
Plant Molecular Biology Reporter **18:** 385–392, 2000

Protocols

A Simplified Protocol for Preparing DNA from Filamentous Cyanobacteria

XIAOQIANG WU, ALIZA ZARKA and SAMMY BOUSSIBA*
Microalgal Biotechnology Laboratory, Blaustein Institute for Desert Research, Ben-Gurion University of the Negev at Sede-Boker, 84990, Israel

Abstract. The preparation of good quality genomic DNA from microalgae and plants is often time-consuming because of the need to remove contaminants that may interfere with the downstream enzymatic manipulation of the DNA. Simpler protocols have been reported but these are applicable only to a few species and in many cases are not effective for removing trace contaminants. In this report, we describe a modification of existing protocols that significantly simplified the preparation of genomic DNA from cyanobacteria and plants. A key step in our protocol is the precipitation of DNA in a high concentration of salt (2-2.5 M NaCl) in the presence of isopropanol, immediately following phenol and chloroform extractions. The preparation and enzymatic digestion of the DNA can be performed in a single day. The DNA was easily digested in 2 h at normal restriction enzyme concentrations, and is highly suitable for PCR and Southern hybridization. We successfully used this simplified protocol to prepare genomic DNA from several filamentous cyanobacteria, such as *Anabaena* sp. PCC 7120, *Anabaena siamensis*, and *Spirulina* strains M2 and Kenya. This protocol may also be useful for preparing genomic DNA from other algae and from higher plants.

Key words: DNA extraction, DNA purification, restriction enzyme digestion, cyanobacteria

Introduction

The elimination of contaminants is a critical step in the preparation of DNA from many organisms. This is particularly true with cyanobacteria, other microalgae, and plants, which are rich in polysaccharides, polyphenols, or other substances that are difficult to remove from DNA preparations and are known to interfere with the enzymatic manipulation of DNA. Cesium chloride gradient centrifugation is often used to purify DNA, but this procedure requires the use of sophisticated equipment and takes one to two days to complete. Simpler procedures have been developed, such as those that employ glassmilk (Golden et al., 1987) or CTAB (hexadecyltrimethyl ammonium bromide) and high salt (Porebski et al., 1997) to purify DNA after ethanol precipitation. However, the ethanol precipitation and drying steps may actually enhance the association of contaminants to the DNA, especially after the DNA has been dried, and may thus constrain the effectiveness

*Author for correspondence. e-mail: sammy@bgumail.bgu.ac.il; fax: 972-7-6596802; ph: 972-7-6596795.

of post-precipitation clean-up steps. Removal of the contaminants prior to ethanol precipitation may thus be more effective. In this report, we describe a simple procedure for purifying DNA that makes possible the removal of contaminants and DNA precipitation in one step. We also describe other techniques useful for efficient DNA extraction from filamentous cyanobacteria and plants.

Material and Methods

Solutions

5 M NaCl
70% ethanol (v/v)
1% and 10% N-lauroylsarcosine (sarkosyl)
Buffer A: 100 mM Tris, 50 mM EDTA, 100 mM NaCl, pH 8
Chaotropic solution: 90.8% NaI and 1.5% Na_2SO_3 in ddH_2O:
One hundred mL of the solution was filtered through a sterile 0.22 μm filter and an additional 0.5 g of Na_2SO_3 was added. The solution was stored in the dark at 4°C (Golden et al., 1987).
Restriction enzymes and buffers, New England Biolabs
Extraction buffer: 100 mM Tris-HCl pH 8, 50 mM Na-EDTA, 1% SDS (W/V), 1% (W/V) polyvinylpyrrolidone (PVP 40,000), 100-200 μg proteinase K mL^{-1} ddH_2O
Lysozyme (50 mg mL^{-1})
Isopropanol
Proteinase K: 20 mg/mL ddH_2O
TE -saturated phenol
RNase A: 10 mg/mL
Taq polymerase and 10x PCR reaction buffer (Sigma)
TE: 10 mM tris-HCl, 1 mM EDTA, pH 8
TES: 50 mM tris, 5 mM EDTA, 50 mM NaCl, pH 8

Materials

100 mL stationary culture of each following species of cyanobacteria:
Anabaena sp. PCC 7120
Transgenic strains of *Anabaena* sp. PCC 7120 containing mosquito-larvicidal genes from *Bacillus thuringiensis* subsp. *israeliensis* (Wu et al., 1997): strain #2 (carrying *cry4A* and *cry11A*), strain #11 (carrying *cry4A*, *cry11A*, *p20*), strain #16A (carrying *cry4A*), and strain #cry11A (carrying *cry11A*)
Anabaena siamensis
Spirulina sp. strains M2 and Kenya
Rice (*Oryza sativa*) roots of 2 week-old seedlings, 2 g

