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TECHNICAL NOTE

Recovery of plant DNA using a reciprocating saw and silica-based columns

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Abstract

The time needed for hand grinding and the cost of commercially available extraction kits remain to be the major limitations in plant DNA extraction for many researchers. We present inexpensive techniques for (i) simultaneously machine grinding large numbers of plant samples for DNA extraction using a commercially available reciprocating saw; and (ii) DNA recovery using silica column-based extractions similar to that used in some commercially available kits. Used together, these allow for the rapid recovery of plant DNA at relatively low cost. Furthermore, these methods appear to be widely applicable within plants with good yields recovered in test extractions across major plant groups (ferns, gymnosperms, monocots and eudicots).

Keywords: DNA, isolation, plant, reciprocating, recovery, saw, silica

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Introduction

Recent advances with commercially available kits have greatly increased the ability for researchers to extract high quality plant DNA. Bead-based homogenization machines in combination with commercially available DNA isolation kits allow efficient high-throughput DNA isolation, but the cost for specialized equipment and purchased disposables limits the use of these approaches to more heavily funded well-equipped laboratories whose research is typically focused on model taxa. After encountering these limitations in various ongoing projects, for which we wished to rapidly grind and extract DNA from large numbers of individuals, we adopted a simple and inexpensive method. This method combines mechanized plant cell disruption using a 'home-modified' readily available reciprocating saw with a silica-gel extraction protocol to allow high throughput recovery of DNA samples at a fraction of the cost for ready made kits. The machine-grinding technique is based on the method used by commercially available (e.g. Dyno-Mill, GlenMills,) and previously published (Michaels & Amasino 2001) methods, but it reduces equipment cost to a small hand-held unit at $US175. The isolation chemistry is based on modifications of the Dellaporta *et al.* (Dellaporta *et al*. 1983) chemistry, using silica gel columns from Epoch BioLabs for *c*. $US0.35 a column or $US13 per 96-well plate. The methods discussed here is amenable to extraction of individual samples in standard 1.5 mL Eppendorf tubes or via 96-well plates.

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Materials and methods

Plant material

Fresh and silica gel-dried leaf (or stem in *Ephedra* and *Rhipsalis*) samples were collected from the following taxa: Pteridophyta: Polypodiales: *Cheilanthes alabamensis*, Ophioglossales: *Ophioglossum vulgatum;* Coniferophyta: Gnetales: *Ephedra torreyana*, Cycadales: *Cycas revoluta;* Anthophyta: Asparagales: *Iris* sp., *Phalaenopsis* sp., *Dracaena* sp.; Arecales: *Chamaedorea elegans;* Zingiberales: *Stromanthe* sp.; Caryophyllales: *Rhipsalis* sp.; Fabales: *Prosopis glandulosa;* Malpighiales: *Chamaesyce* sp.; Brassicales: *Lepidium montanum, Pennellia micrantha, Boechera perennans, Boechera fendleri;* Zygophyllales: *Larrea tridentata;* Asterales: *Heterotheca villosa*.

Reciprocating saw

A Bosch RS20 dual-speed reciprocating saw with a stroke length of 32 mm was used with two different types of

attachments for extraction in 1.5 mL Eppendorf tubes or 96-well plates. The former employs a standard polypropylene 80-well microcentrifuge tube rack (e.g. Laboratory Product Sales T900994) modified for use with the reciprocating saw by plunging a stove-heated Blu-Mol Xtreme wrecking blade through the centre of the plastic rack. The blade was secured with two 1 3/4′ wood screws driven through width of the rack and blade, one from each side (Fig. 1). Holes in the metal blade were predrilled with a standard metal boring drill bit. The wells in the microcentrifuge rack around the screw were filled with polyurethane foam glue (Gorilla Glue) for added stability and longevity. Tubes can be held in place in a variety of ways, but the authors have found electrical tape useful for homogenizing under all conditions (including post-submersion in liquid nitrogen). A 96-deep-well plate-holding bracket was also developed by using two planks of poplar as a clamp mounted to the end of saw blade (Fig. 2). This 96-well adapter is not fit for submersion in liquid nitrogen, but the same design can be easily constructed from materials that are resistant to rapid changes in temperature (e.g. polypropylene).

Grinding

Plant samples (10–20 mg dried or *c*. 100 mg fresh) were placed in 1.5 mL microcentrifuge tubes with three 3.2 mm chrome-steel beads (BioSpec Products, Inc., catalogue no. 11079132c) per tube and oscillated on the saw's 'low' setting for 20–30 s. Oscillation was carried out with fresh plant tissue of all samples except the two species of *Boechera*. All fresh tissue samples were oscillated in 400 mL of grind buffer, and for *P. glandulosa* an additional isolation run was conducted using freezing in liquid N_2 without buffer. *Boechera* isolations used silica-dried material either at room temperature or after freezing in liquid N_2.

