

A Simple and Easy Method for RNA Extraction from the Cyanobacterium *Synechocystis* sp. PCC 6803

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Abstract

Cyanobacteria have been widely used as model organisms in photobiochemical research and have recently been exploited as hosts in numerous pilot studies to produce valuable biochemicals via genetic and metabolic modifications. Analyzing cellular RNA is a suitable method for studying genetic changes in cells. Several methods have previously been reported for cyanobacterial RNA extraction. However, the majority of these methods rely heavily on phenol and chloroform, which are hazardous. Additionally, these methods are time-consuming and difficult to perform. Using *Synechocystis* sp. PCC 6803 as a model, this study developed a novel method for extracting total ribonucleic acid (RNA) using standard centrifugation techniques and laboratory chemicals such as citric acid, ethylenediaminetetraacetic acid, sodium dodecyl sulfate, sodium chloride, and tri-sodium citrate dihydrate to extract RNA from cyanobacterial cells. This method does not necessitate the use of hazardous chemicals, especially phenol and chloroform. Furthermore, it is cost-effective since it does not require expensive chemicals. The results of the quantification, purity, and integrity checks show the effectiveness of this method for extracting good-quality RNA. Furthermore, RT-qPCR results demonstrate that the quality of the extracted RNA is suitable for downstream applications.

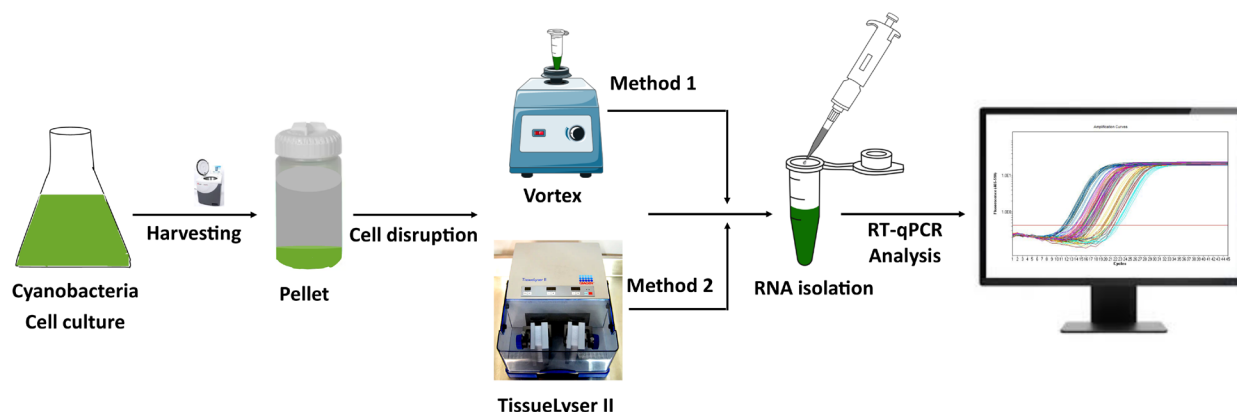
Key features

- Simple and efficient RNA extraction method.
- Requires less than an hour to extract total RNA.
- Provides high-quality RNA suitable for downstream applications.
- This method might be used to extract RNA from other cyanobacteria and algae.

Keywords: Cyanobacteria, *Synechocystis* sp. PCC 6803, RNA extraction, Quantitative reverse transcription polymerase chain reaction (RT-qPCR), Single-stranded complementary DNA (cDNA)

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Graphical overview



Overview of total RNA extraction from cyanobacteria and its downstream application

Background

Cyanobacteria are oxygenic photosynthetic organisms that have been used in various studies to investigate photosynthesis and biochemical production within the cell [1–4]. They have superior natural characteristics, including photoautotrophic growth [5], uptake of exogenous DNA in conjunction with homologous recombination (in some cyanobacteria) [6,7], and minimal growth requirements compared to other photosynthetic organisms [8], making them ideal candidates for biotechnological applications. *Synechocystis* sp. PCC 6803 is a unicellular freshwater cyanobacterium widely used as a model organism in photosynthesis research [9]. It has recently emerged as an efficient host for the production of a variety of valuable chemicals, including biofuels [3], biopharmaceutical proteins [4], and bioplastics [2]. The production of these chemicals within the cell requires effective genetic and metabolic changes. Modifying and introducing foreign genes into the genome to synthesize desired products necessitates their effective expression. RNA analysis is an efficient and reliable method for studying gene expression levels [10]. Several techniques have been developed to analyze RNA and evaluate gene expression, including quantitative polymerase chain reaction (qPCR), RNA-seq, RT-qPCR, complementary DNA (cDNA) libraries, and northern blotting [11–13]. Therefore, the quality and quantity of RNA are particularly critical for the many possible downstream applications.

