

DNA extraction from *Mimulus*

Preparation the day before the extraction

Materials needed

- Waterbath or heat block
- 1 capless tube per sample
- 1.5 ml eppendorfs for final elution
- Liquid N₂ tank
- Metal scoop for sample transfer from mortars
- 70% ethanol for cleaning
- Qiagen DNA extraction kit for plants
- Old plastic bottle for wash buffer waste

Preparation

Freeze 1 mortar/pestle per sample at -20°C.

Extraction day

Setup

1. Prepare liquid N₂, ice box.
2. Clean the bench, trays, pipetes, scoop and centrifuge with 70% ethanol.
3. Prepare high friction paper on table top for mortars.
4. Prepare 1⁽¹⁾ eppendorf per sample with 400 µl **AP1**⁽²⁾.
5. ⁽³⁾Label 1 purple QiaShredder column per sample.
6. Label 1 white DNEasy mini spin column per sample.
7. Prepare 1 eppendorf per sample with 675 µl **AW1**.
8. Label 1 empty eppendorf per sample.

Extraction

1. Take out one mortar/pestle from -20°C⁽⁴⁾. Pour some N₂ and let it evaporate. Cool down pestle end in liquid N₂. Put tissue in mortar and add more N₂. Once most evaporates, start crushing with pestle and increase speed and range of motion once no N₂ remains. Slowly add more N₂ every 20 sec and repeat until ground to a fine powder. Scoop powder to tube with AP1, vortex and put at 65°C. Soak Mortar & pestle and clean scoop with 70% ethanol. Repeat for all samples.

2. Incubate all samples at 65°C for at least 10 min, and vortex them 2-3 times during this period.
3. Add 130 µl **P3**, mix and put on ice for 5 min.
4. Spin for 5 min at 20,000 g.
5. Transfer supernatant into purple QiaShredder column⁽⁵⁾.
6. Spin for 2 min at 20,000 g.
7. Set pipette to 650 µl. Transfer flow through (≈450 µl) to prepared tube with 675 µl **AW1**. Mix by pipetting, and take 650 µl of this mix to white DNEasy column.
8. Spin for 1 min at 6,000 g⁽⁶⁾.
9. Discard flow through in special wash buffer waste bottle. Blot tube rim on paper towel.
10. Transfer remaining mix from step 7 to white DNEasy column.
11. Spin for 1 min at 6,000 g.
12. Place column in new collection tube. Add 500 µl **AW2**. Invert to get **AW2** everywhere.
13. Spin for 1 min at 6,000 g.
14. Discard flow through.
15. Add 500 µl **AW2**.
16. Spin for 2 min at 20,000 g.
17. Put column in labelled empty eppendorf. Add 40 µl **AE**⁽⁷⁾. Incubate at RT for 5 min.
18. Spin for 1 min at 6,000 g⁽⁸⁾.
19. Discard column. Keep tube samples on ice and proceed with QC. Store at -20°C.

⁽¹⁾ If using RNase, can add 4 µl on lid so it only comes in contact with solution when the sample is added.

⁽²⁾ If the solution has precipitates, warm up in waterbath first and swirl until it becomes clear.

⁽³⁾ Steps below here can be done during steps 2-4 of the main protocol.

⁽⁴⁾ Lab gloves should be sufficient to handle mortar/pestle while grinding.

⁽⁵⁾ Do not disturb pellet, take less supernatant if needed.

⁽⁶⁾ This binds the DNA on the column.

⁽⁷⁾ Make sure it is on the membrane, and not walls.

⁽⁸⁾ Optional, apply flow through on membrane again, and repeat the spin to get a more concentrated sample.