Testing protocols for preserving and extracting oak DNA for conservation genetic studies

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Background

Quercus havardii (Fagaceae) is unique in that it primarily reproduces clonally and hybridizes readily with related species. Human disturbances like farming and drilling in its native native southwestern American range are causing rapid population declines. It is important to assess current amounts of genetic variation and determine the most effective management options to prevent further population loss.

Figure 1. *Q. havardii* seedling used for study

To study *Q. havardii* genes, DNA must be isolated. It is difficult to extract DNA from oaks because they contain compounds, like phenolics and tannins, that interfere with normal methods. *Q. havardii* provides additional challenges due to physical properties, like thick, leathery leaves. Different methods of field preservation and homogenization will influence DNA quality. Different DNA extraction protocols may be more or less effective at isolating DNA usable in genetic studies.

Methods

Three species of white oak were tested under all possible combinations of field preservation, tissue homogenization, and extraction method. Each extraction was performed in duplicate, totaling 240 samples.

Species:	**Field Preservation:**
1. *Q. havardii*	1. Pressed 2 days
2. *Q. muehlenbergii*	2. Pressed 7 days
3. *Q. stellata*	3. Chilled 2 days
Extraction Method:	4. Chilled 7 days
1. Qiagen DNeasy kit[A]	5. Dried in silica gel
2. Omega EZNA kit	**Tissue Homogenization:**
3. CTAB[B]	1. Hand ground in liquid N_2 (LN_2)
4. Modified CTAB (CTAB+)[C]	2. FastPrep machine

DNA concentrations were assessed with a fluorometer and molecular weights were measured with agarose gel electrophoresis. Results were analyzed using a multi-factor ANOVA test in JMP.

[A] modified protocol from Hipp Laboratory at The Morton Arboretum
[B] protocol from Pritzker Laboratory at The Field Museum of Natural History and Romero-Severson Laboratory at Notre Dame University
[C] protocol from McCauley Laboratory at Fort Lewis College

Objective

To determine which combination of **(1)** field preservation, **(2)** tissue homogenization, and **(3)** extraction method yields the highest concentration of high molecular weight DNA in **(4)** multiple white oak species.

Results

(1) Preservation: Chilling leaves yields the highest average DNA concentrations, averaging 16.9 ng/µL for 2 day and 19.3 ng/µL for 7 day ($p < 0.05$; Figure 2). Pressing leaves for 7 days yields the lowest average DNA concentrations, averaging 13.6 ng/µL ($p < 0.05$; Figure 2).

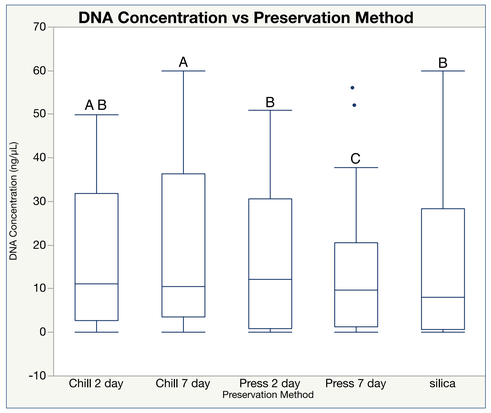

Figure 2. Chilling leaves produces more DNA than drying them with a plant press or silica gel. Letters denote conditions with significant difference.

(2) Homogenization: For every extraction method, FastPrep yields higher DNA concentrations than LN_2 ($p < 0.05$; Figure 3). FastPrep yields DNA at lower molecular weights and with more smear than LN_2 (Figure 4). The highest average DNA concentration is seen in EZNA with FastPrep, at 37.5 ng/µL.

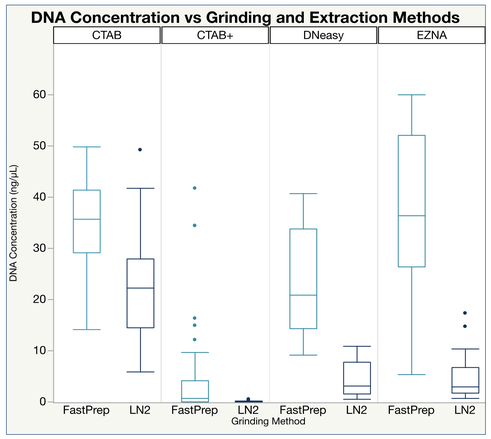

Figure 3. Each extraction method is broken down into grinding methods. Differences are significant between all conditions.

Results Cont.

(3) Extraction method: All methods differ from each other significantly. CTAB shows the greatest DNA concentrations, EZNA and DNeasy show moderate DNA concentrations, and CTAB+ results in the lowest concentrations ($p < 0.05$; Figure 3; Table 1).

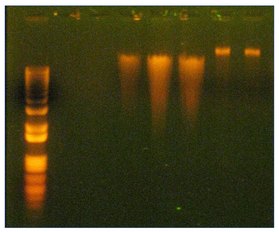

Figure 4. Leftmost band–ladder; three middle bands–FastPrep; rightmost two bands– LN_2

Extraction	Average (ng/µL)
CTAB	28.9
EZNA	21.1
Dneasy	13.6
CTAB+	2.5

Table 1. Total average DNA concentration for each extraction method. All values are significant ($p < 0.05$).

(4) Species: Though all different from each other ($p < 0.05$), all species follow the same trends under all conditions.

Conclusions

- **Overall highest DNA concentration from:** chilled leaves, FastPrep, EZNA
- Chilled leaves and CTAB yields highest DNA concentrations when both grinding methods are combined
- LN_2 grinding yields low quantities of high molecular weight DNA. FastPrep yields high quantities of lower molecular weight DNA
- Different future studies will require different DNA quantities and qualities. Researchers should choose methods best suited to their specific experimentation needs
- Future tests should isolate and compare quantities of high molecular weight DNA from LN_2 and FastPrep using gel extraction
- Further studies should use restriction enzymes to confirm that extracted DNA is suitable for sequencing and test microsatellite markers so DNA can be used in conservation genetic studies
- Future work could perform similar tests in red oaks
- The results of this study will be applied to collection and study of clonality and hybridization in *Q. havardii* to best conserve its population

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