Extracting high-quality RNA from cyanobacteria is always challenging. Cyanobacteria have a complex, gram-negative-type cell wall [14], which poses challenges to the currently available RNA extraction methods. Furthermore, cyanobacteria contain a variety of secondary metabolites, including polysaccharides and phenolic compounds, which hinder the effectiveness of RNA extraction methods in producing high-quality RNA [15]. Several methods, including chemical, physical, and hot and cold treatments, have been successfully used to extract RNA from cyanobacteria; however, they produce low yields [15–18]. Extracting RNA with chemicals such as organic solvents and phenol has been shown to work [15,19]; however, these chemicals constitute possible health hazards [20–22]. Physical and mechanical methods, on the other hand, produce higher yields than chemical ones, but require more time and processes to break the cells [18]. Commercial kits have been used to circumvent the multiple challenges of extracting large amounts of high-quality RNA in a short period of time. Although these commercial kits have a standard procedure for extracting high-quality RNA, they can be prohibitively expensive. Furthermore, the majority of commercially available RNA purification kits are not intended for organisms that contain polysaccharides and phenolic compounds, such as plants, algae, and cyanobacteria, because these compounds irreversibly bind to nucleic acids and co-precipitate them [23–27].

This study developed a novel method for extracting total RNA from *Synechocystis* sp. PCC 6803, using simple laboratory chemicals and no hazardous reagents. This new method is a combination of physical and chemical procedures. It is simple, quick, inexpensive, and produces a high yield. We used two different approaches to break the cells in order to avoid the use of expensive equipment and compared the effectiveness and yields of the two approaches. The cyanobacterium *Synechocystis* sp. PCC 6803 was used as a model organism for this study, but the protocol might also be applied to other cyanobacteria and algae. The method using a TissueLyser to break the cells showed better RNA yield than the method using a vortex mixer.

Materials and reagents

Biological materials

1. Cyanobacterium *Synechocystis* sp. PCC 6803
2. PCR primers (Table 1)

Table 1. List of primers used in RT-qPCR

Protein name	Gene name	Primer name	Sequence (5'-3')	Amplicon size (bp)
Photosystem II protein D1	<i>psbA2</i>	<i>psbA2</i> _qPCR-Fwd	TGTAATCGGCATCTGGTTCAC	141
		<i>psbA2</i> _qPCR-Rev	ATGTTGGCTCGGTCAATACA	
Photosystem II protein D1	<i>psbA3</i>	<i>psbA3</i> _qPCR-Fwd	TCATCAAGGTACCGAACCAAC	203
		<i>psbA3</i> _qPCR-Rev	TCTCTGAGCTTGAGGCCAA	
Photosystem II CP47 chlorophyll-binding protein	<i>psbB</i>	<i>psbB</i> _qPCR-Fwd	TGCTGCTGGTATTGTCGGTAT	124
		<i>psbB</i> _qPCR-Rev	AGCGGCAATACTACTGGACAA	
Photosystem II CP43 chlorophyll-binding protein	<i>psbC</i>	<i>psbC</i> _qPCR-Fwd	TGGGTTGTGGTGTCTGTTA	126
		<i>psbC</i> _qPCR-Rev	AGATAATCGCCGGGTTGAG	
Photosystem II D2 protein	<i>psbDI</i>	<i>psbDI</i> _qPCR-Fwd	TGCGGTGTTTGTCAAGTGTCT	196
		<i>psbDI</i> _qPCR-Rev	ACCGTGGATAGCACAGAGGA	
Photosystem II D2 protein	<i>psbDII</i>	<i>psbDII</i> _qPCR-Fwd	AGACGGTGAAGATTCCAACAC	184
		<i>psbDII</i> _qPCR-Rev	ACCCACAGAACTCATCCACAA	
Cytochrome <i>b</i> ₅₅₉ α subunit	<i>psbE</i>	<i>psbE</i> _qPCR-Fwd	TGTCACCAGCATTCGCTACT	208
		<i>psbE</i> _qPCR-Rev	TTATTGATTAAACTCTTGAATTTCC	
Cytochrome <i>b</i> ₅₅₉ β subunit	<i>psbF</i>	<i>psbF</i> _qPCR-Fwd	GTTGGTAATTAACAATGGCAACC	150
		<i>psbF</i> _qPCR-Rev	CCTAGCGTTGAATAAATTGCATC	
Photosystem II protein J	<i>psbJ</i>	<i>psbJ</i> _qPCR-Fwd	CAATTTAGGAGGCATGGTATGTTC	144
		<i>psbJ</i> _qPCR-Rev	CCTCGATTACATGGAAGAACCTAA	
Photosystem I chlorophyll-binding reaction center protein A	<i>psaA</i>	<i>psaA</i> _qPCR-Fwd	CACCAGATCCACGTCTCCAT	116
		<i>psaA</i> _qPCR-Rev	TAGAGTTCCGCCATCTTGCT	
Photosystem I chlorophyll-binding reaction center protein B	<i>psaB</i>	<i>psaB</i> _qPCR-Fwd	TTCTAAGTTGATGCCGGACAA	120
		<i>psaB</i> _qPCR-Rev	AAGCAGAGATGTCGCAGGTA	
Bacterial RNase P	<i>rnpB</i>	<i>rnpB</i> _qPCR-Fwd	AGAGCGCACCAGCAGTATC	118
		<i>rnpB</i> _qPCR-Rev	ATTCCTCAAGCGGTCCA	

