A rapid and low-cost method for genomic DNA extraction from the cyanobacterium *Synechocystis*

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Abstract

A two-step method is reported for preparation of genomic DNA from the model cyanobacterium *Synechocystis* that can be performed with minimal equipment and reagents in about an hour. High yields of genetic material can be obtained (200–450 ng/µl) with reasonable purity. A further ethanol precipitation step can be included but is not necessary if template is simply required for PCR or digestion. This new protocol is helpful for amplification of genes of interest in early-stage research projects and for low throughput screening of transformants. It is more reliable than colony PCR of *Synechocystis* cultures, and less involved and cheaper than existing clean-DNA preparation methods. It represents an unusually simple and reliable extraction protocol for the growing body of research making use of this cyanobacterium.

INTRODUCTION

The increasing interest in cyanobacteria for biotechnology follows their long history as models for the chloroplast (Bryant, 1994). *Synechocystis* sp. PCC 6803 became a pre-eminent model organism in photosynthesis research as it was the first photoautotrophic organism to have its complete genome sequence published (Kaneko et al., 1996) and, in addition, it is naturally transformable (Wendt and Pakrasi, 2019). This, and early crystal structures of photosystems from the closely related cyanobacterium *Thermosynechococcus elongatus*, were key to better understanding of photosynthesis and its regulation (Jordan et al, 2001; Zouni et al., 2001). Following the early arrival of *Synechocystis* in molecular biology and photosynthetic research, it maintains its position as an experimentally amenable photoautrotroph in the lab by virtue of its increasing use in systems biology and biotechnology. An unusually large research base of genomic, biochemical and physiological data mean that cyanobacteria are considered to provide an excellent genetic framework for synthetic biology (Jones et al., 2009; Kehr et al., 2011) and for drug development (Tan, 2007; Singh et al., 2011; Vijaykumar and Menakha, 2015) by virtue of their native anti-cancer and pro-apoptotic compounds, along with their overproduction of phenylpropanoid precursors (Brey et al., 2020; Costa-Rodrigues et al., 2012). Their use in sustainable bioenergy research has been an area of particular activity (Lindberg et al., 2010; Parmar *et al.,* 2011; Wijffels et al., 2013) including production of bioethanol (de Farias Silva and Bertucco, 2016) or

hydrogen (Sakurai *et al*., 2015), and they have been explored as workhorses for bioplastic production (for review, see Katayama et al., 2018).

Because hundreds of studies using this model organism have been published annually for decades, it is also possible to evaluate and compare data from different laboratories and strains for informed planning and scale-up. Meanwhile, methods for use with *Synechocystis* have been optimised for many years. DNA extraction remains a practical challenge for many people engaged in cyanobacterial research, however. Sufficient yield and quality is required for repeated use of genomic DNA as template in PCR, in order to feed amplicons through to mutations, insertions or deletions in cloning vectors. A rapid and efficient mechanism is also required for the analysis of DNA from transformants.. It is noticeable in performing rapid DNA extraction from transformed *Arabidopsis thaliana* compared with transformed *Synechocystis* that the former has more reliable 'quick and dirty' methods (Edwards et al., 1991; Jing et al., 2005). Rapid and reliable extraction of genetic material, ideally with low time and financial commitment and limited chemical hazards, would be of benefit to many *Synechocystis* projects. Existing cyanobacterial DNA extraction procedures, however, tend to use harmful solvents, labile enzyme stocks, and time-consuming protocols.

The need to break the resistant *Synechocystis* cell adds an extra step to kit-based methods. The multi-layered cell wall and S layer (Trautner and Vermaas, 2013) is disrupted in existing procedures by enzymatic lysis (e.g. lysozyme; Ermakova Gerdes and Vermaas, 1999), multicomponent buffers (Singh et al., 2011) or physical means (e.g., glass beads; Ferreira et al., 2018). The procedure outlined below therefore minimises the number of steps for the process, and avoids costly reagents and multicomponent buffers, by reducing glass bead breaking steps, then adapting one of the simplest methods used for DNA extraction from plants (the 'Shorty' prep; Jing *et al.,* 2005). PCR and restriction digests on the extracts tested showed it would be possible to use this straightforward protocol to increase efficiency within many *Synechocystis* research projects.

