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Plant RNA extractions using TRI reagent

Peter Crisp[1], Diep R Ganguly[2], Aaron ABS Smith[3]

[1]University of Queensland; [2]The Australian National University; [3]Australian National University

1 Works for me dx.doi.org/10.17504/protocols.io.bt8wnrxe

Pogson Genomics Group


Diep Ganguly
The Australian National University

ABSTRACT

Extraction of total RNA from plant tissue using TRI reagent.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Crisp, Ganguly, Smith, Murray, Estavillo, Searle, Ford, Bogdanović, Lister, Borevitz, et al (2017) Rapid Recovery Gene Downregulation during Excess-Light Stress and Recovery in Arabidopsis. Plant Cell 29:1836–1863

ATTACHMENTS

TRIreagent_SDS.pdf chloroform_SDS.pdf

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PROTOCOL CITATION

Peter Crisp, Diep R Ganguly, Aaron ABS Smith 2021. Plant RNA extractions using TRI reagent. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bt8wnrxe

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

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GUIDELINES

TRI reagent and chloroform are hazardous - handle with care, operate in fume hood, wear vinly gloves and safety glasses.

MATERIALS TEXT

- TRI reagent
- Chloroform (or chloroform : IAA [24:1])
- Isopropanol (or 5 M LiCl)
- 75 % Ethanol
- Nuclease-free H_2O (e.g. 0.01% DEPC-treated H_20)
- 2 mL safe-lock Eppendorf tubes
- Tissue lyser
- Nuclease-free 10 mM TRIS-Cl (pH 6.5), 0.1 mM EDTA

SAFETY WARNINGS

TRI reagent - hazardous
Chloroform - hazardous

Ensure you read SDS documents (attached) and organise appropriate waste vessels (fume hood).

BEFORE STARTING

Ensure benches and equipment are RNase free.

1 Collect 50-100 mg of plant tissue and freeze immediately in liquid N_2.

2 ⚠

Grind tissue to fine powder under liquid N_2 using tissue lyser or mortar + pestle, then immediately add 1 mL TRI reagent (1 mL per 100 mg tissue).

Note, achieving a fine grind is critical to high yields of intact RNA.

3 Invert each tube by hand ~20x and incubate at room temperature for 5 minutes (DO NOT vortex samples as it may result in RNA degradation).

4 Add of chloroform (200 µL per mL TRI reagent), cap tubes, shake vigorously (by hand) at room temperature for 15 seconds (solution should become cloudy), then incubate at room temperature for 3 minutes.

5 Centrifuge at 14,000 rcf for 10 minutes at 4°C.

6 Transfer the upper aqueous phase to a new microfuge tube (approx. 600 µL).

7 Repeat steps 4 and 5 (approx. 400 µL).

Note, if you are observing buffer and salt carryover in your purified RNA (high 230 nm absorbance), reduce volume of upper-phase recovered. Alternatively, replace chloroform (step 3) with chloroform : IAA (24:1).

8 Add equal volume *ice-cold* 100% isopropanol, then mix by inverting tubes ~20x by hand.

Note, if you want to avoid capturing shorter RNA molecules (small RNAs, degradation intermediates), replace

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Isopropanol with 5 M LiCl (room temperature).

9 Incubate at -20°C for 1 hour. Alternatively, incubate O/N for small RNA precipitation or higher yields.

10 Centrifuge samples at 14,000 rcf for 20 minutes at 4°C.

11 Remove the supernatant.

Note, you should observe a white pellet at this step, a yellow pellet is indicative of buffer carryover and you may need to do an additional clean-up.

12 Add 1 mL of 75 % ethanol and invert tube ~10x.

13 Centrifuge samples at 7, 500 rcf for 5 minutes at room temperature.

14 Remove supernatant (careful, pellet often becomes dislodged at this step).

15 Air-dry pellet at room temperature for 5 minutes.

16 Resuspend pellet in 25 - 100 µL of RNase-free (e.g. 0.01% DEPC-treated) buffer, depending on expected yield and desired concentration.

Buffer = water or 10 mM TRIS-Cl (pH 6.5), 0.1 mM EDTA.

17 If pellet is not dissolving straight away, incubate in buffer at room temperature for 5 minutes then resuspend by pipetting.

18 Visualise approx. 50 - 100 ng of purified RNA for each sample on a semi-denaturing 1% agarose TBE gel.

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