PCR

- Primers (12.5 μmole): Un4(d) and un4(r); EE-11A(d) and EE-11A(r) (Ben-Dov et al., 1997).
- PCR mixtures: each 25 μL reaction volume contained 2.5 μL 10x reaction buffer for *Taq* DNA polymerase, 2.5 μL dNTP (2 mM of each), 1 μL of each primer, 0.5 units of *Taq* DNA polymerase, 10 ng of DNA.

- PCR cycles: 30 cycles: 94°C, 1 min; 56°C, 1 min; 72°C, 1 min. 1 cycle: 72°C, 3 min.

DNA Extraction

a) Cell breakage[1]
   - Axenic culture of *Anabaena* sp. (100 mL) was centrifuged at 6,000 *g*, 4°C for 10 min.
   - The pellet was resuspended in 10 mL Buffer A, pH 8, and kept at room temperature for 10 min.
   - Sarkosyl was added to a final concentration of 0.1%; the sample was kept at 4°C for 30-90 min (depending on the strain)[2].
   - The filaments were collected by centrifugation at 8,000 *g* for 10 min (the supernatant looked bluish)[3,4], washed with 20 mL TES, pH 8, and resuspended in 2.5 mL of TES.
   - Lysozyme was added to a final concentration of 0.5 mg/mL. The sample was incubated at 37°C for 30 min[5].
   - SDS was added to a final concentration of 1%. The sample was stirred thoroughly and kept at 37°C for 10 min.
   - Proteinase K[6] was added to 50 µg/mL and the sample incubated at 37°C for 1 h.

Notes

1. Various methods, either physical or enzymatic or a combination of both, can be used to break the cells. Cell breakage (which can be checked under a microscope) is necessary to obtain high yields.
2. The incubation with 0.1% sarkosyl can be extended for several hours at 4°C; alternatively, the samples may also be shaken for 30-60 min at 30°C (Kallas et al., 1985).
3. Filaments of most filamentous cyanobacteria are often surrounded by a gelatinous sheath (polysaccharide) (Philippis and Vincenzini, 1998), which may interfere with the enzymatic digestion of the cell wall. The use of 0.1% sarkosyl to clean part of these materials (lipid polysaccharide) is essential to cell lysis. A bluish supernatant after centrifugation is a good indication of the removal of the sheath. This step can be repeated.
4. If the above protocol is not effective, other procedures may be used for tough materials:

   i. Cells can be incubated at 60-65°C for 20 min, or treated with penicillin (200 µg/mL) overnight (Joset, 1987). These procedures will make the cell wall fragile.

   ii. Cells can be washed with NaI or pretreated with 4% SDS at 75°C for 15 min to preclude the use of proteinase K in the following steps. Each gram of cell pellet is resuspended in 2 mL of saturated NaI (2 g/mL H_2O) and incubated at 37°C for 20 min (Williams, 1987), then washed with a large volume of water. The supernatant will be bluish. Then the pellet is resuspended in 8 mL of TES and treated with lysozyme (70 mg/g cells) at

37°C for 20 min. Sarkosyl is added to a final concentration of 1%; the samples are incubated at 37°C for 20 min. The solution becomes sticky, indicating cells lysis. If the cells are not broken, proteinase K can be added as described in the main protocol.

iii. The use of a chaotropic salt solution and incubation at 50°C for 30-60 min can also facilitate the breakage of *Spirulina* filaments and cells.

iv. For plant materials and some tough strains like *Spirulina* and green algae, grinding in liquid nitrogen to break the cells may be necessary. To break the filaments into short filaments or single cells, sonication or grinding in a blender may also be necessary. Repeated osmotic shock may also facilitate the fragmentation of the filaments. The pellet is resuspended in 2-5 M NaCl or 25% sucrose (Kallas et al., 1985; Kallas and Malkin, 1988) for 10 min, precipitated, and washed with a large volume of water.