Reagents

1. EDTA (Sigma-Aldrich, catalog number: E9884)
2. Anhydrous citric acid (Sigma-Aldrich, catalog number: 1.93026)
3. Sodium chloride (NaCl) (Merck, catalog number: 1.06404)
4. Sodium dodecyl sulfate (SDS) (Sigma-Aldrich, catalog number: 436143)
5. Tri-sodium citrate dehydrate (Sigma-Aldrich, catalog number: 1.06448)
6. RNaseOUT (Invitrogen™, catalog number: 10777019)
7. DNase I (Invitrogen™, catalog number: 18080044)
8. Superscript III (Invitrogen™, catalog number: 18068015)
9. qPCRBIO SyGreen Blue Mix Separate Rox (PCRBIO SYSTEMS, catalog number: PB20.17)
10. Liquid nitrogen

11. Diethyl pyrocarbonate (DEPC)-treated MilliQ water (Thermo Fisher Scientific, catalog number: AM9922)
12. Agarose (Sigma-Aldrich, catalog number: A2576)
13. Tris-borate-EDTA (TBE), 10× buffer (Thermo Scientific Chemicals, catalog number: J62788.K2)
14. Deoxyribonucleotide triphosphate (dNTP) mix (Thermo Scientific, catalog number: FERR0192)
15. 96-well plates (Bio-Rad, catalog number: HSP9601)
16. Dithiothreitol (DTT) (Thermo Scientific Chemicals, catalog number: AC327190010)
17. Phusion DNA polymerase (NEB, catalog number: M0530)
18. 1 kb Plus DNA ladder (Thermo Fisher Scientific, catalog number: 10787018)
19. RNA loading dye (NEB, catalog number: B0363S)
20. DNA loading dye (NEB, catalog number: B7024S)
21. BG-11 media (Gibco, catalog number: A1379901)
22. Isopropanol (Thermo Scientific Chemicals, catalog number: 327272500)

Solutions

1. Lysis buffer (see recipes)
2. Precipitation buffer (see recipes)

Recipes

1. Lysis buffer

Reagent	Final concentration	Quantity or volume
EDTA	0.01 M	0.372 g
Anhydrous citric acid	0.132 M	2.536 g
Tri-sodium citrate dihydrate	0.068 M	2.82 g
SDS, pH 5.0	0.069 M	1.99 g
DEPC-treated MilliQ water	n/a	to 100 mL

2. Precipitation buffer

Reagent	Final concentration	Quantity or volume
NaCl	4 M	23.37 g
Anhydrous citric acid	0.033 M	0.634 g
Tri-sodium citrate dihydrate	0.017 M	0.50 g
DEPC-treated MilliQ water	n/a	to 100 mL

Note: Lysis and precipitation buffer can be stored at room temperature up to one year. Filtration/autoclaving is not needed.

Laboratory supplies

1. Nuclease-free microfuge tubes (Eppendorf, catalog number: 022364111)
2. Nuclease-free pipette tips (Eppendorf, catalog number: 0030078748)

Equipment

1. Pipettes (GILSON, catalog numbers: F167500, F167300)
2. Vortex (USA Scientific Inc, catalog number: 74045600)
3. Refrigerated tabletop micro centrifuge (Eppendorf centrifuge 5415 R, with rotor, catalog number: 022621431)
4. Mini centrifuge (Thermo Scientific™, catalog number: 75004061)
5. Thermocycler (Eppendorf Mastercycler® nexus X2, catalog number: 6337000043)
6. TissueLyser II (QIAGEN, catalog number: 85300)
8. Agarose gel electrophoresis system (Analytik Jena, model: Biometra Horizon 58)
9. LightCycler® 480 system (Roche, catalog number: 05 015 278 001)
10. Gel Doc XR+ System (Bio-Rad, catalog number: 170–8195)

11. NanoDrop (Thermo Fisher Scientific, catalog number: ND-ONE-W)
12. Steel beads (5 mm) (QIAGEN, catalog number: 69989)
13. UV-Visible spectrophotometer (JASCO, model: V-550)

Procedure

A. Sample collection

1. Grow 150 mL of liquid culture of cyanobacteria in BG-11 medium until it reaches an OD₇₃₀ of 0.8–1.0.
2. Harvest the cells by spinning them in a Beckman centrifuge at 5,000× g for 10 min at room temperature.
3. Discard the supernatant and resuspend the pellet in 5 mL of BG-11 medium.
4. Transfer 1 mL of sample into a 1.7 mL microfuge tube.