Materials and Methods

Cyanobacterial culture

Synechocystis sp. PCC 6803 (GT strain; gift from Prof. CW Mullineaux, Queen Mary University of London) was cultured using BG11 (Castenholz, 1988) supplemented with 10 mM sodium bicarbonate, and, for plates, with 10 mM 2-[(2-hydroxy-1,1-bis[hydroxymethyl]ethyl) amino]ethanesulphonic acid, 3 g/l sodium thiosulphate and 15 g/l agar, , with incubation conditions of 30 °C, 148 rpm, 24 h light (intensity, 10 umol photons/m^2/s).

Rapid DNA extraction

40 ml of overnight and long-term *Synechocystis* cultures (of approx. 2 x 10^8 cells/ml) were pelleted in 50 ml sterile centrifuge tubes (Fisher, Hampton, USA) at 4000 *g* for 5 mins. The

supernatant was removed from each tube and the pellet was resuspended in sterile deionised water, and centrifugation repeated to remove residual medium. The tube containing the washed cell pellet was placed on ice and resuspended in 5 ml extraction buffer [200 mM Tris-HCl pH8.0 (Sigma, Darmstadt, Germany); 0.4 M lithium chloride (Fisher); 25 mM EDTA pH 8 (Sigma); 1 % w/v SDS (Applichem, Ottoweg, Germany); pH 9.0]. Approximately 200 µl of sterile acid-washed glass beads (150-212 µm; Sigma) were added to the resuspended pellet, the tube was vortexed for 30 s and then returned to ice for 30 s. This step was repeated five times. After centrifuging at 3000 *g* for 15 min at 4 °C, the supernatant was gently taken up into a sterile 10 ml syringe (Beckton Dickinson, Franklin Lakes, USA) and filtered through a sterile 0.2 µm filter (Minisart; Sartorius, Göttingen, Germany). From 5ml pellet in buffer, approximately 4 ml of filtrate was collected in a 5 ml tube. This was split into five aliquots of 800 µl in 1.5 ml micro-centrifuge tubes for alcohol precipitation of DNA, when required. This was achieved by adding 600 µl of ice-cold isopropanol (Fisher) and immediate mixing by pipetting. Samples were then centrifuged at 16,000 *g* for 20 min at 4 ˚C, and the supernatant removed carefully so as not to disturb the pellet. Tubes were left to air dry for 15 min then 200 µl of TE buffer (2 mM Tris-HCl pH 8.0; 1 mM EDTA pH 8.0) was added for resuspension of the pellet. Resuspended material was transferred from each tube to the next in turn, to resuspend each pellet sequentially, and all DNA was collected in one 200 µl aliquot.

Optional purification step

10 µl of sterile 3 M sodium acetate (pH 5.2; Sigma) was added to 100 µl of genomic DNA extract and vortexed to mix. 300 µl of ice-cold absolute ethanol (Fisher) was added before vortexing again and incubating the tube at -20 ˚C for 2 h. Samples were centrifuged at 16,000 *g* for 30 min at 4 ˚C, and the supernatant was removed. The pellet was washed by adding 200 µl of room temperature 70 % ethanol, centrifuging at 10,000 *g* for mins at 4 ˚C, and removing the supernatant. The pellet was again left to air dry for 10 min, before resuspending it in 50 µl of TE pH 8.0. Samples, once resuspended, were centrifuged for 3 mins at 5,000 *g* and the supernatant was carefully transferred to a fresh, sterile 1.5 ml tube.

