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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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Keywords: RNA, de-etiolated seedlings, Arabidopsis, Plant, extracting rna, rna extraction, recovering rna, rnase, using ctab, useful for tough tissue, pine tree tissue, effective alternative to trizol, etiolated tissue, ctab, tough tissue, based extraction

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)

Troubleshooting

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.