.



- 16 While on ice, re-suspend the pellet in  500 μL RNase-free H_2O using gentle pipetting.

Note

Ensure the pellet is completely re-suspended before proceeding to step 17.



- 17 Add  500 μL of  4 Molarity (M) LiCl. Precipitate the RNA overnight at  4 $^{\circ}\text{C}$.

- 17.1 Add  500 μL of  4 Molarity (M) LiCl.  4 $^{\circ}\text{C}$.

- 17.2 Precipitate the RNA overnight at  4 $^{\circ}\text{C}$.

- 18 Centrifuge at  16000 x g for  00:30:00 at  4 $^{\circ}\text{C}$ in a microcentrifuge.

- 19 Remove supernatant by pipette.

- 20 Wash the pellet three times with  200 μL of chilled 70 % ethanol diluted in RNase-free H_2O . Centrifuge at  4 $^{\circ}\text{C}$.

Note

Remove the pellet from the wall of the tube to wash the pellet more completely. Pellet will now dislodge easily and care must be taken when removing the supernatant between washes.



21 Spin the pellet. Remove ethanol and dry as in step 15. [⇒ go to step #15](#) Centrifuge at 4 °C .

21.1 Spin the pellet.

21.2 Remove ethanol and dry as in step 15. [⇒ go to step #15](#)

21.3 Centrifuge at 4 °C .

22 Dissolve pellet in RNase-free H₂O on ice.

Note

The volume of the water is dependent on the size and clarity of the pellet, as well as the viscosity of the re-suspension, but 50 µL can be used as a standard starting point.

23 Centrifuge RNA extract for 00:01:00 at 4 °C .

Check concentration and integrity using a NanoDrop 2000 spectrophotometer.

23.1 Centrifuge RNA extract for 00:01:00 at 4 °C .

23.2 Check concentration and integrity using a NanoDrop 2000 spectrophotometer.

24 Purify and DNase treat up to 100 µg of RNA on a RNeasy Mini Kit spin column (pink) by Qiagen.

Note

Follow the 'RNA Cleanup' protocol as published in the **RNeasy Handbook**, including the on-column DNase digestion with the RNase-Free DNase Set by Qiagen.



- 25 Elute RNA twice from the column using $40\ \mu\text{L}$ of $95\ ^\circ\text{C}$ RNase-free water to a total of $80\ \mu\text{L}$.

Note

However, if the mass of RNA applied to the column is less than $50\ \mu\text{g}$, then use only $40\ \mu\text{L}$ to elute.