DNA analysis

Purity of DNA was assessed using A_{260}/A_{230} and A_{260}/A_{280} values (NanoDrop 2000C spectrophotometer; ThermoFisher, Waltham, USA; Supplementary Figure 1). DNA quality and quantity were also checked by gel electrophoresis and compared with a bacteriophage lambda digest.

PCR and enzyme digestion

Whether DNA quality was appropriate for use as a template in PCR was assessed in reactions for a standard housekeeping gene (130 bp of the 16s rRNA gene; 5’ AGCGTCCGTAGGTGGTTATG 3’ and 5’ CTACGCATTTCACCGCTACA 3’), and two further test open reading frames with cloning primers containing mismatches for enzyme cut sites (1024

bp product from 5' GCCggattcAGGCCCGTGAATTTCTTAAA 3' and 5' CAAggtaccGATATAGTCCGATAATTTGCT 3'; 620 bp product from 5' CTAgaattcATTTTTGCTGTAGTAATGC 3' and 5' AAAGTCAcggccgGCCCCTTCT 3'). PCR was carried out using 1 µl of the extracted DNA (with or without purification), DreamTaq polymerase (ThermoFisher) and RNase/DNase free water (HyPure; GE Life Sciences, Marlborough, USA) in a 25 µl total volume in 0.2 ml PCR tubes (Starlabs). Cycles were designed according to standard practice, with initial 5 min denaturation at 95 °C, annealing for 1 min at temperatures set according to primer Tm, and a final extension period of 7 min at 72 °C.

Restriction digests were set up according to standard practice, using *Nhe*I and appropriate buffer (New England Biolabs, Ipswich, MA, USA).

Results

DNA was successfully isolated using the rapid extraction method from new and stock cultures. Three out of four low purity extracts (by A_{260}/A_{230} and A_{260}/A_{280} ratios; Table 1, Figure 1) were of sufficient quality for PCR amplification of products of various sizes including using primers with mismatches (Figure 2). PCR was also satisfactory from extractions from non-exponentially growing stock cultures (Supplementary Figure 2).

There was also good recovery of genomic DNA after purification, quantified by spectrophotometry (Table 2) with the desired improvement in A_{260}/A_{230} and A_{260}/A_{280} ratios in most cases (desired A_{260}/A_{280} of 1.8; Sambrook and Russell, 2006). Gel electrophoresis of all samples, with and without purification steps (Figure 3), showed large genomic DNA fragments and no smear (Figure 3, Supplementary Figure 3). Purified samples were tested in PCR as above, with all extracts now serving as templates for successful amplification (Figure 4). This included PCR from stored (frozen) extractions (Supplementary Figure 3). Digests were also successful with DNA from all extracts (Supplementary Figure 3).

Discussion

Numerous methods exist for cyanobacterial genomic DNA extraction which achieve high-quality samples suitable for sequencing. This standard use of high quality cyanobacterial extracts, indicated by ratios of A_{260}/A_{230} of 2.0 and A_{260}/A_{280} of 1.8 (Sambrook and Russell, 2006; Morin *et al.,* 2010), is not necessary for PCR-based cloning, screening transformants, or early investigations. Cyanobacterial colony PCR is often refractory, and material cannot be retained for future PCR reactions. Here, DNA was quickly prepared from new and longstanding *Synechocystis* cultures, avoiding delicate, harmful or expensive reagents such as chloroform, lysozyme, or kit columns. The optimum density of *Synechocystis* cultures for rapid extraction was approx. 8.36×10^8 c.f.u./ml but this was not critical.

The simplest method provided material effective as template for PCR in the majority of cases (Figure 2). A further purification step could achieve samples with A_{260}/A_{280} close to 1.8 although, even when there was little improvement in spectrophotometric purity, PCR was more successful (extract "α" had lowest A_{260}/A_{280} and a low A_{260}/A_{230} indicating residual carbohydrate contamination; Table 2; Figures 2, 4). DNA visualised by gel electrophoresis revealed integrity of genomic DNA, suggesting minimal degradation. Digests were successful on all extracts, including fresh or frozen preparations, and could be useful for library construction, for example. Therefore, this is an inexpensive and straightforward method to produce and archive genetic material, which requires minimal equipment and reagents, and can start with any extant culture of this model cyanobacterium. This should aid all early studies in *Synechocystis* biology and biotechnology.

