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 N_2 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 N_2, ice box.
2. Turn on waterbath to 65°C.
3. Clean the bench, trays, pipetes, scoop and centrifuge with 70% ethanol.
4. Prepare high friction paper on table top for mortars.
5. Prepare 1[1] eppendorf per sample with 400 µl **AP1**[2].
6. [3]Label 1 purple QiaShredder column per sample.
7. Label 1 white DNEasy mini spin column per sample.
8. Prepare 1 eppendorf per sample with 675 µl **AW1**.
9. Label 1 empty eppendorf per sample.

Extraction

1. Take out one mortar/pestle from -20°C[4]. Pour some N_2 and let it evaporate. Cool down pestle end in liquid N_2. Put tissue in mortar containing some N_2 and add more if needed. Once most evaporates, start crushing with pestle and increase speed and range of motion once no N_2 remains. Slowly add more N_2 every 20 sec and repeat until tissue is 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. Set pipette to 650 µl.
5. Transfer supernatent into purple Qlashredder column[5].
6. Spin for 2 min at 20,000 g.
7. 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 (top) 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.
20. Clean up, leave lab in better state than you found it.

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[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 supernatent 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.