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**NOTE**

# A SIMPLE AND RAPID TECHNIQUE FOR THE ISOLATION OF DNA FROM MICROALGAE[1]

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**A simple method for the purification of PCR-quality DNA from microalgae is presented. This method uses the detergent dodecyltrimethylammonium bromide coupled with cell breakage by agitation in the presence of glass beads and chloroform. A final purification step involves a commercial cartridge system. The procedure requires only about 1–2 mL of algal culture and can be completed in about 20 min. DNA suitable for PCR has been obtained from several algal lineages using this method, including numerous green algae and stramenopiles.**

***Key index words:*** **DNA isolation; microalgae**

***Abbreviations:*** **CTAB, hexadecyltrimethylammonium bromide; DTAB, dodecyltrimethylammonium bromide**

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Diversity studies of microalgae require the purification of PCR-ready DNA from a large number of isolates. In our ongoing investigation of the diversity of chlorophyte microalgae, we are purifying DNA from over 1000 isolates. With such a large number of isolates, it is essential that the method used for DNA purification is rapid, inexpensive, requires little cellular material, results in DNA that can be used directly for PCR, and is effective across a broad range of algal lineages. Moreover, the use of highly toxic material such as phenol should be avoided, and the procedure should be simple enough for undergraduate students to perform.

DNA purification techniques that are commonly used for microalgae are mostly based on the hexadecyltrimethylammonium bromide (CTAB) method of Doyle and Doyle (1990), which requires grinding a fairly large quantity of cells in liquid nitrogen. Several modifications of the basic CTAB method have been published, but they are generally fairly time consuming. At the other extreme, some studies have foregone the isolation step altogether and instead have used whole cells in the PCR (Hoham et al. 2002), a process that can only work for some organisms.

Some coccoid microalgae, such as *Chlorella vulgaris*, can present special problems for DNA isolation, primarily because of the mechanical strength of their cell walls coupled with their very small size. A technique useful for some coccoid algae was developed by Friedl (1995). We previously modified this technique to make it somewhat simpler (Fawley et al. 1999, Phillips and Fawley 2000). This technique used an initial extraction with the detergent dodecyltrimethylammonium bromide (DTAB) with cell disruption using a MiniBead-Beater (Biospec Products, Bartlesville, OK, USA), a chloroform extraction, followed by precipitation using CTAB in low salt solution, and a final ethanol precipitation. Very little starting material was required for this procedure, and several samples could be processed simultaneously. However, several steps were used and required about an hour to complete. We also found that results with this technique were not consistent for many algal isolates (unpublished observations), although we were able to purify PCR-quality DNA from isolates from the Chlorophyceae, Trebouxiophyceae, and Prasinophyceae using this technique (Fawley et al. 1999, 2000, Phillips and Fawley 2000). This technique has also been successfully used for at least some streptophytes (Turmel et al. 2002).

Our new technique takes some of the procedures from Friedl's (1995) method and our (Fawley et al. 1999, Phillips and Fawley 2000) modification and replaces the precipitation steps with a single step using a standard DNA purification spin cartridge. The result is a DNA purification procedure that typically requires only 1.5–2 mL of algal culture, works with a wide range of algal lineages, and takes about 20 min to complete. Multiple samples can be processed simultaneously, and the process is simple enough that students learn it in only one session. The resulting DNA is suitable for PCR with a variety of primers and provides enough DNA for at least 30 25-μL reactions.

*DNA purification procedure.* One to 2 mL of algal culture was centrifuged in a conical-bottom 2-mL screw-top microcentrifuge tube at approximately 16,000 *g* for 1 min. The supernatant was discarded and 200 μL extraction buffer (1 M NaCl, 70 mM Tris, 30 mM $Na_2EDTA$, pH 8.6) added and vortexed briefly. This suspension was then centrifuged at 16,000 *g* for 1 min, the supernatant discarded, and 200 μL fresh extraction buffer added. A quantity of glass beads (G-8772, Sigma Chemical Co., St. Louis,

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Table 1. Algal isolates used to evaluate the new DNA purification system.

| Taxon | Isolate |
|---|---|
| Eustigmatophyceae | |
| *Nannochloropsis limnetica* Krienitz | KR1998/3 |
| Bacillariophyceae | |
| *Nitzschia palea* (Kütz.) W. Smith | FDCC L996 |
| *Nitzschia sigma* (Kütz.) W. Smith | FDCC L1546 |
| Phaeophyceae | |
| *Ectocarpus siliculosus* (Dillw.) Lyng. | UTEX LB2008 |
| Xanthophyceae | |
| *Gloeobotrys* sp. | Tow 6/3 P-16w |
| Chlorophyceae | |
| *Chlamydomonas reinhardtii* Dang. | CGC1691 |
| *Pseudodictyosphaerium* sp. | CCMP2217 |
| *Scenedesmus* sp. | |
| *Monoraphidium* sp. | Itas 9/21 14-7w |
| Trebouxiophyceae | Tow 8/18 P-2w |
| *Chlorella vulgaris* Beij. | UTEX 30 |
| *Choricystis* sp. | CCMP2207 |
| *Nannochoris* sp. | CCMP2225 |
| *Oocystis* sp. | CCMP2249 |
| Pedinophyceae | |
| *Pedinomonas minor* Korsh. | UTEX 1350 |
| Prasinophyceae | |
| *Nephroselmis pyriformis* (Carter) Ettl. | CCMP717 |
| *Prasinococcus capsulatus* Miyashita et Chihara | CCMP1407 |
| *Pyramimonas parkeae* Norris et Pearson | CCMP725 |
| Unidentified coccoid | CCMP1413 |
| Rhodophyta | |
| *Porphyridium aerugineum* Geitler | UTEX 755 |

*Scenedesmus* sp., *Monoraphidium* sp., and *Gloeobotrys* sp. are from our collection. CCMP, Provasoli-Guillard National Center for Culture of Marine Phytoplankton (Andersen et al. 1997); CGC, Chlamydomonas Genetics Center (Harris 1984); FDCC, Freshwater Diatom Culture Collection (Czarnecki 1994); UTEX, Culture Collection of Algae at the University of Texas at Austin (Starr and Zeikus 1993).

