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Preparing High-Quality DNA From Moss (*Physcomitrella patens*)

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Abstract. *Physcomitrella patens*, a moss, is the only land plant that performs high rates of homologous recombination, making it a valuable tool for functional genomics. Unfortunately, commercially available plant DNA preparation kits are ineffective with *Physcomitrella*. Furthermore, labor-intensive CTAB preparations produce low yields, and the DNA is degraded and contaminated. We present a protocol that is faster and doubles the DNA yield obtained from standard procedures. The high-quality DNA prepared is suitable for PCR reactions and Southern blot analysis.

Full text†: This manuscript, in detail, is available only in the electronic version of the *Plant Molecular Biology Reporter.*

Key words: genomic DNA, method, moss, PCR, *Physcomitrella*, Southern blot

Introduction

Physcomitrella patens, an easily transformable moss, is the only plant known to exhibit a high rate of homologous recombination, ranging from 40-100% among transgenics (Reski, 1998; Schaefer, 2001). When the rate of homologous recombination in *Arabidopsis thaliana* (~0.01%) is compared, the advantage of *Physcomitrella* in functional genomics is obvious. It has been used as a model system in recent years (Schaefer and Zryd, 1997; Girke et al., 1998; Strepp at al., 1998; Girod et al., 1999; Hofmann et al., 1999; Nishiyama et al., 2000; Imaizumi et al., 2002). To yield a saturated mutant collection, we established a reliable high-throughput platform for production, quality control, and characterization of transgenic *Physcomitrella* plants (Egener et al., 2002). Labor-intensive CTAB preparations (Murray and Thompson, 1980; Reski et al., 1991; Hofmann et al., 1999) have been performed to isolate genomic *Physcomitrella* DNA for molecular analyses, but these preparations often contain polysaccharides, phenolic compounds, and other secondary metabolites that inhibit restriction enzymes and

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polymerases. Additionally, the DNA yield is variable and sometimes very low, and degradation is common.

Materials and Methods

Plant material

P. patens (Hedw.) B.S.G. was grown in liquid cultures in modified Knop medium (Reski and Abel, 1985) supplemented with 10-50 g/L of glucose either on a rotary shaker (120 rpm) or bubbled with CO_2-enriched air. Cultures were grown at 25°C and illuminated with a light intensity of 50 µE (light/dark regime of 16/8 h).

PCR and Southern blot analyses

PCR amplifications were performed with 100 ng of genomic DNA using *Taq* polymerase (Promega, Mannheim, Germany). Primer length and GC content were 36 bp/52.8% GC and 31 bp/58.1% GC. The 1633-bp product was amplified in a Biometra T1 Thermocycler (Goettingen, Germany) as follows: 1 min denaturation at 95°C and then 25 cycles of 30 s at 95°C, 30 s at 67.9°C, and 2 min at 72°C. For Southern blot analysis, 1 µg of DNA was cut with 20 U of *Hin*d III (MBI Fermentas, St. Leon-Roth, Germany) for 5 h and probed with a single gene probe using Dig-labeling and Anti-Dig antibody (Roche, Mannheim, Germany) and CDP-Star detection (Promega) according to manufacturer's instructions.

DNA preparation

Reagents

- CTAB buffer: 2% CTAB, 100 mM Tris, 1.4 M NaCl, 20 mM Na_2EDTA (pH 8), autoclaved
- *N*-lauroylsarcosine buffer: 10% *N*-lauroylsarcosine, 100 mM Tris, 20 mM Na_2EDTA (pH 8), autoclaved
- Proteinase K (Sigma, Taufkirchen, Germany) stock: 10 µg/µL in sterile water
- Pronase (Calbiochem, Schwalbach, Germany) stock: 10 µg/µL in sterile water
- RNase A (Sigma, Taufkirchen, Germany) stock: 10 µg/µL in sterile water