Footnotes

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Investigation and analysis, writing, reviewing, editing: DH, EPT; supervision, project administration, funding acquisition, resources: EPT.

Data availability
Additional spectroscopic and electrophoresis data are available in supplementary material.

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Table 1. DNA concentration and quality following rapid extraction.

Sample name	A260/A230	A260/A280	Yield (ng/µl)
α	0.62	1.43	197.6
β	0.53	1.48	212.6
γ	0.41	1.53	445.4
δ	0.51	1.61	281.6

Table 2. DNA concentration and quality following rapid extraction with purification.

Sample name	Input DNA (µg)	Quality following purification				
		A260/A230	A260/A280	Concentration (ng/µl)	Total recovery (µg)	Proportion recovery (%)
α	19.76	0.75	1.48	130.0	13.00	66
β	21.26	0.72	1.58	157.0	15.70	74
γ	44.54	0.79	1.68	176.0	17.60	40
δ	28.16	1.03	1.88	188.8	18.80	67

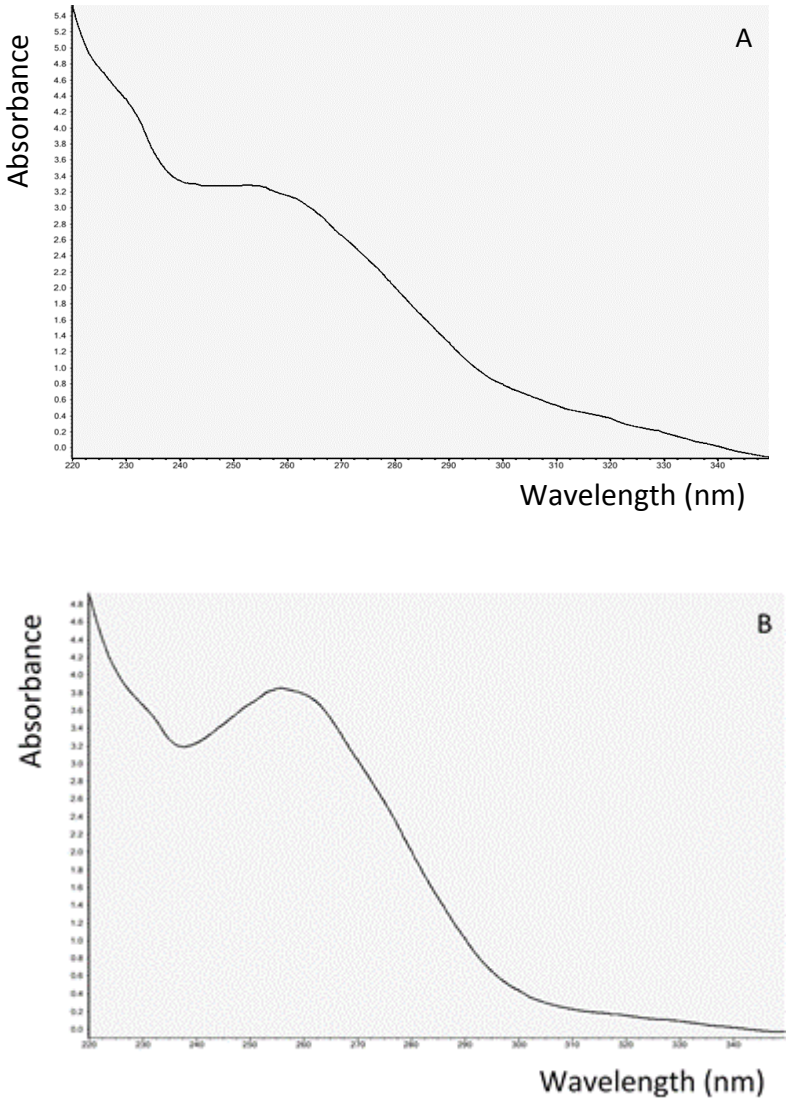


Figure 1. Analysis of DNA isolated from *Synechocystis.* (A) δ extract; (B) δ purified.

