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Plant RNA purification using TRIzol (TRI reagent) V.2

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ABSTRACT

Extraction of total RNA from plant tissue using TRIzol (or TRI reagent) followed by DNA nuclease treatment.

ATTACHMENTS

TRIreagent_SDS.pdf chloroform_SDS.pdf

GUIDELINES

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

MATERIALS

- TRIzol (or TRI reagent)
- Chloroform (or chloroform : IAA [24:1])
- Isopropanol
- 80% Ethanol
- Nuclease-free H_2O (e.g. 0.01% DEPC-treated H_20) or Tris-EDTA (10 mM Tris-Cl, pH 6.5, 0.1 mM EDTA)
- 2 mL safe-lock Eppendorf tubes
- 1.5 mL microcentrifuge tubes
- Tissue lyser or mortar and pestle
- RNase-Free DNase Set (Qiagen)
- RNA loading dye, 2X (NEB)

Protocol status: Working
We use this protocol and it's working

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SAFETY WARNINGS

TRI reagent - hazardous
Chloroform - hazardous

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

BEFORE START INSTRUCTIONS

Ensure benches and equipment are RNase free.

For RNase-Free DNase Set (Qiagen): Prepare DNase I stock solution by dissolving the lyophilized DNase I (1500 Kunitz units) in 550 µl of the RNase-free water. Mix gently by inverting the vial. Divide into single use aliquots and store at -20 °C. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.

RNA purification

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 1/5 volume of pre-mixed chloroform:isoamyl alcohol (24:1), cap tubes, shake vigorously (by hand) 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. 400-600 µL).

7 Repeat steps 4 and 5 (approx. 300-400 µL).

Note, if you are observing buffer and salt carryover in your purified RNA (high 230 nm absorbance), reduce volume of upper-phase recovered.

8 (Optional) If the expected RNA concentration is ≤10 µg/mL, add 1/10 volume of 3M NaOAc (pH 5.5) and/or 100 µg/mL GlycoBlue (or glycogen).

9 Add equal volume of 100% isopropanol, then mix by inverting tubes ~20x by hand.

10 Incubate at -20°C for 1 hour. Alternatively, incubate overnight to capture small RNAs.

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

12 Remove the supernatant, you should observe a white pellet.

13 Add 1 mL of 80 % ethanol and invert tube ~10x.

14 Centrifuge samples at 10,000 rcf for 5 minutes at room temperature.

15 Remove supernatant, carefully since the pellet often becomes dislodged at this step.

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

17 Resuspend pellet in RNase-free water (e.g. 0.01% DEPC-treated water) or Tris-EDTA (10 mM Tris-Cl, pH 6.5, 0.1 mM EDTA).

DNA nuclease treatment and ethanol precipitation

18 Make up volume of RNA solution to 87.5 µL with nuclease-free water. This can be performed with an aliquot or total sample from the previous step.

19 Add 10 µL Buffer RDD and 2.5 µL DNase I stock solution (Qiagen RNase Free DNase Set) and mix with gentle pipetting.

20 Incubate at room temperature for 5-10 minutes.

21 Add 500 µL of 100% ethanol.

22 (Optional) Add 3 µL glycogen or GlycoBlue, and 10 µL NaOAc (pH 5.5) to aid RNA precipitation.

23 Mix by gentle inversion.

24 Incubate samples for at least 1 hour at -20 °C (overnight, if purifying small RNAs).

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

26 Remove supernatant and rinse pellet with 1 mL of 80% ethanol.

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

28 Remove supernatant without disturbing pellet and air-dry for 2 minutes.

29 Resuspend pellet in RNase-free water (e.g. 0.01% DEPC-treated water) or Tris-EDTA (10 mM Tris-Cl, pH 6.5, 0.1 mM EDTA).

Quality control

30 Take 50-100 ng aliquot of RNA and mix 1:1 with 2X RNA loading dye (NEB).

31 Incubate RNA at 65 °C for 5 minutes.

32 Load and run samples on a 1% agarose TBE gel.

33 Nanodrop RNA to check for purity and quantity.