5. Because the structure of the cyanobacterial cell membrane is similar to that of gram negative bacteria, treatment with lysozyme is usually effective in promoting protoplast formation or cell lysis except for a few tough species.
6. The combination of SDS and proteinase K is an important factor in cell lysis. The stability of proteinase K allows the samples to be incubated at 50°C for 90 min (as in other protocols, Golden et al., 1988). Incubation at 4°C overnight could also be used as an alternative gentle lysis procedure. The solution should appear sticky when cells are completely lysed. To maximize the yield, it is important to ensure that the cells are completely lysed before proceeding to the next steps.

b) Purification of DNA

- The sample containing the lysed cells was extracted sequentially with equal volume of phenol[1,2] and equal volume of chloroform:isoamyl alcohol (1:1).

Notes

1. The phenol extraction may be done only once (mixing at 37°C for 5-10 min or incubating the samples at 4°C overnight both resulted in satisfactory yields). However, some standard protocols recommend repeated phenol extractions until the interface between the phenol and aqueous layers is clear.
2. Before centrifugation, adding a small volume (from ½ volume up to an amount equal to the volume of phenol) of chloroform will help separation of the interface and facilitate the recovery of the aqueous phase.

c) Precipitation of DNA

- The top aqueous phase was transferred to a new 50 mL tube using a wide-bore pipette tip, and mixed sequentially with 2/3 volume 5 M NaCl[1] and then with 1 volume isopropanol. The DNA formed visible clumps.
- Using a 1 mL blue pipette tip or a disposable glass pipette, the DNA was transferred into 70% ethanol in a 1.5 mL Eppendorf tube. The DNA was collected by centrifugation and air-dried in a biological hood[2].
- The DNA was dissolved in ddH_20 or TE (200 to 500 μL).

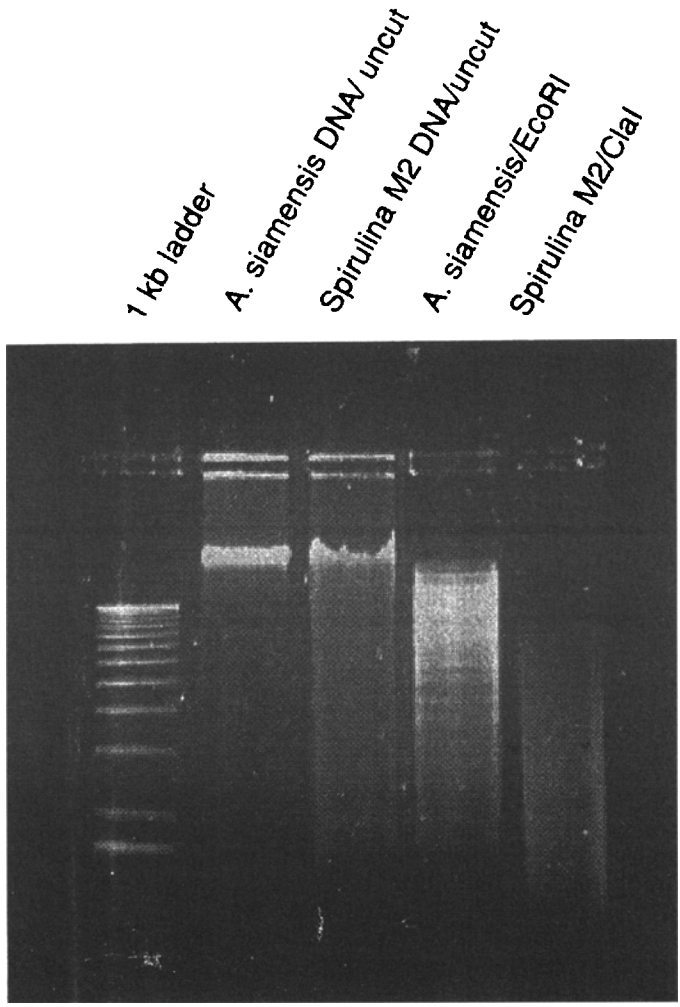

Figure 1. Agarose gel electrophoresis of DNA (uncut and cut with restriction enzymes) prepared from *A. siamensis* and *Spirulina* sp. strain M2 using the simplified protocol.

Notes

1. This one step precipitation of DNA with high concentration of NaCl has been modified from the basic DNA precipitation protocol described by Sambrook et al. (1989), which includes a step for enzymatic degradation of RNA and another round of phenol-chloroform extractions.
2. Using our protocol, we found that the use of RNase is no longer necessary.