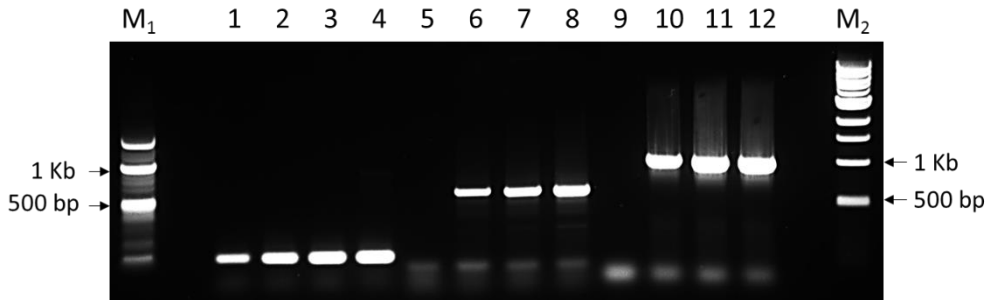


Figure 2. Replicate PCR using α, β, γ, and δ from crude extract as template, respectively. Lanes 1– 4, PCR for 130bp 16S rRNA product; 5 – 8, PCR for 620 bp product; PCR for 9 – 12, 1024 bp product. $M_{1,}$ 100 bp ladder, $M_{2,}$ 1 Kb ladder.

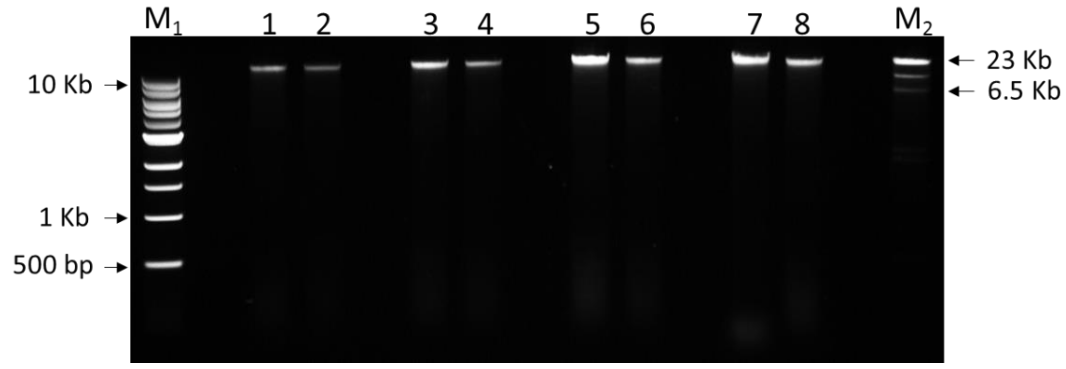


Figure 3. Extracts pre- and post-ethanol precipitation. 1, 2, α crude and purified respectively; 3, 4, β crude and purified; 5, 6, γ crude and purified; 7, 8, δ crude and purified. $M_{1,}$ 1Kb ladder; $M_{2,}$ Lambda *Hin*dIII digest (23 Kb band, 47.7 ng DNA).