B. RNA extraction

B1. Method 1

1. Vigorously vortex the sample for 5 min at maximum speed.
2. Add 300–400 µL of lysis buffer to the cell suspension and gently mix by pipetting up and down 5 times using a 1,000 µL pipette.
3. Add 150 µL of precipitation buffer to the cell lysate and mix by inversion (10 times).
4. Incubate cell lysate on ice for 5–7 min.
5. After incubation, centrifuge the sample at 15,000× g for 3 min on a small benchtop centrifuge to pellet the unbroken cells and membranes.
6. Transfer the supernatant to a new microfuge tube without disturbing the pellet, then centrifuge at 16,000× g for 4 min to remove any remnants.
7. After centrifugation, transfer the supernatant to another clean microfuge tube and mix with 100% isopropanol in a 1:1 ratio.
8. Incubate the sample for 7 min on ice, followed by 10 min at room temperature.
9. Following incubation, centrifuge the sample mixture at 15,000× g for 5 min using a small benchtop centrifuge.
10. Discard supernatant and wash the pellet with 400 µL of cold 70% ethanol. Centrifuge again at 15,000× g for 5 min.
11. Remove the ethanol carefully without disturbing the RNA pellet.
12. Air dry the pellets by inverting the tubes on a clean paper towel with the lid open for 10–15 min.
13. Gently resuspend the dried pellet in 40 µL of DEPC-treated Milli-Q water and centrifuge at 16,000× g for 5 min to remove impurities and remnants. Impurities and remnants go to the pellet.
14. Transfer the RNA-containing supernatant to a clean microfuge tube and store it at -80 °C for later analysis.

B2. Method 2

1. Snap-freeze resuspended cells in liquid nitrogen and break them using one stainless steel bead per tube in a TissueLyser II (bead bashing for 1 min at 30 rps). Transfer the cell lysates to a new microfuge tube and proceed with step B1.2 and subsequent steps from method 1. See Figure 1 for the graphical presentation of RNA extraction methods.