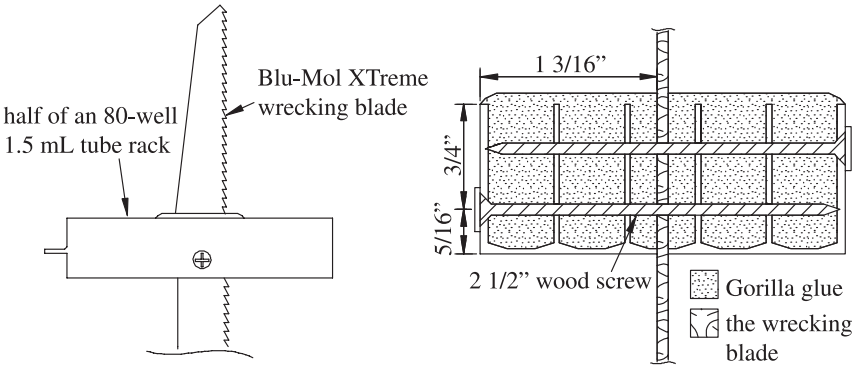

Fig. 1 A diagram showing the modification of a tube rack for attachment to the reciprocating saw.

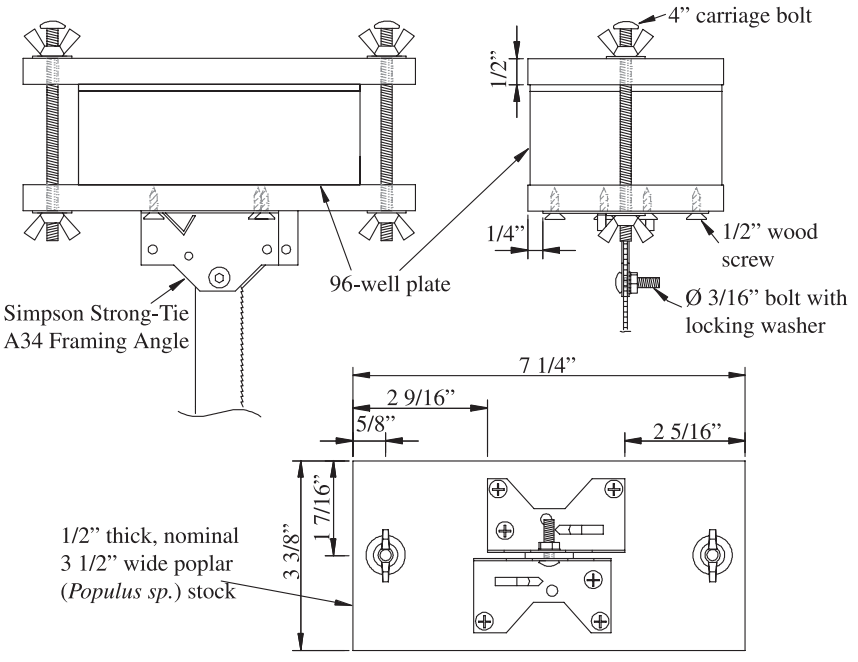

Fig. 2 A diagram showing an adapter to hold a 96-well plate attached to the reciprocating saw.

DNA isolation

DNA of all species was isolated according to the protocol given in Table 1, and duplicate isolations of all except the two *Boechera* species were conducted using the QIAGEN DNeasy protocol. Recovered DNA yield and quality were assessed through comparison with 100-bp DNA mass ladder (New England BioLabs N3231L) on 0.9% agarose gels stained with ethidium bromide.

Results and discussion

Machine grinding

Machine-based tissue grinding methods use rapid oscillation of 96-well plates or centrifuge tubes containing both the tissue samples and dense inert objects as a substitute for hand grinding with mortar and pestle or a micropestle. Essentially, all that is required for these methods is a motor geared to produce rapid linear oscillations of a stroke length equal to or greater than the length of the tubes or wells used. A heavy-duty hand-held reciprocating saw appears to be the simplest and least expensive readily available device that produces oscillations of appropriate rapidity and stroke-length. The only modification needed is the addition of a receptacle capable of holding microcentrifuge tubes or 96-well plates (see Materials and methods). In the present study, 1.0 mm zirconia beads (BioSpec Products, Inc., catalogue no. 11079110zx) were also tested, but these did not appear to be as effective as 3.2 mm chrome-steel beads. Other authors have used less expensive materials, such as #7 1/2 bismuth bird shot, with good results (Krysan 2004).

Grinding can be done with fresh or dried material. With fresh material, grinding in the grind buffer gives best results. However, with dried material grinding at room temperature without buffer is preferred, since with dried material the grind buffer is not necessary for prevention of DNase activity and simply adds weight and impedes bead movement. With either fresh or dry material, freezing in liquid nitrogen without grind buffer is an alternative approach that appears to give equivalent results.

