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RNA extractions from de-etiolated Arabidopsis seedlings using CTAB

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Protocol status: Working
We use this protocol in our group and it is working, though it will be continually developed.

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Abstract

A CTAB based method for extracting RNA, which is particularly useful for tough tissues. This has been adapted from a protocol that was originally used for pine tree tissue, which is difficult due to the high concentrations of polysaccharides, phenolics, and RNase (Chang, S., Puryear, J. & Cairney, J. Plant Mol Biol Rep (1993) 11: 113. https://doi.org/10.1007/BF02670468). The protocol described herein was an effective alternative to TRIzol based extractions for recovering RNA from juvenile de-etiolated tissues.

Guidelines

Ensure that you use RNA friendly practices (e.g. clean surfaces with RNAesy or 80% ethanol, use RNAase-free filter tips, use DEPC-treated water for buffers, adjust RNA buffer pH to be slightly acidic [e.g. ~6] in which RNA is more stable).

Materials

MATERIALS

- Isoamylalcohol
- Beta-mercaptoethanol
- Chloroform
- DEPC
- Hexadecyltrimethylammonium bromide (CTAB) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**H9151
- 80% Ethanol
- Lithium chloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**793620
- Polyvinylpyrrolidone K 30 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**81420

Extraction buffer

1. 2% CTAB
2. 2% PVP K30
3. 100 mM Tris-HCl (pH 8.0)
4. 25 mM EDTA (pH 8.0)
5. 2 M NaCl
6. Autoclave
7. 2% 2-mercaptoethanol (add before use = 100 uL per 5 mL buffer)

Safety warnings

Perform steps with 2-mercaptoethanol in a fume hood.

Before start

Prepare extraction buffer (requires autoclaving) and lithium chloride solution before starting. You may consider filter-sterilizing these to be extra cautious.

1 Harvest tissues into liquid nitrogen and grind (under liquid nitrogen) using mortar and pestle to obtain a fine powder. Ground tissue should be kept in safe-lock tubes in liquid nitrogen (or returned to -80C storage) until all samples are processed. This step is critical for efficient extractions so take your time here.

2 Add 1 mL of prewarmed (65°C) extraction buffer to each tube, mix well and incubate for 5 mins at 65°C (can be longer if needed e.g. 10-15 mins)

3 Add 200 ul of chloroform:IAA (24:1), mix well and spin @ 14,000 rcf for 10 mins. Remove upper aqueous phase to a new tube. Make sure not to disturb or pipette any material from the interface. Repeat chloroform:IAA step twice, being increasingly conservative when recovering the aqueous phase.

4 Add equal volume of 5 M LiCl to aqueous layer, mix well and incubate overnight @ -20°C.

5 Spin tubes @ 14,000 rcf for 20 mins @ 4°C.

6 Remove supernatant with pipette, add 1 mL 80% ethanol and invert tube ~10X. Centrifuge @ 7,500 rcf for 5 minutes @ RT. Remove ethanol and repeat.

7 Remove as much ethanol as possible using a pipette. Air-dry tubes with lid open for 1 min. Resuspend in 30 - 50 ul (depending on expected concentration and purpose, e.g. we aim for ~ 500 ng / ul) DEPC-treated water or low EDTA TE buffer (0.1 mM EDTA, 10 mM Tris base, pH 6.5).

8 Check quality of RNA e.g. visualize ~50 ng RNA on a 1% agarose gel or use BioAnalyser / LabChip GXII.