R. Shi
201202

RNA extraction for total RNA (not for analysis small RNA)

Supplies & reagents:

Kit:

Plant RNeasy® mini kit

RNase-Free DNase Set

Additional reagent

β-Mercaptoethanol (β-ME)

Ethanol (96–100%)

Important Notes:

- There are several methods for RNA extraction from xylem tissue of P. trichocarpa. The protocol using Plant RNeasy Mini Kit is one of our methods to extract total RNA which will be used in gene transcript quantitation. This method requires less tissue sample, and capable yield enough high quantity total RNA for quantitation of transcript level, such as qRT-PCR, or gene cloning, including RT-PCR, 5' or 3' RACE. Average RNA yields from 100mg stem developing xylem tissue of Populus trees are up to 1ug/ul in final concentration (50ul final elution).
- Stem developing xylem of P. trichocarpa for analysis in our NSF project and other projects are collected around 10:00-11:30am.

Protocol:

1. Determine the amount of plant material. Do not use more than 100 mg.

   Weighing tissue is the most accurate way to determine the amount.

2. Immediately place the weighed tissue in liquid nitrogen, and grind thoroughly with a mortar and pestle. Decant tissue powder and liquid nitrogen into an RNase-free, liquid-nitrogen–cooled, 2 ml microcentrifuge tube (not supplied). Allow the liquid nitrogen to evaporate, but do not allow the tissue to thaw. Proceed immediately to step 3.

   RNA in plant tissues is not protected until the tissues are flash-frozen in liquid nitrogen. Frozen tissues should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible.

3. Add 450 µl Buffer RLT to a maximum of 100 mg tissue powder, and then vortex vigorously.

A short 1–3 min incubation at 56°C may help to disrupt the tissue. However, do not incubate samples with a high starch content at elevated temperatures, otherwise swelling of the sample will occur.

4. Transfer the lysate to a QIAshredder spin column (lilac) placed in a 2 ml collection tube, and centrifuge for 2 min at full speed. Carefully transfer the supernatant of the flow-through to a new microcentrifuge tube (not supplied) without disturbing the cell-debris pellet in the collection tube. Use only this supernatant in subsequent steps.

It may be necessary to cut off the end of the pipet tip to facilitate pipetting of the lysate into the QIAshredder spin column. Centrifugation through the QIAshredder spin column removes cell debris and simultaneously homogenizes the lysate.

While most of the cell debris is retained on the QIAshredder spin column, a very small amount of cell debris will pass through and form a pellet in the collection tube. Be careful not to disturb this pellet when transferring the lysate to the new microcentrifuge tube.

5. Add 0.5 volume of ethanol (96–100%) to the cleared lysate, and mix immediately by pipetting. Do not centrifuge. Proceed immediately to step 6.

6. Transfer the sample (usually 650 µl), including any precipitate that may have formed, to an RNeasy spin column (pink) placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 15 s at ~8000×g (~10,000 rpm). Discard the flow-through.*

Reuse the collection tube in step 7.

If the sample volume exceeds 700 µl, centrifuge successive aliquots in the same RNeasy spin column. Discard the flow-through after each centrifugation.*

Optional: If performing optional on-column DNase digestion:

D1. Add 350 µl Buffer RW1 to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at ~8000×g (~10,000 rpm) to wash the spin column membrane. Discard the flow-through.

Reuse the collection tube in step D4.

D2. Add 10 µl DNase I stock solution (see The RNase-Free DNase Set) to 70 µl Buffer RDD. Mix by gently inverting the tube, and centrifuge briefly to collect residual liquid from the sides of the tube.

D3. Add the DNase I incubation mix (80 µl) directly to the RNeasy spin column membrane, and place on the benchtop (20–30°C) for 15 min.

Be sure to add the DNase I incubation mix directly to the RNeasy spin column membrane. DNase digestion will be incomplete if part of the mix sticks to the walls or the O-ring of the spin column.

D4. Add 350 µl Buffer RW1 to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at ~8000×g (~10,000 rpm). Discard the flow-through. Continue with the first Buffer RPE wash step (8) in the relevant protocol.

7. Add 700 µl Buffer RW1 to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at ~8000×g (~10,000 rpm) to wash the spin column membrane. Discard the flow-through.

   Reuse the collection tube in step 8.

8. Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at ~8000×g (~10,000 rpm) to wash the spin column membrane. Discard the flow-through.

   Reuse the collection tube in step 9.

9. Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 2 min at ~8000×g (~10,000 rpm) to wash the spin column membrane.

   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.

   Note: After centrifugation, carefully remove the RNeasy spin column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.

10. Optional: Place the RNeasy spin column in a new 2 ml collection tube (supplied), and discard the old collection tube with the flow-through. Close the lid gently, and centrifuge at full speed for 1 min.

    Perform this step to eliminate any possible carryover of Buffer RPE, or if residual flow-through remains on the outside of the RNeasy spin column after step 9.

11. Place the RNeasy spin column in a new 1.5 ml collection tube (supplied). Add 30–50 µl RNase-free water directly to the spin column membrane. Close the lid gently, and centrifuge for 1 min at ~8000× g (~10,000 rpm) to elute the RNA.

12. If the expected RNA yield is >30 µg, repeat step 11 using another 30–50 µl RNasefree water, or using the elution from step 11 (if high RNA concentration is required). Reuse the collection tube from step 11.

    If using the eluate from step 11, the RNA yield will be 15–30% less than that obtained using a second volume of RNase-free water, but the final RNA concentration will be higher.