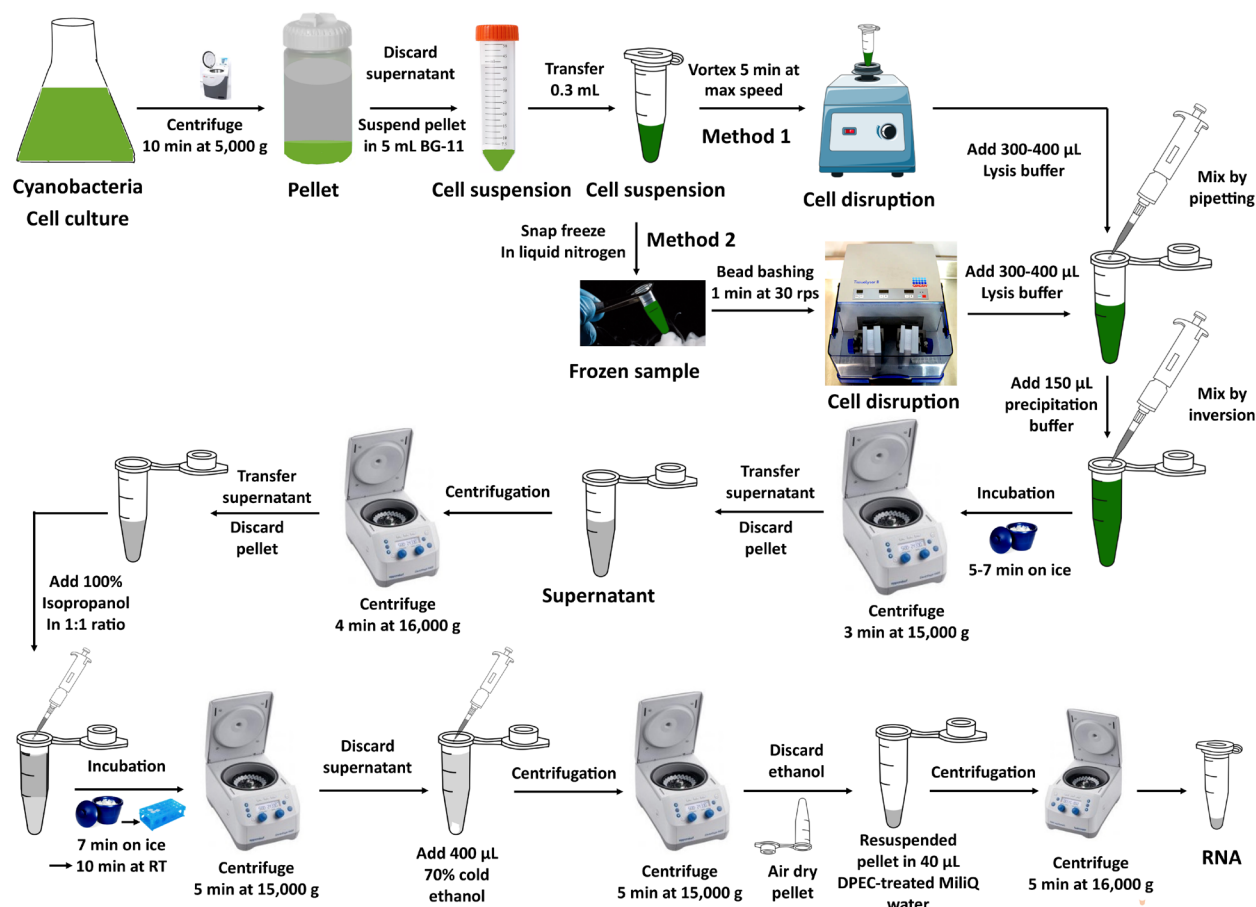


Figure 1. Overview of RNA extraction method

C. Quantification and purity check

Note: Data corresponding to this section are presented in Table 2.

1. Assess the purity and quantity of extracted RNA samples with a NanoDrop. Take 1 μ L of isolated RNA and measure its concentration and purity.
2. The absorbance values at 260 and 280 nm ($A_{260}/280$) indicate the purity and concentration of the isolated RNA.
3. The $A_{260}/280$ and $A_{260}/230$ ratios should be in the range of 1.9–2.1 and 2.0–2.2, respectively. The purity check results for the extracted RNA samples show the $A_{260}/280$ and $A_{260}/230$ ratios in the above range, indicating that the extracted RNA is of good quality and devoid of contaminants.

Table 2. Concentration and purity of RNA samples

Sample number	Method 1			Method 2		
	$A_{260}/280$ nm	$A_{260}/230$ nm	Concentration (μ g/ μ L)	$A_{260}/280$ nm	$A_{260}/230$ nm	Concentration (μ g/ μ L)
1	2.05	2.10	0.125	1.99	2.19	1.53
2	2.00	2.08	0.109	2.04	2.16	1.98
3	1.99	2.01	0.150	2.06	2.0	1.89
4	1.95	1.89	0.138	2.03	2.08	1.25
5	1.98	2.15	0.132	1.97	1.98	1.77
6	1.99	2.13	0.145	1.95	1.90	1.99
7	1.96	1.95	0.149	1.99	2.13	1.36

D. Integrity check

Note: Data corresponding to this section are presented in Figure 2.

1. Use agarose gel electrophoresis to determine the integrity of RNA.
2. Load 1 μ L of extracted RNA, 2 μ L of loading dye, and a molecular marker onto a 1% agarose gel. Run for 50 min at 150 V using TBE (1 $\times$) buffer.
3. Use a Gel Doc XR+ System to capture and analyze the gel image. A good RNA sample should display obvious, distinct bands on the gel that correspond to 23S, 16S, and 5S RNA. If the RNA sample shows bands or smears below the 5S RNA band, this indicates that the RNA has been degraded or contaminated.

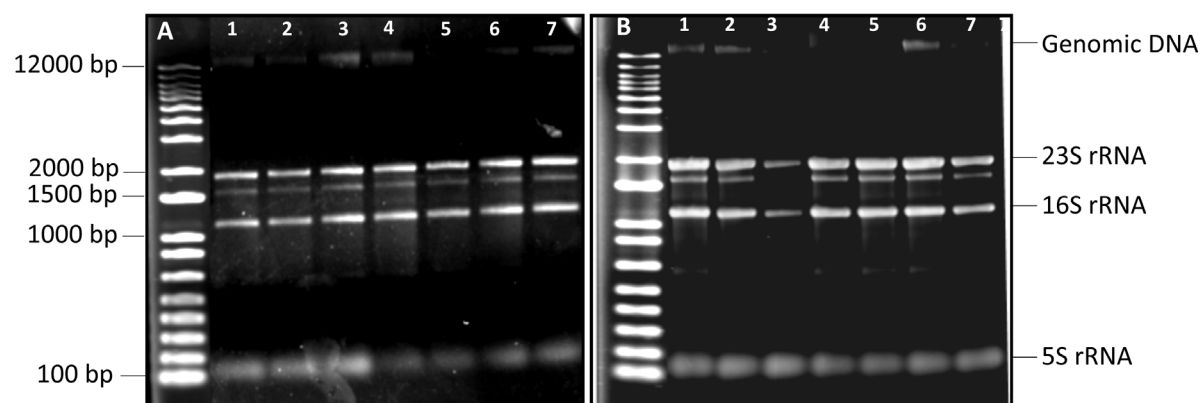


Figure 2. Electrophoretic mobility of extracted RNA on a 1% agarose gel. (A) Total RNA extracted from samples using Method 1 (section B1). (B) Total RNA extracted from samples using Method 2 (section B2). Samples 1–7 represent independent biological repeats. A 1 kb Plus DNA ladder was used in each gel.