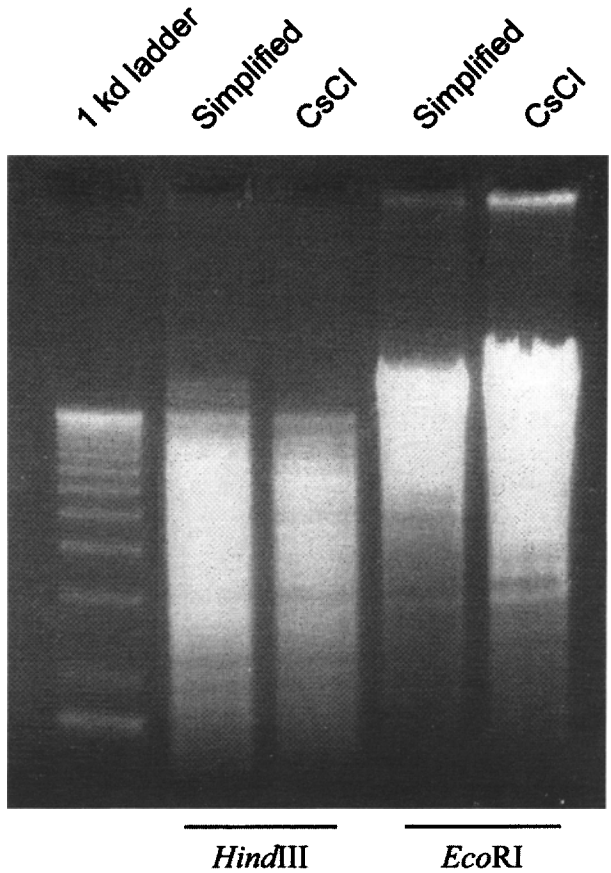

Figure 2. Agarose gel electrophoresis of *Eco*R I- and *Hin*d III-digested DNA prepared from *Anabaena* sp. PCC 7120 using the simplified protocol or the CsCl gradient method.

Results and Discussion

Using the simplified protocol, we successfully prepared high quality DNA from several species of filamentous algae, such as *Anabaena* sp. PCC 7120, *Anabaena siamensis*, strains of *Spirulina*, and from several transgenic lines of *Anabaena* sp. PCC 7120. All the DNA preparations showed satisfactory quality and yield (with average of 200 μg/g fresh weight pellet). For example, DNA extracted from *A. siamensis* and *Spirulina* sp. strain M2 using the modified protocol was of high molecular weight and was digested by *Cla* I and *Eco*R I in 2-4 h (Figure 1). The DNA prepared from *Anabaena* sp. PCC 7120 was also digested in 2-4 h by *Eco*R I and *Hin*d III, and in this respect is comparable to CsCl gradient-purified DNA (Figure 2). The DNA prepared from a number of transgenic lines of *Anabaena* sp. PCC 7120 was suitable for PCR (Figure 3), strongly hybridized to probes (portions of mosquito-larvicidal genes from *Bacillus thuringiensis* subsp. *israelensis*) in Southern hybridizations (data not shown), and has a A_{260} / A_{280} ratio of about 1.7.

