A modified CTAB-Qiagen column for DNA extraction in ferns

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Materials

3X CTAB buffer (autoclaved and kept at room temperature)
- 0.1 M Tris HCl pH 8.0
- 1.4 M NaCl
- 0.02 M EDTA
- 30 mg/mL CTAB

PCI solution (molecular grade, keep at 4°C, avoid light)
- Phenol: Chloroform: IAA, 25:24:1 mixture in pH 7.5-8.0

CI solution (molecular grade, keep at 4°C, avoid light)
- Chloroform: IAA, 24:1 mixture

Qiagen DNeasy Plant Mini Kit

Filter tips are recommended

Protocol

1. Add 5 µL β-mercaptoethanol and 4 mg PVPP for per 1 mL of 3X CTAB buffer just before use. You will use 1 mL of buffer per sample.
2. For fresh/silica-dry material, flash freeze the appropriate amount of sample, and grind with liquid nitrogen. For herbarium material, fragments ≤ 20 mg into 2.0 mL tube with beads, and homogenize with a bead blaster. Ensure that you are left with a fine powder as lysis will be most efficient without chunks of tissue.
4. Add 1 mL of CTAB solution to sample powder immediately, vortex to homogenize, and incubate samples at 65°C for 30 mins.
5. Add 500 µL PCI solution into each sample, vortex to homogenize, and then shake/invert for 2.5-3 hours at room temperature. Note: Using CI solution in place of PCI works better for some taxa in this step.
6. LUNCH! <optional>
7. Centrifuge samples at 13,000 rpm for 10 min. Transfer the aqueous phase (upper layer) into a new 2.0 mL Eppendorf.
8. Add 1.5 µL of RNAse A (100mg/mL), mix gently, and incubate at room temperature for 15 min.
9. Add 500 µL CI solution, vortex to homogenize, and shake/invert samples for 0.5-1 hour at room temperature. (I do a 45 min inversion on the first round, and then a 30 min inversion for the second round)
10. Centrifuge samples at 13,000 rpm for 10 min. Transfer the aqueous phase (upper layer) into a new 2.0 mL Eppendorf.
11. Repeat step 9-10 again, but substitute the CI solution for Chloroform (>99%).
12. Transfer the aqueous phase into new 5 ml Eppendorf(s) and mix with 3-5 times volume of Qiagen binding buffer (Buffer AW1). The following steps are slightly

modified from Qiagen protocol.

13. Transfer appropriate volume (about 650 µL) of the mixture into DNA binding column (DNeasy Mini spin column) with 2 ml collection tube, then centrifuge samples at ≥ 8000 rpm for 1 min. Discord the flow-through.
14. Repeat step 13 until all the remaining mix solution has been put through the column.
15. Add 500 µl washing buffer (Buffer AW2) to DNA binding column, and then centrifuge at ≥ 8000rpm for 1 min. Discord the flow-through. If the flow-through or membrane of DNA binding column is discolored, it is recommended to repeat this step until the color disappears or is diluted. 100% Ethanol can be used to substitute washing buffer for more efficient clearing.
16. Put the column into a new collection tube and dry the DNA binding column at 13,000 rpm for 5 mins in table centrifuge.
17. Discard the collection tube and apply DNA binding column to a new 1.5 ml Eppendorf labeled with sample information.
18. Dry the DNA binding column in vacuum centrifuge for 10 mins. <optional>
19. Apply 50 µL Elution Buffer (10mM Tris-HCl) directly onto DNA binding column, incubate at 65°C for 2-3 min, and then centrifuge at 12,000 rpm for 1 min.
20. Place the column in a new 1.5 mL Eppendorf and repeat step 19, discard the DNA binding column. <optional>
21. QC with Nanodrop and Qubit. Store the extracted DNA at 4°C for short-term use or -20°C for long-term storage.

Related references

DNeasy Plant Handbook — October 2012. http://www.qiagen.com/resources/download.aspx?id=95dec8a9-ec37-4457-8884-5dedd8ba9448&lang=en

Varma A, Padh H, Shrivastava N. 2007. Plant genomic DNA isolation: an art or a science. *Biotechnol J.* **2**: 386-392.

Appendix 1. Comparison of DNA quantity and quality by different DNA extraction methods from 20 mg frond fragments of *Deparia lancea* specimens in TAIF. (Without RNAse treatment)

DNA concentration of total 100 µL extracted solution:

Specimen No.	G	C+G	Q	C+Q
212849	21 ng/µl	85 ng/µl	126 ng/µl	170 ng/µl
221676	21 ng/µl	113 ng/µl	95 ng/µl	137 ng/µl

OD 260/280 value of total 100 µL extracted solution:

Specimen No.	G	C+G	Q	C+Q
212849	1.750	1.932	1.969	2.024
221676	1.400	1.948	1.939	2.015

OD 260/230 value of total 100 µL extracted solution:

Specimen No.	G	C+G	Q	C+Q
212849	1.651	3.400	1.482	1.910
221676	0.525	1.794	1.357	1.827

G: Geneaid plant mini kit process; C+G: CTAB DNA extraction + Geneaid genomic mini kit (plant) column purification; Q: Qiagen Plant mini kit process; C+Q: CTAB DNA extraction + Qiagen Plant mini kit column purification

Appendix 2. Extraction quality metrics for several species of ferns.

Species	Weight (mg)	Concentration (ng/uL)	OD260/280	OD260/230
Lygodium japonicum	19.3	143	1.92	2.03
Callistopteris baldwinii	6.3	15.2	1.83	1.78
Mryiopteris lanosa	29.4	38.5	1.85	1.90
Homalosorus pycnocarpos	18.2	41.0	1.87	1.93
Dryopteris ludoviciana	22.7	37.8	1.86	1.84