E. DNase I treatment

Note: Data corresponding to this section are presented in Figure 3.

1. In a thermocycler, incubate 1 μ g of extracted RNA with 1 μ L of DNase I for 30 min at 37 $^{\circ}$ C.
2. To assess genomic DNA degradation, run 1 μ L of DNase I-treated RNA on a 1% agarose gel alongside 1 μ L of the untreated sample.
3. To further verify DNA degradation, run a PCR using samples with and without DNase I treatment as template. Add the following to each tube: 1.25 μ L of forward and reverse primers (10 μ M), 0.5 μ L of dNTP mix (10 mM), 5 μ L of 5 $\times$ GC buffer, 0.75 μ L of $MgCl_2$ (50 mM), 0.25 μ L of Phusion DNA polymerase, 1 μ L of template, and H_2O up to 25 μ L. PCR conditions are as follows:
 - a. Initial denaturation: 98 $^{\circ}$ C for 30 s
 - b. Denaturation (35 cycles): 98 $^{\circ}$ C for 10 s
 - c. Annealing: (according to the primers) for 30 s
 - d. Extension: 72 $^{\circ}$ C for 30 s/kb
 - e. Final extension: 72 $^{\circ}$ C for 10 min
4. Run 1 μ L of PCR product from DNase I-treated sample on a 1% agarose gel alongside 1 μ L of PCR product from DNase I-untreated sample. The treated sample should not provide any PCR products on the gel, whereas an amplified product from the untreated sample is expected.

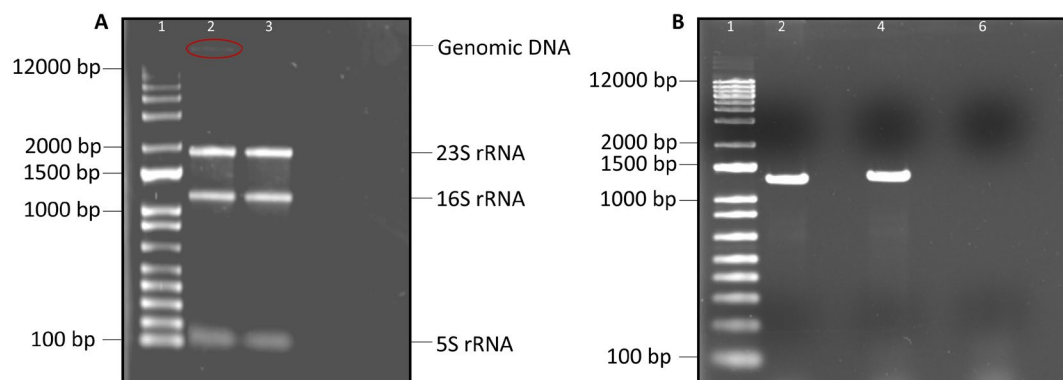


Figure 3. Gel electrophoresis of DNase I-treated RNA sample and the amplified PCR products. (A) Gel represents the electrophoretic mobility of DNase I-treated and untreated RNA samples in lanes 3 and 2, respectively. (B) Amplified PCR products from DNase I-untreated samples are presented in lanes 2 and 4; treated sample is in lane 6. The *psbDII* gene was amplified using fwd: AGTGTGTTTAGCGGCCCAAG, and rev: GTGGTGCAAACAGCGCAACA primers. Lane 1 represents the molecular marker (1 kb plus DNA ladder).

F. Complementary DNA (cDNA) synthesis

Note: Data corresponding to this section are presented in Figure 4.

1. Transfer approximately 500 ng of DNase I-treated RNA to a clean, RNase-free microfuge tube, leaving the remaining 500 ng of RNA in the original tube. Keep the tubes on ice. Label one tube as “RT” and the other as “NO RT”.
2. Add the following to each tube: 0.65 μ L of random primers (250 ng/ μ L) (6:2:1:1 mix of 7, 8, 9, and 10-mers), 1 μ L of dNTP mix (40 mM), 4 μ L of 5 $\times$ first-strand synthesis buffer, 2 μ L of 0.1 M DTT (ensure all DTT is resuspended before use), and 0.75 μ L of RNaseOUT.
3. Mix them by inversion followed by a quick spin.
4. Incubate the mixture at 25 $^{\circ}$ C for 5 min prior to the addition of Superscript III reverse transcriptase.
5. Add 1 μ L of Superscript III to the “RT” tube and 1 μ L of water to the “NO RT” tube. Mix them with gentle pipetting and inversion only, followed by a quick spin.
6. Incubate in a thermocycler for 60 min at 50 $^{\circ}$ C (55 $^{\circ}$ C if using a gene-specific primer).
7. After incubation, heat-inactivate the sample for 15 min at 70 $^{\circ}$ C.
8. Keep the cDNA at -20 $^{\circ}$ C for later use.
9. Check the cDNA synthesis on a 1% agarose gel. The cDNA sample should show multiple bands on the agarose gel, confirming the presence of cDNA.