Protocol

- Grind plant material in liquid nitrogen.
- Prewarm CTAB and *N*-lauroylsarcosine. Add per gram fresh weight: 3 mL CTAB buffer, 300 µL *N*-lauroylsarcosine buffer, 60 µL Proteinase K stock, 90 µL Pronase stock (always prepare fresh), and 120 µL β-Mercaptoethanol.
- Incubate at 60°C for 1 h in a shaking water bath.
- Cool to room temperature.
- Add 1 vol of sterile 5 M ammonium acetate, mix, and incubate for 5 min.
- Centrifuge for 10 min at 15,000 *g* at room temperature.
- Precipitate the DNA from the supernatant with 1 vol of isopropanol at -20°C for 30 min.
- Centrifuge for 30 min at 12,000 *g* at 4°C.
- Wash pellet with 70% ethanol, dry, and redissolve in 3 mL TE buffer.

- Add 100 µL of RNAse stock. Incubate at 37°C for 15 min.
- Increase the vol to 5 mL with TE. Perform 2 extractions with 1 vol of chloroform-isoamylalcohol (24:1).
- Precipitate the DNA from the aqueous phase with isopropanol. Centrifuge as above.
- Wash pellet with 70% ethanol, dry, and redissolve in 500 µL TE buffer.

Results and Discussion

Existing DNA preparation protocols using CTAB are unreliable when considering yield and quality. Our average yield was 14.2 µg/g fresh weight, with a high standard deviation (Table 1). The highest and lowest DNA yields with the standard CTAB method (Reski et al., 1991) were 40 µg and 3 µg/g fresh weight. Part of this variation might be due to variable amounts of water caught between the moss protonema filaments, resulting in an inaccurate determination of the fresh weight. Additionally, CTAB preparations contain compounds that inhibit PCR reactions (data not shown) and restriction enzymes (Figure 1A). Furthermore, we tested the protocol of Dellaporta et al. (1983) for moss, which produced purer DNA but a low yield (average 3.6 µg/g fresh weight). We also tested 5 kits available for plant DNA extraction: NucleoSpin plant XL (Macherey and Nagel, Dueren, Germany), DNeasy plant maxi (QIAGEN, Hilden, Germany), Nucleon Phytopure (Amersham Pharmacia Biotech, Freiburg, Germany), peqGOLD DNA pure PT (peqlab, Erlangen, Germany), and an upscale of the Wizard® magnetic plant system (Promega, Mannheim, Germany). The kits produced clean but insufficient amounts of DNA (yields of 4-10 µg/g fresh weight). We developed a new protocol because these kits are too inefficient to be used as standard protocols for DNA isolation from *Physcomitrella*.

We used CTAB/*N*-lauroylsarcosine lysis because lysis of moss cells with guanidine hydrochloride is inefficient. Proteinase K is usually used in plant DNA preparations, but we observed varying DNA yields even when cell lysis appeared to be complete. We concluded that some DNA remained attached to proteins and was therefore lost in the chloroform extractions. We tested 2 additional nuclease-free proteases to improve the yield of pure DNA. Bacterial type VIII protease (subtilisin A, Sigma, Taufkirchen, Germany) did not positively affect the preparation. Pronase protease (*Streptomyces griseus,* Calbiochem, Schwalbach, Germany) did increase DNA yield. Lysis is followed by means of protein precipitation with ammonium acetate and 2 chloroform extractions.

Our protocol doubled the DNA yield produced with the standard CTAB method (Table 1). The highest and lowest values were 41.3-µg/g and 14.3-µg/g fresh weight. Thus, the lowest yield with our method equals the average yield of the standard CTAB protocol. DNA prepared with our protocol showed no degradation (Figure 2A) and was successfully used in PCR reactions (Figure 2B). Furthermore, restrictions were complete, and no background smear was visible in Southern blot analyses (Figure 1B). We are confident that this method will be adaptable to other plants.

Table 1. Comparison of DNA yields.