Isolation chemistry

The isolation protocol presented in Table 1 is derived from the method of Dellaporta *et al*. (Dellaporta *et al*. 1983) as modified by the Nasrallah laboratory at Cornell (Castleman

Table 1 Protocol for DNA isolation using a reciprocating saw and silica columns

1. Prepare enough grind buffer for 400 µL per sample and preheat to 65 °C. For each sample, the grind buffer is composed of 320 µL of homogenization buffer (0.1 M NaCl, 0.2 M Sucrose, 0.01 M EDTA, 0.03 M Tris-HCl pH 8.0), 80 ul lysis buffer (0.25 M EDTA, 2.5% SDS, 0.5 M Tris pH 9.2), and 4 µL (100 mg/µL) RNase. The homogenization and lysis buffers can be stored at room temperature indefinitely after filter sterilization. 80 ul
2. Add two to four sterile 3.2-mm chrome steel beads to 1.5 mL Eppendorf tubes (rated to withstand 30 000 *g* centrifugation) and 10–20 mg dried or *c*. 100 mg fresh plant material to each tube. When using fresh material, add 400 µL preheated grind buffer to each tube.
3. Load the tubes into the modified reciprocating saw rack and mount the rack to the saw. Reciprocate on the 'low' setting for 20–30 s or until homogenization is complete. Use the minimum duration needed to disrupt the plant material.
4. For dry material, add 400 µL preheated grind buffer to each tube now.
5. Incubate buffered grindate at 65 °C for 10 min, mixing the tube by inversion every 2–3 min.
6. Add 130 µL 3 M pH 4.7 potassium acetate, invert several times and incubate on ice for 5 min. Preparation of potassium acetate — add 14.72 g to 20 mL sterile water, slowly add sufficient glacial acetic acid to make 45 mL, check pH, and add sterile water or glacial acetic acid to make 50 mL. Do not autoclave or filter sterilize.
7. Spin tubes in a microcentrifuge at maximum force for 10 min. If a more powerful centrifuge is available, use higher centrifugal force for even better separation of supernatant and precipitate.
8. Transfer supernatant to sterile 1.5 mL centrifuge tube. Avoid transfer of any precipitate.
9. If any precipitate is transferred with the supernatant, repeat steps 7 and 8.
10. Add 1.5 volumes binding buffer (2 M guanidine hydrochloride in 95% EtOH; note, guanidine hydrochloride is a hazardous chaotropic salt — store at room temperature). Typically ~400 µL is recovered from previous steps, in which case 600 µL binding buffer is added.
11. Apply 650 µL of mixture from step 10 to Epoch spin column and centrifuge for 1–10 min (until all liquid has passed through) at maximum force in a microcentrifuge and discard flow-through (contains guanidine hydrochloride).
12. Repeat step 11 with the remaining volume from step 10.
13. Wash the DNA bound to the silica membrane by adding 500 µL of 70% EtOH to the column and centrifuge until all liquid has passed to the collection tube (2–8 min). Discard the flow-through.
14. Repeat step 13.
15. After discarding the flow-through from step 14 centrifuge the columns at maximum force for an additional 5 min to remove any residual ethanol.
16. Discard the collection tubes and place the columns in sterile 1.5-mL centrifuge tubes.
17. Add 100 µL preheated (65 °C) sterile 10 mM Tris to each tube. Let stand 1–5 min and then centrifuge for 1–2 min at maximum force to elute the DNA.