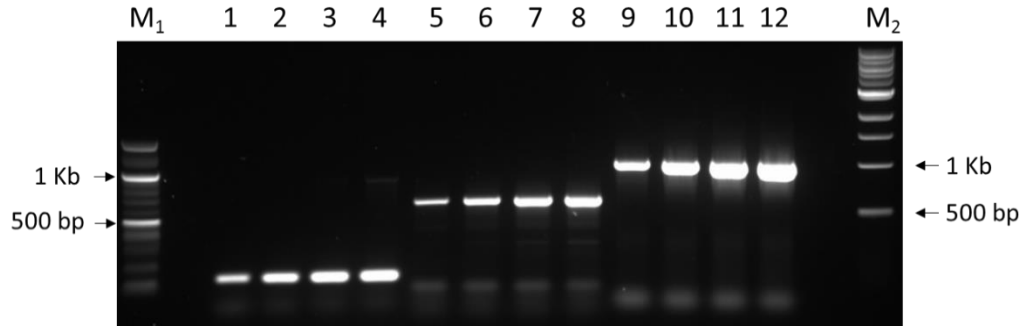
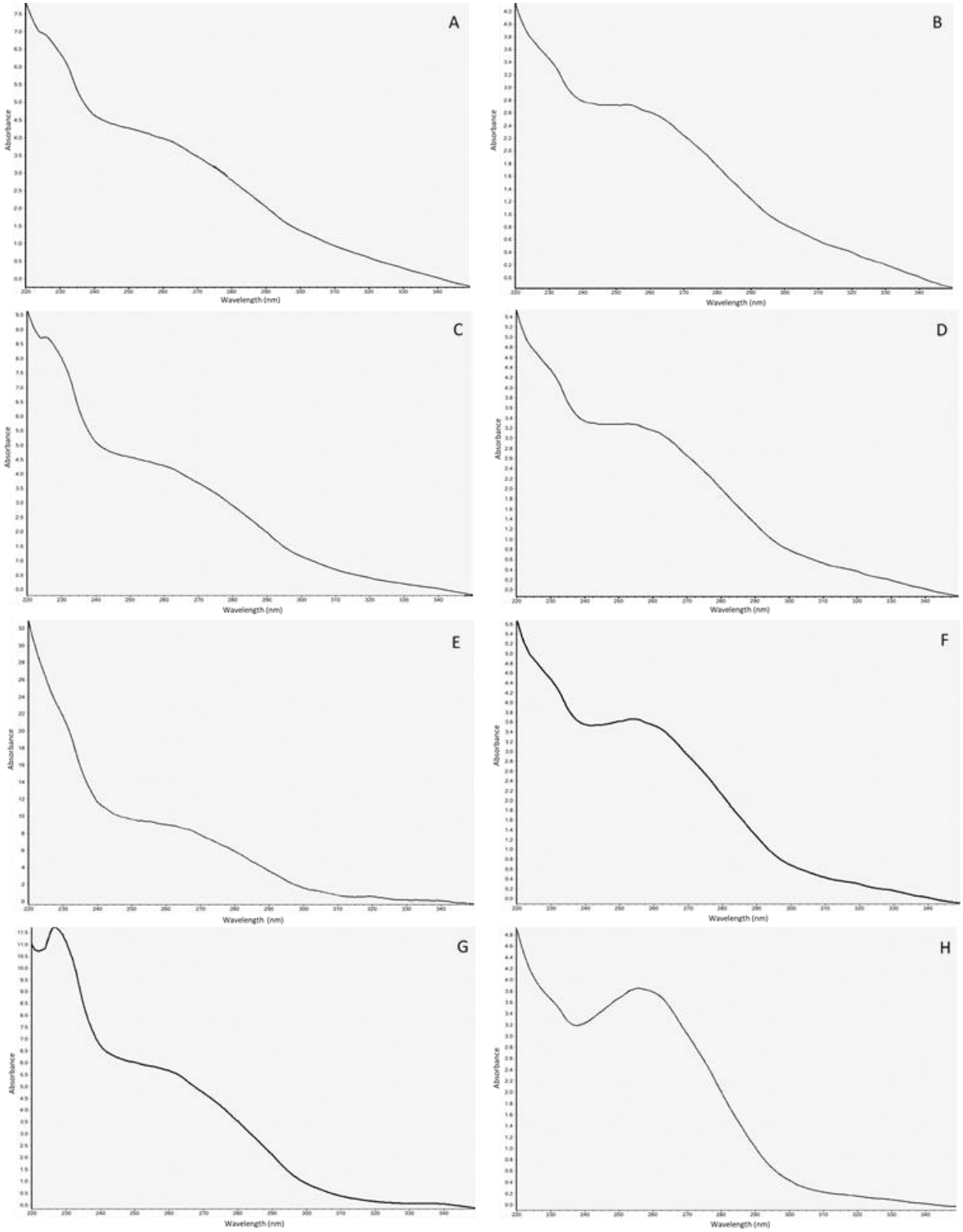