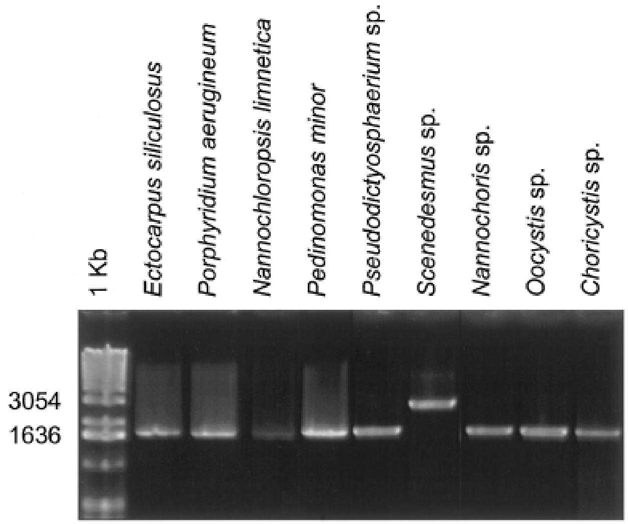


Fig. 1. Agarose gel electrophoresis of 18S rDNA PCR products from select algal isolates (see Table 1). Sizes (in base pairs) of pertinent markers from the 1-kb DNA standard (Life Technologies, Rockville, MD, USA) indicated to the left.

MO, USA) sufficient to fill the conical portion of the centrifuge tube was then added, followed by 25 µL 10% DTAB (Sigma Chemical Co.) and 200 µL chloroform. A MiniBeadBeater was then used to disrupt the cells, with agitation for 20 s at top speed. The mixture was then centrifuged at 2000 *g* for 2 min to separate the phases. If the cells were not well broken, as evidenced by a cell layer at the interface of the aqueous and organic phases and a lack of green pigment in the organic phase, the MiniBeadBeater step and centrifugation were repeated. The MiniBeadBeater step was never repeated more than once. One hundred microliters of the aqueous phase was removed to a 1.5-mL microcentrifuge tube, 500 µL GeneClean salt (QBiogene, Carlsbad, CA, USA) was added and mixed, and the resulting solution was applied to a GeneClean Turbo (QBiogene) cartridge. The cartridge was then used to purify the DNA according the manufacturer's instructions.

*Test organisms.* We used this DNA purification technique with a wide range of organisms, including green algae from the Chlorophyceae, Trebouxiophyceae, Pedinophyceae, and Prasinophyceae; xanthophytes; eustigmatophytes; a red alga; a brown alga; and diatoms. Most of these organisms are our own isolates from our diversity studies, but we also tested several organisms from culture collections. Table 1 presents a partial list of organisms that have been examined. These organisms were grown using standard methods for our laboratory (Fawley et al. 1990, Fawley 1992).

*PCR.* Universal primers specific for 18S rDNA were used to evaluate the DNA purification procedure. Primers and conditions were essentially those of Phillips and Fawley (2000) or Fawley et al. (1999, 2000), except that the Qiagen Master Mix PCR Kit was used (Qiagen, Valencia, CA, USA).

The 18S rDNA was successfully amplified using template DNA isolated from all the test organisms by the new procedure. Figure 1 shows an agarose gel of the amplification products from a sampling of these organisms. We also used this technique successfully with over 300 additional isolates from our present diversity studies. Additional loci, including *rbc*L and the ribosomal internal transcribed spacer regions, were also PCR amplified from many isolates using template DNA purified with this procedure (data not shown). The procedure also works with some organisms (*Porphyridium* and *Ectocarpus*) that possess polysaccharides that can inhibit PCR amplification (Kitade et al. 1996). Although *Porphyridium* DNA can be isolated using a simple procedure that includes a standard kit (Yoon et al. 2002), existing protocols for DNA isolation from the Ectocarpales are quite involved (Siemer et al. 1998). The largest PCR product we obtained using template DNA isolated with this procedure was about 4.3 kb, the 18S rDNA from a *Choricystis* sp. that possessed multiple putative group I introns. However, amplification of larger fragments may also be possible.

With the new procedure, we typically purify DNA from four to eight algal isolates at the same time, with approximately 30 min required for purification. If desired, the purification procedure could be scaled up to process over 100 isolates per day. The main advantages to this method over previous methods are as follows:

1. Ease and simplicity. This DNA purification procedure requires far fewer steps than most other procedures. The process is easily mastered by anyone able to use a micropipetter and microcentrifuge.

2. General applicability. Although we have not attempted this procedure with some types of algae, it has been applied successfully to different lineages of green algae and stramenopiles and one rhodophyte and is likely to work with many other kinds of algae.

3. Low exposure to hazardous materials. Only a single chloroform extraction is used in this procedure.

4. Low cost. The only specialized equipment required is a low cost MiniBeadBeater, and the materials used are inexpensive compared with commercial DNA isolation kits.

This DNA purification procedure should enable phycologists to examine many algal isolates by molecular techniques much more rapidly than using existing protocols. It should have wide applicability for any studies involving PCR-based techniques, such as PCR-RFLP, rapid analysis of polymorphic DNA, and sequencing.

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