RNA extraction from *Mimulus*

Preparation the day before the extraction

Materials needed

- Micropestles
- 2 ml eppendorfs for sample leaves
- Liquid N_2 tank
- Long forceps to fish out tubes from liquid N_2 tank
- 100% ethanol
- (2) β-mercaptoethanol and fume hood access
- Qiagen RNA extraction kit for plants
- Filter tips (1000 µl and 10 µl)
- RNase-free tubes
- Glass beaker for β-mercaptoethanol waste
- Old plastic bottle for other buffer waste

Preparation

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

Collect 2 leaves in 2 µl tubes, flash freeze them in liquid N_2. Transfer to -80°C freezer and keep in liquid N_2 until disruption.

Extraction day

Setup[1]

1. Prepare liquid N_2, ice box.
2. Clean the bench, trays, pipetes, centrifuge and gloves with 70% ethanol.
3. Clean all micropestles in bleach, wash in water and dry on paper towel.
4. Prepare 1 tray with purple QiaShredder columns and one micropestle per sample, keep in fume hood. Label with sample names.
5. 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.
6. Prepare 1 tray with kit eppendorfs and extra eppendorfs (quality control subsample) and label with sample names.
7. Prepare RLT buffer: per 4 samples, in 2 ml tubes, 1,780 µl RLT + 20 µl β-mercaptoethanol (or 2 samples: 890 µl + 10 µl).
8. Prepare glass beaker in fume hood (RLT buffer) and old kit plastic bottle for other waste[2].

[1] 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.

Extraction

1. Normal gloves are sufficient. Cool down micropestle end in liquid N_2. Fish out sample tube with forceps, open tube, cool down micropestle again. Holding tube in rack to avoid warming up, crush leaves, first with up-down motion, then with circular motion. Holding the eppendorf by the lid with the pestle inside, periodically half immerge in liquid N_2. Remove micropestle in water jar and add 450 µl RLT buffer[3]. Close the lid, whirlmix and keep in tray in fume hood. Repeat for all samples.
2. Transfer the samples in RLT[4] into the QiaShredder columns. Spin for 2 min at top speed.
3. Transfer the supernatant without disturbing the cell debris into the prepared eppendorf with ethanol, mix by pipetting[5] and transfer with the same tip to RNeasy column.
4. Spin for 15 sec at 10,000 RPM.
5. Remove flow-through into glass beaker[6]. Add 700 µl RW1 solution[7].
6. Spin for 15 sec at 10,000 RPM.
7. Discard flow-through in plastic bottle. Add 500 µl RPE.
8. Spin for 15 sec at 10,000 RPM.
9. Discard flow-through in plastic bottle. Add 500 µl RPE.

[2] Could wash down sink but they react badly with bleach, so better to evaporate in fume hood.

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

[4] Set the pipette to 700 µl to have peace of mind for the following 2 steps. Do not worry about leftover tissue.

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

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

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

10. Spin for 15 sec at 10,000 RPM.
11. Discard flow-through in plastic bottle. Transfer column in new collection tube.
12. Spin for 1 min at top speed to fully dry membrane.
13. Transfer column to labelled eppendorf from kit. Add 30 µl[8] RNase-free H_20 from kit, directly on the membrane.
14. Clean centrifuge lid and tube-holding area.
15. Spin for 1 min at 10,000 RPM[9].
16. Pipette 3 µl to tube "Q" to be used for qbit and the tape station. Keep on ice. Order in a box and transfer samples to -80°C until use.

[8] 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.

[9] I further 30 µl step could be added for higher yield and lower concentration, but single elution yields 140 - 450 ng/µl in 30 µl which is plenty.