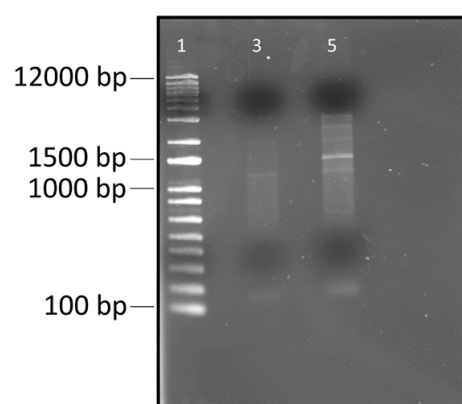


Figure 4. Electrophoretic mobility of RT-PCR products (cDNA) on 1% agarose gel. Lane 1 represents 1 Kb plus DNA ladder. Lane 3 represents PCR product without Superscript III reverse transcriptase. Lane 5 represents PCR products with Superscript III reverse transcriptase.

G. Quantitative reverse transcription polymerase chain reaction (RT-qPCR)

Note: Data corresponding to this section are presented in Figures 5 and 6 and Table 3.

1. Dilute cDNA 1:4 in DEPC-treated MilliQ water.
2. Transfer 4 μ L of diluted cDNA to a 96-well plate. Add 1 μ L of gene-specific 10 μ M primer mix (forward and reverse primers) and 5 μ L of 2 $\times$ qPCRBIO SyGreen Blue Mix to the well containing cDNA.
3. PCR conditions:
 - a. Pre-incubation: 95 $^{\circ}$ C for 5 min
 - b. Amplification (45 cycles): 95 $^{\circ}$ C for 10 s
58 $^{\circ}$ C for 10 s
72 $^{\circ}$ C for 08 s
 - c. Melting curve: 95 $^{\circ}$ C for 5 s
65 $^{\circ}$ C for 1 min
97 $^{\circ}$ C [acquisition mode: continuous; acquisition (per $^{\circ}$ C): 5]
 - d. Cooling: 40 $^{\circ}$ C for 30 s
4. RT-qPCR results for the non-template control and no-reverse transcriptase control should not show any amplification curves, whereas the sample containing template and enzyme should exhibit one amplification curve and one melting peak for a particular gene of interest. Expect an RT-qPCR efficiency of 90%–100% for high-quality RNA and cDNA samples.

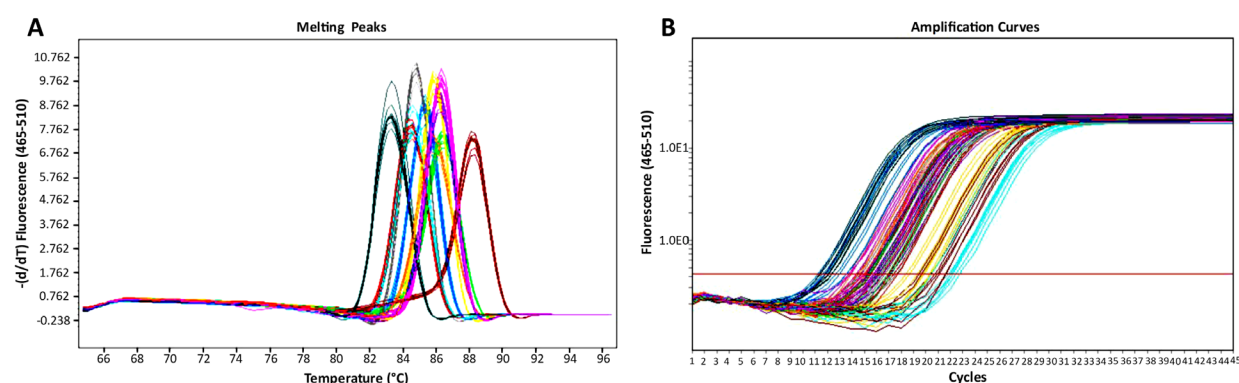


Figure 5. qPCR analysis results. (A) Melting peaks. (B) Amplification curves. Data are shown for *psbA2* (dark blue), *psbA3* (purple), *psbB* (yellow green), *psbC* (orange), *psbDI* (red), *psbDII* (magenta), *psbE* (yellow), *psbF* (maroon), *psbJ* (cyan), *psaA* (light blue), *psaB* (pink), and *rnpB* (green).

