Pericarp RNA Extraction

Alex Rajewski

Abstract

This protocol is adapted from the QIAGEN RNeasy Plant mini kit (Jan 2011). Specific modifications have been made to apply this RNA extraction to tobacco pericarp or fruit tissue.

Citation: Alex Rajewski Pericarp RNA Extraction. **protocols.io**
dx.doi.org/10.17504/protocols.io.nw4dfgw
Published: 19 Mar 2018

Materials

RNeasy Mini Kit 74104 by Qiagen
2-Mercaptoethanol View by Sigma Aldrich
Polyvinylpyrrolidone (PVP-40) PB0436.SIZE.250g by Bio Basic Inc.
✓ nuclease free water by Contributed by users
RNase-Free DNase Set 79254 by Qiagen

Protocol

Prepare

Step 1.

Make up a lysis buffer with the following compenents. Vortex to mix.

AMOUNT
500 µl Additional info: Buffer RLC
AMOUNT
5 µl Additional info: 2-mercaptoethanol
AMOUNT
12.5 mg Additional info: PVP

Prepare

Step 2.

Make up a batch ofRNase-free 80% ethanol. You will need 500µL for each RNA extraction sample. This can be made in a single large batch for all samples.

AMOUNT
400 µl Additional info: Absolute ethanol
AMOUNT
100 µl Additional info: RNase-free water

Prepare

Step 3.

Prepare one eppendorf with 0.5 volumes of absolute ethanol for each RNA extraction sample. If you are using 450µL of prepared lysis buffer, this would be 225µL of absolute ethanol. Label the tube and close the lid to prevent evaporation.

Prepare

Step 4.

Make up a batch of DNAse solution for each RNA sample and keep on ice.

AMOUNT
10 µl Additional info: Resuspended DNase
AMOUNT
70 µl Additional info: Buffer RDD

NOTES
Alex Rajewski 19 Mar 2018

These components are from the RNase-free DNase kit. The resuspended DNase is made up from lyophilized powder and stored frozen in aliquots of ~50µL. Once thawed, the resuspended DNase cannot be refrozen, but may be used within 6 months if kept refrigerated. Buffer RDD is also kept refrigerated.

Prepare

Step 5.

Arrange tubes an dcolumns in order of use: QIAshredder (lilac), absolute ethanol tube, RNeasy spin column (pink), empty collection tube, final collection/storage tube. Label the final collection/storage tube with the sample name, date, and your initials. All other tubes can be given a shorthand label/number.

EXPECTED RESULTS

Final Label: 1 DPA Pericarp RNA AR 19 Mar 18

Prepare

Step 6.

Ensure that ethanol is added to Buffer RPE before use.

Grinding

Step 7.

Determine the amount of plant material. Do not use more than 100mg. The tissue should have been flash frozen in liquid nitrogen and stored at -80º.

Grinding

Step 8.

Place the frozen tissue in a cold 2mL, round-bottom eppendorf tube with 2-3 stainless steel balls. Using a homogenizer-shaker, grind the tissue with the stainless steel balls for 45 seconds at 30 Hz. Frozen tissues should not be allowed to thaw during handling.

Grinding

Step 9.

Examine the ground tissue for visible clumps, and, if necessary, re-cool them in liquid nitrogen and repeat the grinding.

GOTO

Visible clumps? -> go to step #8

Lysis

Step 10.

Add 450µl of prepared lysis buffer to a maximum of 100mg tissue powder. Vortex vigorously. A short 1–3 min incubation at 56°C may help to disrupt the tissue.

AMOUNT

450 µl Additional info: Prepared lysis buffer

Lysis

Step 11.

Transfer the lysate to a QIAshredder spin column (lilac) placed in a 2 ml collection tube, and centrifuge for 2 min at full speed.

RNA Precipitation

Step 12.

Carefully transfer the flow-through to the eppendorf tube with 0.5 volumes of absolute ethanol without disturbing the cell-debris pellet in the collection tube. You may discard the QIAshredder column and collection tube.

RNA Precipitation

Step 13.

Mix the flow-through and ethanol together by pipetting gently up and down until the solution looks homogenous.

RNA Precipitation

Step 14.

Transfer the sample (usually 650µl), including any precipitate that may have formed, to an RNeasy spin column (pink) placed in a 2mL collection tube and centrifuge briefly. Discard the flow-through.

Washing

Step 15.

Add 350µl Buffer RW1 to the RNeasy spin column and centrifuge to wash the spin column membrane. Discard the flow-through.

AMOUNT
350 µl Additional info: Buffer RW1

Washing

Step 16.

Add 80µL of the prepared DNase solution directly to the membrane and incubate at room temperature.

AMOUNT
80 µl Additional info: Prepared DNAse solution

Washing

Step 17.

Add 350µl Buffer RW1 to the RNeasy spin column and centrifuge to wash the spin column membrane. Discard the flow-through.

AMOUNT
350 µl Additional info: Buffer RW1

Washing

Step 18.

Add 500µl Buffer RPE to the RNeasy spin column and cetrifuge to wash the membrane. Discard the flow-through.

AMOUNT
500 µl Additional info: Buffer RPE

Washing

Step 19.

Add 500µl Buffer RPE to the RNeasy spin column and centrifuge to wash the membrane.

AMOUNT
500 µl Additional info: Buffer RPE

Washing

Step 20.

Add 500µL of 80% ethanol to the RNeasy spin column and centrifuge to wash the membrane. Discard the flow-through.

AMOUNT
500 µl Additional info: 80% ethanol

Washing

Step 21.

After centrifugation, carefully remove the RNeasy spin column from the collection tube so that the column does not contact the flow-through. Place the RNeasy spin column in a new 2 mL collection

tube, and centrifuge with the cap open to dry the membrane. Discard the old collection tube with the flow-through.

NOTES

Alex Rajewski 19 Mar 2018

The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution. Residual ethanol may interfere with downstream reactions.

Elution

Step 22.

Place the RNeasy spin column in a labeled 1.5mL collection tube. Add 50µl RNase-free water directly to the spin column membrane and centrifuge to elute the RNA.

AMOUNT

50 µl Additional info: RNase-free water

Step 23.

Measure the concentration of the sample along with the 260/280 and 260/230 ratios. Record the concentration on the side of the tube. Store the sample at -80ºC.