RNA extraction from *Silene*

Preparation the day before the extraction

Materials needed

- Ceramic mortar and pestles
- Metal scoops to transfer processed powder
- Liquid N_2 tank
- Long forceps to fish out tubes from liquid N_2 tank
- RNAse Zap
- 100% ethanol
- β-mercaptoethanol and fume hood access
- Large tubes (5 µl) to hold the plant material
- Qiagen RNA extraction kit for plants
- Qiagen optional DNase
- Filter tips
- RNase-free tubes for samples
- Glass beaker for β-mercaptoethanol waste
- Old plastic bottle for other buffer waste

Preparation

Add H_2O from the DNase kit to the glass tube containing the DNase powder. Gently mix and aliquot to solutions to store at 2-8°C.

Add 44µl ethanol to RPE buffer, and tick the box on the lid.

Wipe mortar and pestles with RNase Zap, and store at -20°C[1].

Collect plant material in 5 µl tubes, flash freeze them and keep on dry ice (-80°C). Transfer to -80°C freezer and keep on dry ice until disruption in liquid N_2.

Extraction day

Setup[2]

1. Prepare liquid N_2, dry ice box with samples, ice box.
2. Clean the bench, trays, pipetes and gloves with 70% Ethanol and RNase zap.
3. Prepare 1 tray with purple QiaShredder columns and one eppendorf per column. Label both with the sample names. The eppendorfs will receive the first buffer.
4. Prepare 1 tray with pink RNEasy mini spin columns, and one eppendorf per column. Label the RNEasy mini spin columns, and pipette 225 µl ethanol to the eppendorfs.
5. Prepare RLT buffer: for 10 samples and working in a fume hood, add 45 µl β-mercaptoethanol to 4.5 ml buffer RLT and aliquot 450 µl to the labelled eppendorfs on the tray with the QiaShredder columns. Keep the RLT buffer rack on ice.
6. Lay out paper towels to place the mortar and pestles on[3].
7. Clean metal scoop with RNase zap.

Extraction

1. Take mortar and pestle out of freezer. Add some liquid N2 to cool down, add plant material and slowly crush with pestle. As soon as the liquid evaporates, crush vigorously into powder. Add more liquid N_2 and repeat as soon as it evaporates. Add more liquid N_2 to gather powder and transfer using metal scoop into eppendorf containing RLT buffer[4]. Close the lid, shake to mix, and add more powder if needed[5]. Put used mortar and pestles in sink with water, wash, dry and RNase zap the scoop. Repeat for all samples.

2. Prepare DNase: for 10 samples mix 100 µl DNase stock and 700 µl RDD buffer. Mix by gentle pipetting and inverting only. Keep on ice.

3. Transfer the RLT buffer[6] into the QiaShredder columns. Spin for 2 min at top speed (13,200 RPM).

4. Transfer the supernatant without disturbing the cell debris into the prepared eppendorf with ethanol, mix by

[1] It is essential to have the mortar at low temperature to avoid denaturing RNA and so that the plant powder does not stick to the ceramic and it is easy to recover.

[2] Since RNA is sensitive, the more time is spent preparing the lab before the RNA is in a sensitive stage, the better the extraction quality.

[3] This prevents spilling liquid N_2 from travelling far, and keeps the emerging plant powder in control.

[4] The samples should be stable in RLT buffer.

[5] Do not use more than the recommended 100 mg (4 flower buds, 2-3 small leaves). Do not let use powder that is turning green and mushy as it is already too warm.

[6] Set the pipette to 700 µl to have peace of mind for the following 2 steps.

pipetting[7] and transfer with the same tip to RNeasy column.

5. Spin for 15 sec at 10,000 RPM.

6. Remove flow-through into glass beaker[8]. Add 350 µl RW1 solution[9].

7. Spin for 15 sec at 10,000 RPM.

8. Apply 80 µl pre-made DNase solution on membrane, and incubate for 15 min at room temperature.

9. Label 1.5 µl tubes that come with the kit with sample names. Label[10] 2x more tubes and order them in a tray on ice.

10. Wash[11] all mortar and pestles, and place on paper towels to dry.

11. Add 350 µl RW1 solution.

12. Spin for 15 sec at 10,000 RPM.

13. Discard flow-through in plastic bottle. Add 500 µl RPE.

14. Spin for 15 sec at 10,000 RPM.

15. Discard flow-through in plastic bottle. Add 500 µl RPE.

16. Spin for 15 sec at 10,000 RPM.

17. Discard flow-through in plastic bottle. Transfer column in new collection tube.

18. Spin for 1 min at 13,200 RPM to fully dry membrane.

19. Transfer column to labelled eppendorf from kit. Add 30 µl[12] RNase-free H_20 from kit, directly on the membrane.

20. Clean centrifuge lid and tube-holding area with RNase zap.

21. Spin for 1 min at 10,000 RPM.

22. Add 30 µl RNAse-free H_20 from kit, directly on the membrane.

23. Spin for 1 min at 10,000 RPM.

24. Pipette 30 µl to tube "1", 3 µl to tube "QC". Order in a box and transfer samples to -80°C until use.

[7] Pipette solution on wall of eppendorf for better mixing. You may see nucleic acid precipitate already.

[8] Leave beaker to evaporate in fume hood. Wash salt remains.

[9] For all these washes, close the lid and roll and invert the tubes to achieve a thorough wash.

[10] The aim is for the kit tubes that go into the centrifuge to be a backup sample "2", and one of the eppendorf copies to be the main sample "1" and the other to hold a small amount (3 µl) for sample quantification that does not risk degrading the samples "Q". You can write these numbers with a different marker colour and keep them in separate frozen boxes.

[11] There should be time during the DNase incubation. It is important to dry them so they are ready for RNase zap and freezing at the end of the extraction, in case the protocol is repeated in the same day.

[12] This is the minimum and ensures a high final concentration. If a larger dilution is required for library preparation, it is easy to do after QC.