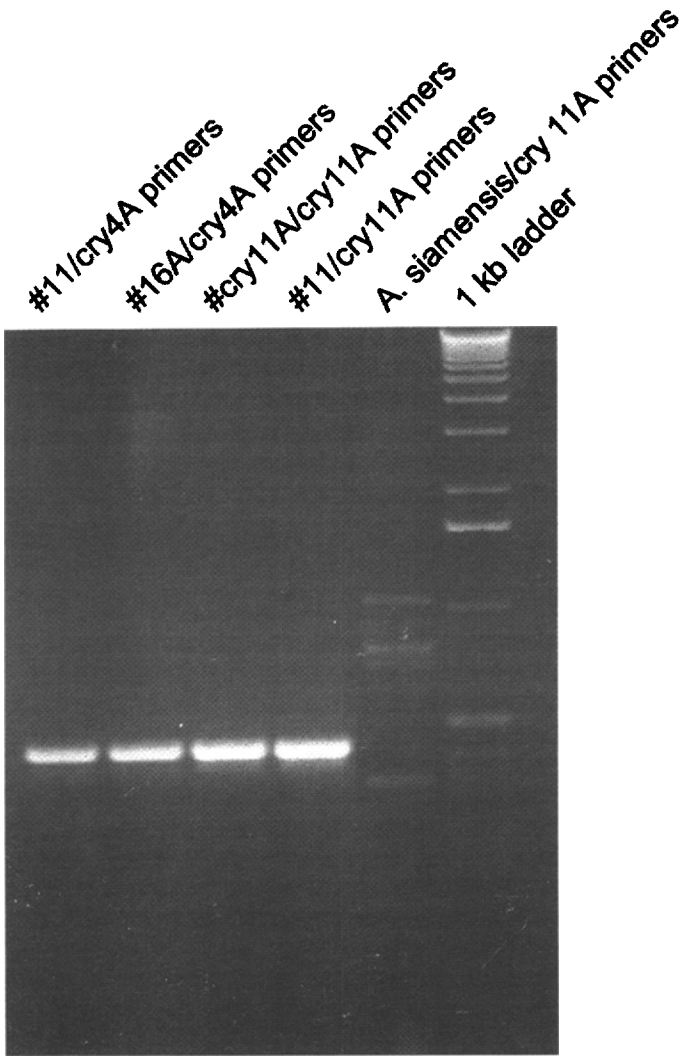

Figure 3. PCR amplification of *Bti* cry gene fragments from DNA extracted from transgenic lines of *Anabaena* sp. PCC 7120 and from wild type *A. siamensis* using the simplified protocol.

In our hands the previously described methods (e.g. Williams, 1987; Ausubel et al., 1998) required a longer time to complete and some of them, such as the NaI method, failed to produce digestible DNA, even after intensive dialysis for several days. Our improved protocol substantially reduces the time for DNA preparation and seems suitable for DNA preparation from many other strains. The major advantage of our protocol is its simplicity, as it requires only one phenol extraction and no further clean-up steps. This allows all the DNA preparation and enzymatic digestion steps to be performed in one day.

In addition to streamlining the DNA purification process, we also examined ways of improving the other DNA extraction steps, particularly cell breakage,

which are critical to DNA extraction from cyanobacteria and plants. DNA yield is particularly dependent on cell breakage. We have successfully used a number of techniques, which we described in the main protocol and in a number of alternative protocols.

Because the basic principle for DNA preparation used in this simplified protocol should be universally applicable, our protocol may be used for extracting DNA from organisms belonging to other taxonomic groups. Indeed DNA prepared from rice root cells with this method was well digested by a number of restriction enzymes (data not shown).

Acknowledgements

We thank Arturo Lluisma for his help with the preparation of the manuscript.

References

Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA and Struhl K (1998) Current Protocols in Molecular Biology. J. Wiley & Sons, New York, NY.

Ben-Dov E, Zaritsky A, Dahan E, Barak Z, Sinai R, Manasherob R, Khameraev A, Troyetskaya A, Dubitsky A, Berezina N and Margalith Y (1997) Extended screening by PCR for seven cry-group genes from field-collected strains of *Bacillus thuringiensis*. Appl Environ Microbiol 63: 4883-4890.

Golden J, Carrasco C, Mulligan M, Schneider G and Haselkorn R (1988) Deletion of a 55-kilobase-pair DNA element from the chromosome during heterocyst differentiation of *Anabaena* sp. strain PCC 7120. J Bacteriol 170: 5034-5041.

Golden SS, Brussian J and Haselkorn R (1987) Genetic engineering of the cyanobacterial chromosome. Methods Enzymol 153: 215-231.

Joset F (1987) Transformation in *Synechocystis* PCC 6714 and 6803: Preparation of chromosomal DNA. Methods Enzymol 167: 712-714.

Kallas T, Coursin T and Rippka R (1985) Different organization of *nif* genes in non-heterocystous and heterocystous cyanobacteria. Plant Mol Biol 5: 321-329.

Kallas T and Malkin R (1988) Isolation and characterization of genes for cytochrome *b6/f* complex. Methods Enzymol 167: 779-794.

Philippis RD and Vincenzini M (1998) Exocellular polysaccharides from cyanobacteria and their possible applications. FEMS Microbiol Rev 22: 151-175.

Porebski S, Bailey LG and Baum BR (1997) Modification of a CTAB DNA extraction protocol for plants containing high polysacharide and polyphenol components. Plant Mol Biol Reptr 15: 8-15.

Sambrook J, Fritsch EF and Maniatis T (1989) Molecular Cloning: A Laboratory Manual. 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Williams JGK (1987) Construction of specific mutations in photosystem II photosynthetic reaction center by genetic engineering methods in *Synechocystis* 6803. Methods Enzymol 167: 766-778.

Wu XQ, Vennison SJ, Huirong L, Ben-Dov E, Zaritsky A and Boussiba S (1997) Mosquito larvicidal activity of *Anabaena* sp. strain PCC 7120 with a combination of delta-endotoxin genes from *Bacillus thuringiensis* subsp. *israelensis*. Appl Environ Microbiol 63: 4971-4975.