Silique RNA Extraction

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Abstract

This protocol was based on a 2010 paper by Meng and Feldman for extraction of RNA from arabidopsis siliques. While the protocol remains unchanged, I have made some slight edits to streamline it in the lab and also added some notes regarding my results applying this protocol to tobacoo fruit tissue.

Citation: Alex Rajewski Silique RNA Extraction. **protocols.io**
dx.doi.org/10.17504/protocols.io.nzfdf3n
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Guidelines

All solutions should be made with DEPC-treated water and all work should be completed on an RNase-free work surface with RNase-free tools

Before start

Make up the extraction buffer:

- 100mM Tris-HCl, pH 9.5
- 150mM NaCl
- 1.0% sarkosyl
- 5mM DTT (or 0.5% β-ME)

Make up ~3.2mL of 75% ethanol (RNase-free) and at least 200µL 3M sodium acetate (pH 5.2) for each sample to be extracted.

Set a centrifuge to 4ºC and allow to cool while setting up.

Protocol

Lysis

Step 1.

Add three stainless steel BBs to each 2-mL tube with tissue in liquid nitrogen and homogenize the samples in a Retsch mixer mill for 60 seconds at 25 Hz.

EQUIPMENT
Equipment brand:
Retsch MM400
SKU:
20.745.0001
Specifications:
91a02b88002a8c7eeb245eb2c7c2c845.png

Lysis

Step 2.

Add 1.2mL of Extraction Buffer (with βME or DTT added) to the frozen tissue and mix.

AMOUNT
1.2 ml Additional info: Extraction buffer

Lysis

Step 3.

After brief mixing, vortex vigorously for 5 min, and spin for 5 min at 11 000×g.

RNA Partitioning 1

Step 4.

Transfer the supernatant to a new 2-mL Eppendorf tube, add 0.5 volume of chloroform (500µL) and vortex for 2 min.

AMOUNT
500 µl Additional info: Chloroform

RNA Partitioning 1

Step 5.

Add 0.5 volume of acid phenol (water-saturated; 500µL) and vortex for another 2 min.

AMOUNT
500 µl Additional info: Acid Phenol

RNA Partitioning 1

Step 6.

Centrifuge at 11,000g for 15 min.

Nucleic Acid Precipitation 1

Step 7.

Carefully remove the upper aqueous phase (avoiding the interface) to a new RNase-free 2-mL Eppendorf tube, and add 90µL 3 M sodium acetate (pH 5.2) and 600µL isopropanol. Mix briefly.

AMOUNT
90 µl Additional info: 3M Sodium Acetate

AMOUNT

600 µl Additional info: Isopropanol

Nucleic Acid Precipitation 1

Step 8.

Incubate at room temp for 10 min then spin at 11,000g for 10 min.

Nucleic Acid Precipitation 1

Step 9.

Discard the upper aqueous phase and wash the pellet with 1 mL 75% ethanol (RNase-free).

AMOUNT

1 ml Additional info: 75% Ethanol

Nucleic Acid Precipitation 1

Step 10.

Spin at 11,000g for 10 min, and discard the supernatant. Air dry the pellet briefly (<5 min).

RNA Partitioning 2

Step 11.

Resuspend the pellet in1 mL TRIzol reagent and vortex until dissolved.

AMOUNT

1 ml Additional info: TRIzol

RNA Partitioning 2

Step 12.

Add 200µL chloroform and mix by shaking vigorously for 15s.

AMOUNT

200 µl Additional info: Chloroform

RNA Partitioning 2

Step 13.

Incubate for 2–3 min, then centrifuge at 11,000g at 4°C for 15 min.

Nucleic Acid Precipitation 2

Step 14.

Transfer the upper aqueous phase to a new well-labeled RNase-free 1.5-mL Eppendorf tube containing 500µL isopropanol. Mix, then incubate for 10 min. This is the tube that will be used for final storage. A white mat may form in the aqueous phase, and should also be transferred.

AMOUNT

500 µl Additional info: Isopropanol

Nucleic Acid Precipitation 2

Step 15.

Spin at 11 000×g at 4°C for 15 min

Nucleic Acid Precipitation 2

Step 16.

Wash the pellet with 1.2 mL 75% ethanol RNase-free), and spin at 11 000×g at 4°C for 10 min.

AMOUNT
1.2 ml Additional info: 75% Ethanol

Nucleic Acid Precipitation 2

Step 17.

Discard the supernatant and dry the pellet.

Nucleic Acid Precipitation 2

Step 18.

Dissolve the RNA pellet in 100µL DEPC-treated, sterilized ddH2O.

AMOUNT
100 µl Additional info: RNase-free Water

Nucleic Acid Precipitation 3

Step 19.

Add 10µL DEPC-treated 3 M sodium acetate (pH 5.2) and 250µL RNase-free 100% ethanol and mix.

AMOUNT
10 µl Additional info: 3M Sodium Acetate
AMOUNT
250 µl Additional info: Absolute Ethanol

Nucleic Acid Precipitation 3

Step 20.

Incubate for 20 min, then spin at 11,000g at 4°C for 15 min.

Nucleic Acid Precipitation 3

Step 21.

Wash the pellet with 1.2 mL 75% ethanol, then spin at 11,000g at 4°C for 10 min.

AMOUNT
1.2 ml Additional info: 75% Ethanol

Nucleic Acid Precipitation 3

Step 22.

Discard the supernatant and dry the RNA pellet.

Resuspension and Storage

Step 23.

Dissolve the pellet in 50µL DEPC-treated, sterilized ddH2O, and store at – 80°C.

AMOUNT
50 µl Additional info: RNase-free Water

Warnings

Many components of this extraction are hazardous (phenol, chloroform, TRIzol) and should only be used in a fume hood. Also, make sure to properly discard of consumables that come in contact with these componenets.