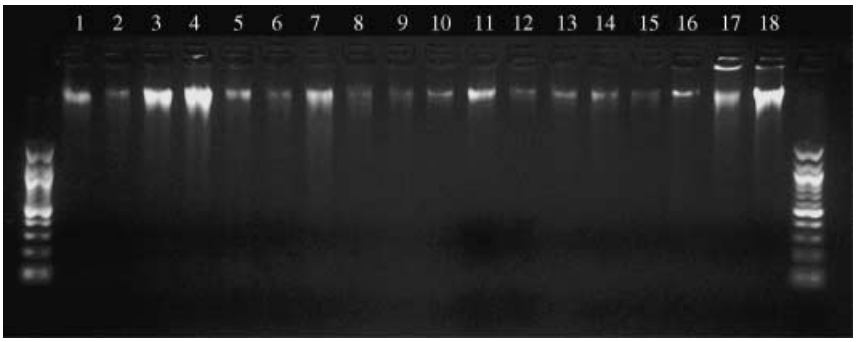

Fig. 3 An agarose gel showing results of DNA isolations with the described techniques across a range of taxa. The lanes at far right and far left each contain 1 μL of 100-bp DNA mass ladder. The numbered lanes include 3 μL of each sample as follows: 1-*Ophioglossum* sp.; 2-*Cheilanthes alabamensis*; 3-*Ephedra* sp.; 4-*Cycas revoluta*; 5-*Iris* sp.; 6-*Phalaenopsis* sp.; 7-*Chamaedorea* sp.; 8-*Stromanthe* sp.; 9-*Dracaena marginata*; 10-*Rhipsalis* sp.; 11-*Prosopis glandulosa*; 12-*Chamaesyce albomarginata*; 13-*Lepidium* sp.; 14-*Pennellia micrantha*; 15-*Boechera fendleri* 16-*Boechera perennans*; 17-*Larrea tridentata*; 18-*Heterotheca* sp.

1998) and then further modified by the authors. The most significant modification is that the ethanol-precipitation and centrifugation steps used for final washing and purification of DNA are replaced with the use of guanidine hydrochloride in ethanol to bind the DNA to a silica-membrane column (Epoch BioLabs Spin Columns). Bound DNA is subsequently washed and eluted from the column (steps 10–18, Table 1). This protocol appears to be very similar to that used in commercially available kits with unpublished chemistry.

Resulting DNA quality

DNA isolations were successful across all taxa, giving yields generally similar to that obtainable from hand-grinding using a QIAGEN DNeasy kit. However, some extractions derived from machine-homogenized material showed some amount of 'smearing' on the gel, indicating that some of DNA is being sheared (Fig. 3). Other papers showing DNA isolates from machine-grinding techniques (Csaikl *et al*. 1998) also show smearing, suggesting that this is likely a result of the violent process involved in bead homogenization. The degree of smearing apparently depends on the chemistry used, as in duplicate extractions using reciprocating saw homogenization with either the presented protocol or the QIAGEN DNeasy Plant Minikit protocol, the DNeasy protocol consistently showed greater smearing and often lower yields (data not shown). Decreasing yield with increasing storage time since grinding has also been observed with *Boechera* (P. Alexander, unpublished data), suggesting that grinding tissues more than a week before DNA isolation is best avoided. Regardless, large numbers of isolations from *Boechera* seem to give consistent results (see Fig. 4) and polymerase chain reaction (PCR) amplifications for sequencing and amplified fragment length polymorphism studies have so far produced good results from DNA extracted using the approaches discussed here (P. Alexander *et al*., unpublished data).

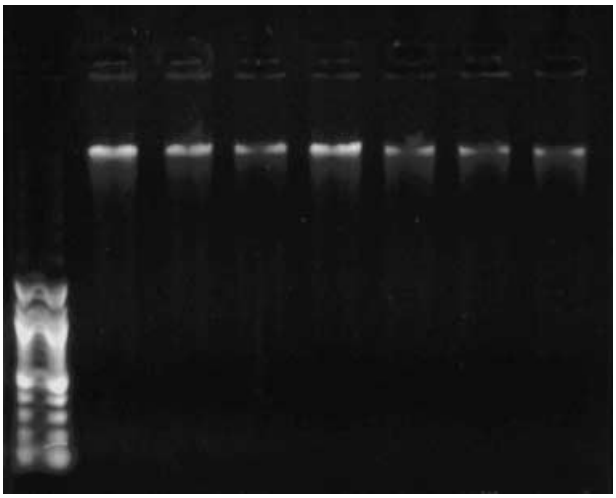

Fig. 4 An agarose gel showing results of DNA isolations with the described techniques from multiple dried samples of *Boechera fendleri*. The lane at far left contains 1 μL of 100-bp DNA mass ladder, the others contain 3 μL each of samples from seven individuals from the same population.

Summary

Efficient methods for high-throughput DNA isolation have been available for some time, yet their use remains limited to a minority of laboratories equipped with appropriate equipment and supplies. The methods presented make low-cost high-throughput extraction of plant material available to most laboratories.

Acknowledgements

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References

Castleman AL (1998) *A Transcriptional, Genetic, and Physical Exploration of the S locus in Brassica*. PhD Dissertation, Cornell University, New York.

Csaikl UM, Bastian H, Brettschneider R *et al.* (1998) Comparative analysis of different DNA extraction protocols: a fast, universal maxi-preparation of high quality plant DNA for genetic evaluation and phylogenetic studies. *Plant Molecular Biology Reporter*, **16**, 69–86.

Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA minipreparation: version II. *Plant Molecular Biology Reporter*, **1**, 19–21.

Krysan P (2004) Ice-cap. A high-throughput method for capturing plant tissue samples for genotype analysis. *Plant Physiology*, **135**, 1162–1169.

Michaels SD, Amasino RM (2001) High throughput isolation of DNA and RNA in 96-well format using a paint shaker. *Plant Molecular Biology Reporter*, **19**, 227–233.