	Standard CTAB	New protocol
Yield in µg/g fresh weight	14.2 ± 7 n = 66	28.5 ± 7.9 n = 14
Quality	variable	high

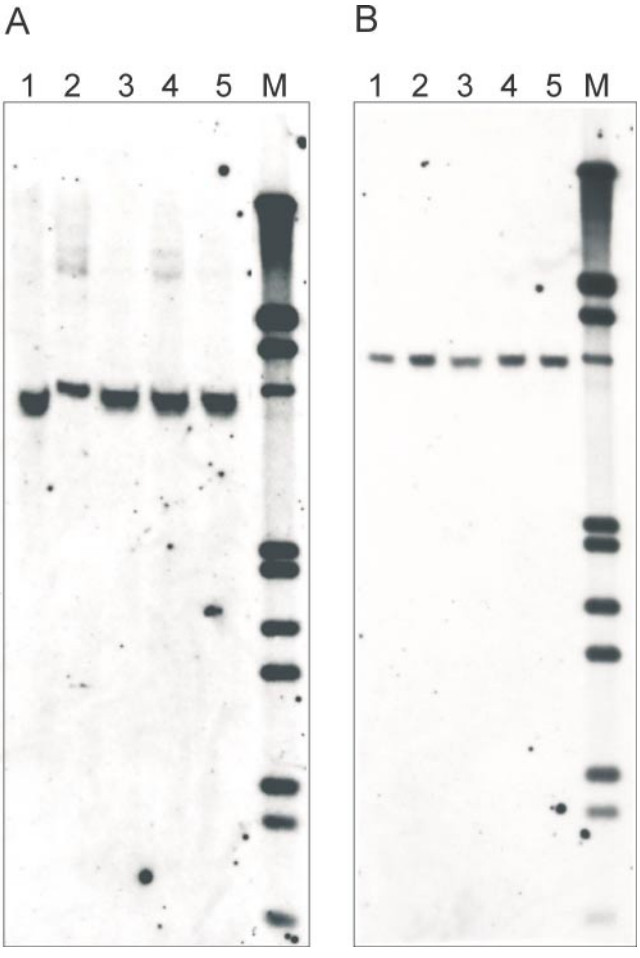

Figure 1. Southern blot analyses of DNA prepared following the standard CTAB protocol and the new method. Each lane was loaded with 1 µg of cut DNA and probed with a single gene probe. (A) Lanes 1-5: standard CTAB-DNA preparations; (B) Lanes 1-5: DNA preparations following the new protocol; M: Marker III Dig-labeled (Roche, Mannheim, Germany).

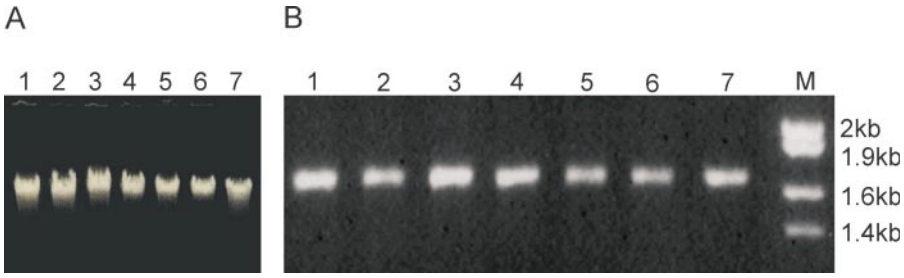

Figure 2. Agarose gel electrophoresis of DNA prepared following the new protocol and performance in PCR reactions. (A) Lanes 1-7: 0.4-µg of different DNA preparations from independent *Physcomitrella* lines on a 0.6% agarose gel; (B) Lanes 1-7: PCR amplification of a 1633-bp product from 100 ng of the genomic DNA preparations shown in Figure 2A, separated on a 1% agarose gel; M: λ DNA *Eco*R I / *Hin*d III (MBI Fermentas, St. Leon-Rot, Germany).

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