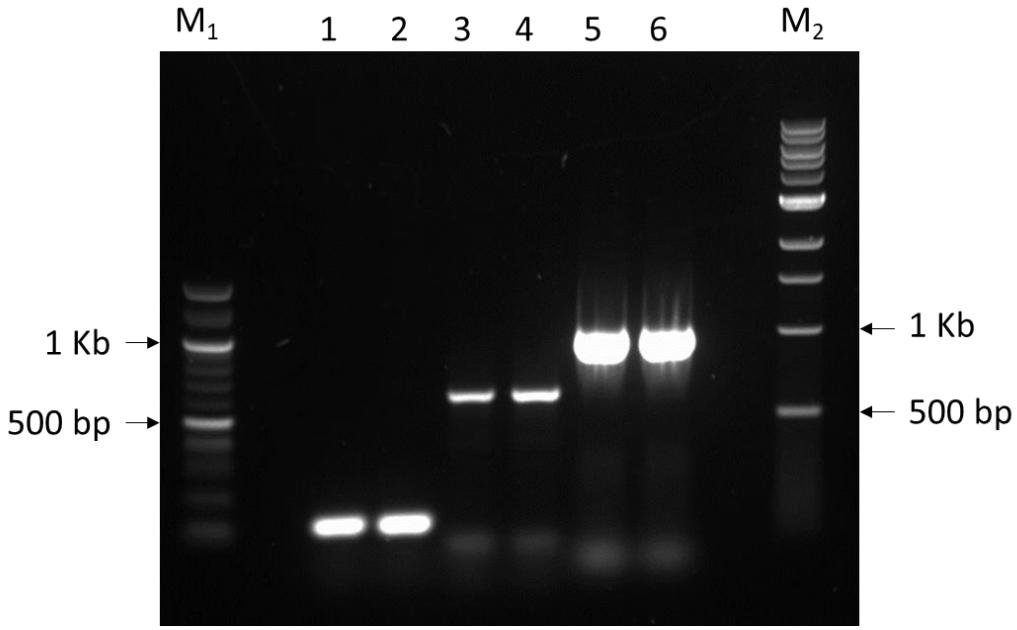