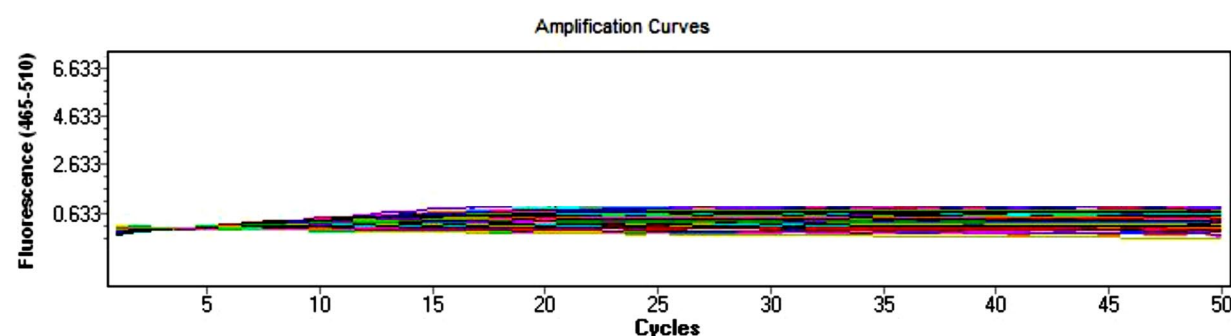


Figure 6. qPCR analysis results of non-template control and no-reverse transcriptase control. The non-template samples are blue, green, olive, cyan, brown, light blue, maroon, gray, purple, lime, and salmon. The no-reverse transcriptase samples are red, pink, yellow, magenta, orange, light green, violet, indigo, navy blue, mustard, and pale green.

Table 3. qPCR analysis results

Protein name	Gene name	Amplicon Tm (°C)	Ct values	Efficiency (%)
Photosystem II protein D1	<i>psbA2</i>	85.31	15.96	100
Photosystem II protein D1	<i>psbA3</i>	83.68	18.46	96
Photosystem II CP47 chlorophyll- binding protein	<i>psbB</i>	84.48	17.51	90
Photosystem II CP43 chlorophyll- binding protein	<i>psbC</i>	86.35	17.05	99
Photosystem II D2 protein	<i>psbDI</i>	86.28	15.92	89
Photosystem II D2 protein	<i>psbDII</i>	84.83	18.71	100
Cytochrome <i>b</i> ₅₅₉ α subunit	<i>psbE</i>	85.92	20.81	99
Cytochrome <i>b</i> ₅₅₉ β subunit	<i>psbF</i>	88.15	21.65	100
Photosystem II protein J	<i>psbJ</i>	84.63	22.25	98
Photosystem I chlorophyll- binding reaction center protein A	<i>psaA</i>	83.35	12.96	100
Photosystem I chlorophyll- binding reaction center protein B	<i>psaB</i>	85.81	15.09	100
Bacterial RNase P	<i>rnpB</i>	86.22	18.49	100

Data analysis

Raw data collected from Nanodrop and RT-qPCR are presented in tables. Gel images were created using Microsoft Publisher imaging software. The RT-qPCR data of the targeted genes can be calculated (relative expression or fold change) using the $2^{-\Delta\Delta CT}$ or comparative C_T method [35–36].

Validation of protocol

This protocol has been used and validated in the following research article:

- Majhi and Eaton-Rye [1]. PsbE:Phe10 of cytochrome b559 modifies inactivation and recovery of Photosystem II in response to high-light stress. *Physiologia Plantarum* (Figure S16 and S17).

In this study, this protocol was used to measure the expression of various photosynthetic genes. Cyanobacterial cells were treated with different light conditions, and the extracted RNA samples were used to assess the expression of genes for photosystem proteins in response to varied light conditions. At least three biological replicates were performed to verify the robustness of this protocol. The results presented in [1] demonstrate the effectiveness of this protocol.

General notes and troubleshooting

General notes

1. RNA is very sensitive to temperature. As such, keep the RNA sample on ice to prevent degradation.
2. Breaking cells using the TissueLyser improves yield by 15–20 times compared to vortexing the cells. Extracted RNA using both methods had a small quantity of remnant genomic DNA.
3. DNase treatment is necessary for downstream applications.
4. The whole extraction process generally takes less than 1 h.

Troubleshooting

Problem 1: Low yield.

Possible cause: Cells were not broken completely.

Solution: Vortex or bead-beat a little longer.

Problem 2: Low or high A260/280 nm values.

Possible causes: Impurities or protein contamination.

Solution: Centrifuge the dissolved RNA pellet again and carefully take out the supernatant without disturbing the pellet.

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Competing interests

The authors declare no conflicts of interest.

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