# RNA extraction from *Mimulus*

## Preparation the day before the extraction

### *Materials needed*

- Lab keys
- Micropestles or beads
- 2 ml tubes 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)
- RNase-free tubes
- Glass beaker for β-mercaptoethanol waste
- Glass beaker for other waste

### *Preparation*

Once per kit: 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 $N_2$.
2. Clean the bench, trays, pipetes, centrifuge and gloves with 70% ethanol.
3. Prepare micropestles or beads.
4. Prepare 1 long[2] tray with purple QiaShredder columns, label with numbers.
5. Prepare 1 long tray with pink RNEasy mini spin columns, label with numbers.
6. Prepare tubes with 225 µl ethanol.
7. Prepare 1 tray with kit tubes.
8. 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 or 24 samples 10,680 µl + 120 µl).
9. Prepare glass beaker in fume hood (RLT buffer + β-mercaptoethanol waste) and glass beacked for other waste[3].

### *Extraction*

Work in fume hood until step 7.

1. Quickly transfer 12 samples from $N_2$ to cut tray that fits chainsaw tissue-lyser. Open, add a bead in each, add pre-made RLT + β-merc to each, close lid. Put to machine with rubber band and grind.

or

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 tube 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[4]. Close the lid, whirlmix and keep in tray in fume hood. Repeat for all samples.
2. Take photo of ordered tubes in tray and send to Paris on the same day. Label kit tubes with those sample names.
3. Transfer the samples in RLT[5] into the QiaShredder columns. Use filter tips. Spin for 2 min at top speed.
4. Wash beads in water and dry after putting to 100% ethanol.
5. Transfer the supernatant without disturbing the cell debris into the prepared tube with ethanol, mix by pipetting and transfer with the same tip to RNeasy column.
6. Spin for 15 sec at 10,000 RPM.

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[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.

[2] Only long trays are compatible with the multichannel pipette.

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

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

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

7. Pour off flow-through into β merc waste glass beaker[6]. Add 700 µl RW1 solution[7].

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

9. Discard flow-through in other waste glass beaker. Add 500 µl RPE.

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

11. Discard flow-through in other waste glass beaker. Add 500 µl RPE.

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

13. Prepare 2 µl collection tube from kit in racks.

14. Discard flow-through in other waste glass beaker. Transfer column to new collection tube from kit.

15. Spin for 1 min at top speed to fully dry membrane.

16. Transfer column to labelled 1.5 µl tube from kit. Add 60 µl[8] RNase-free $H_20$ from kit, directly on the membrane.

17. Clean centrifuge lid and tube-holding area with 70% ethanol.

18. Spin for 1 min at 10,000 RPM[9].

19. (Optional) Pipette 3 µl to additional tube "Q" to be used for qbit and the tape station. Keep on ice. Order in a box and transfer samples to -80ºC until use.

20. Update lab book with extraction names, 2 rows for 24 sample.

21. Take samples to -80ºC.

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[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.

[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] Single elution yields 140 - 450 ng/µl in 30 µl which is plenty.