Figure 4. Replicate PCR using α, β, γ, and δ from purified extract as template, respectively. Lanes 1– 4, PCR for 16S rRNA product; 5 – 8, PCR for 620 bp product; 9 – 12, PCR for 1024 bp product. $M_{1,}$ 100 bp ladder, $M_{2,}$ 1 Kb ladder.

Supplementary Data

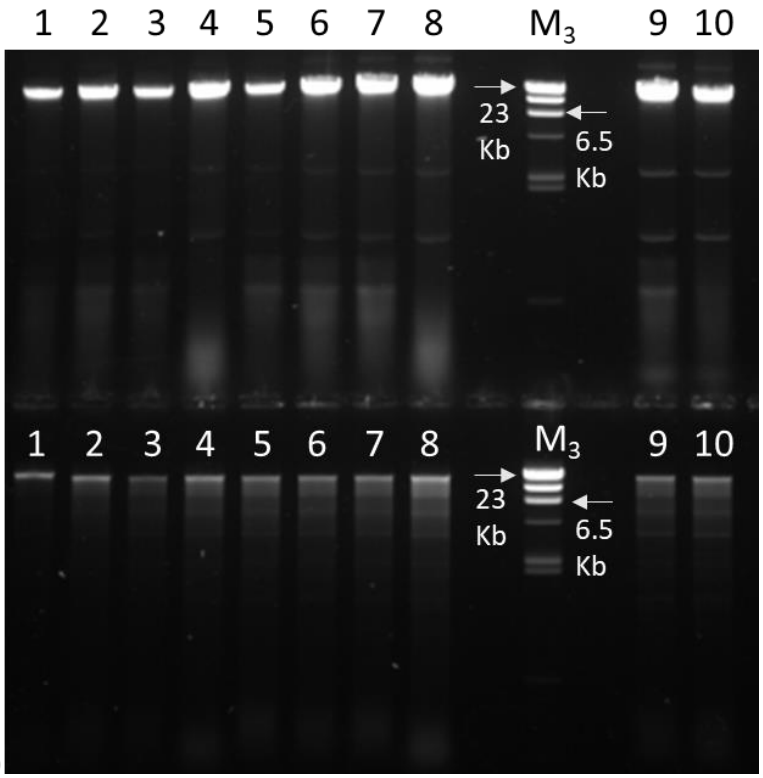


Suppl. Fig. 1. Extracted *Synechocystis* DNA; A, α extract; B, α purified; C, β extract; D, β purified; E, γ extract; F, γ purified; G, δ extract; H, δ purified (ThermoFisher Nanodrop 2000c).

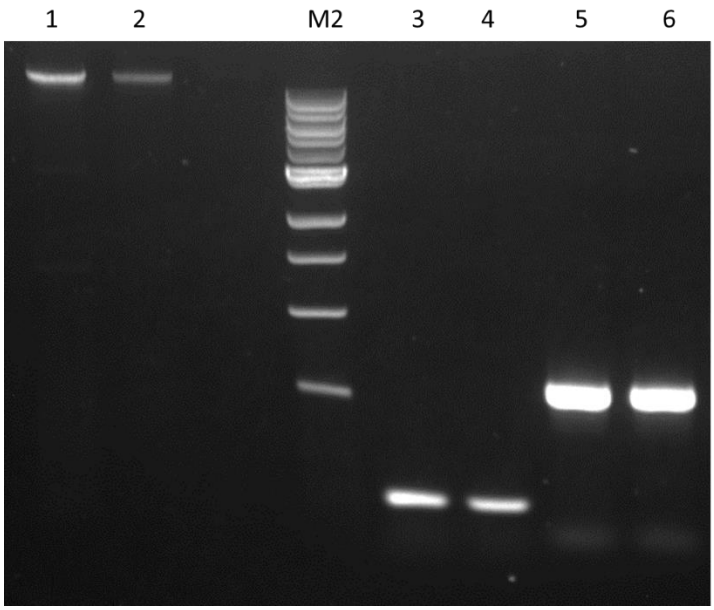


Suppl. Fig. 2. PCR using DNA from stock (old, not in exponential growth) versus new *Synechocystis* cultures: M1, 100bp ladder, 1, 2, old vs new 16S rRNA product; 3, 4, old vs new 620 bp product; 5, 6, old vs new 1024 bp product; M2, 1Kb ladder.

(A)



(B)



Suppl. Fig. 3. Use of extracts stored at -20degC. (A) Undigested controls (top wells) and *Nhe*I digests (lower wells) of new extracts (lanes 1, 3, 5, 7), and frozen, stored extracts (lanes 2, 4, 6, 8). Right panel, Stock culture (not in exponential growth) quick extract (lane 9), pure extract (lane 10): top, undigested; lower panel, *NheI* digests. (B) PCR: 1, fresh, 2, stored template; 3, 4, PCR with 16S rRNA primer pair for fresh and stored template; 5, 6, PCR for 620bp product with fresh and stored template. M2, 1 Kb ladder; M3, Lambda *Hin*dIII digest.