Communication

Transferability of microsatellite markers developed in *Oenothera* spp. to the invasive species *Oenothera drummondii* Hook. (Onagraceae)

Genomic DNA of all samples was extracted from leaf tissue as follows [1]:

1. For each sample, 0.05 g of dried leaf tissue was weighed. Note: youngest leaves allow better extraction than old ones.
2. Leaves were surface sterilized with 70% ethanol, rinsed with distilled water, cut in small pieces, and put into ceramic mortars.
3. Then leaves were pulverized to dust with liquid nitrogen.
4. In a 1.5 ml tube, the tissue was suspended in 1 ml of 2% CTAB extraction buffer (1.4 M NaCl, 100 mM Tris [pH 8.0], 20 mM Na2 EDTA, 2% [w/v] CTAB) and placed in a water bath at 65°C for at least 90 min, mixing carefully before place the tubes in the water. Tubes can stay in a water bath overnight.
5. The 1 ml of extraction buffer was transferred to a clean 1.5 ml tube, saving the tissue for repeated extractions.
6. This solution was extracted twice for 5 min, with 500 µl of chloroform-isoamyl alcohol 24:1 (v/v) and centrifuged at 13000 rpm for 10 min.
7. The upper aqueous layer was mixed with 50 µl of 3 M sodium acetate; absolute cold ethanol (800 µl) was then added to precipitate DNA for at least one hour. Then, centrifuged at 13000 rpm for 10 min.
8. The pellet was collected, rinsed with 300 µl of 70% cold ethanol, vacuum dried, and re-suspended in TE buffer (10 mM Tris-Cl, pH 8.0 1.0 mM EDTA).
9. The tissue was kept in the refrigerator at -4°C for future DNA re-extractions. We recovered suitable quality DNA from the same tissue up to three consecutive extractions.

References

1. González, D.; Vovides, A. Low Intralineage Divergence in Ceratozamia (Zamiaceae) Detected with Nuclear Ribosomal DNA ITS and Chloroplast DNA trnL-F Non-coding Region. *Systematic Botany* **2002**, *27*, 654–661.