

*Full Length Research Paper*

# Effects of different light treatments on the natural transformation of *Synechocystis* sp. strain PCC 6803

Zongjuan Long, Jiaohong Zhao, Jingsong Zhang, Lanzhen Wei, Quanxi Wang and Weimin Ma\*

College of Life and Environment Sciences, Shanghai Normal University, Guilin Road 100, Shanghai, 200234, People's Republic of China.

Accepted 16 March, 2019

Although many factors that affect the frequency of natural transformation of the unicellular cyanobacterium *Synechocystis* have been extensively reported, little is known regarding the effects of changes in the spectral quality and intensity of light on its natural transformation. The frequency of natural transformation was significantly enhanced or inhibited by the treatments with low light (LL) or high light (HL) under the incubation condition of cells and DNA before plating and/or on the plates in comparison with that by the treatment with growth light (GL); the changes in the spectral quality of light did not remarkably affect the transformation efficiency of *Synechocystis*. Further, the lengths of the appearance time of transformants were shortened or retarded by HL or LL illumination when cells and DNA were incubated on the plates relative to that by GL illumination. Further, the transformation efficiency of *Synechocystis* was closely associated with the permeability of the cell membranes. Treatment with LL significantly enhances the frequency of natural transformation whereas HL illumination remarkably shortens the appearance time of *Synechocystis* transformants. These phenomena can be extensively applied to future studies according to the specific demands of the transformation experiments. Possible mechanisms underlying these phenomena are discussed.

**Key words:** Appearance time of transformants, light treatments, natural transformation, *Synechocystis*, transformation efficiency.

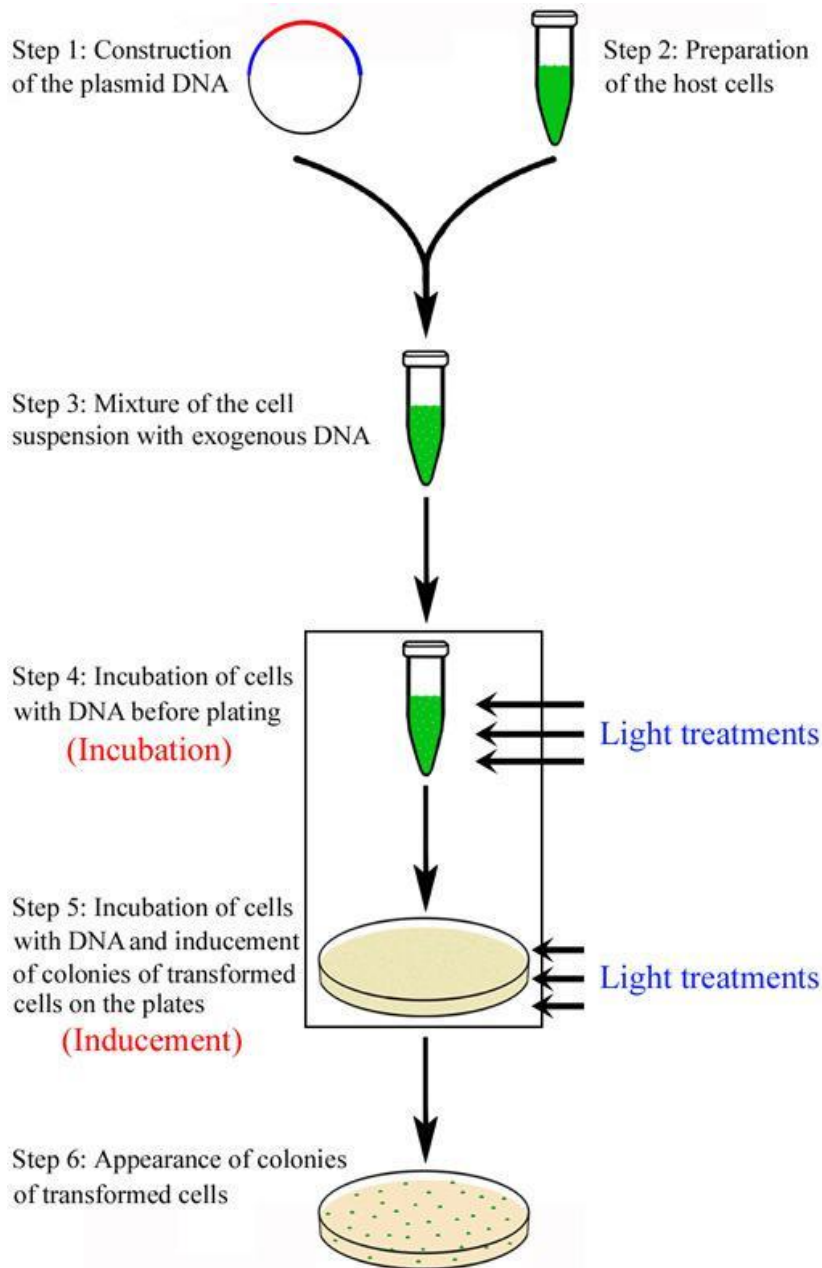
## INTRODUCTION

*Synechocystis* sp. strain PCC 6803 (hereafter referred to as *Synechocystis*) is a unicellular cyanobacterium that shows highly efficient homologous double recombination between the genome and the incorporated exogenous DNA (Grigorieva and Shestakov, 1982; Williams, 1988). Up to now, this system has been used successfully to study the molecular organization and function of the photosynthetic apparatus and other physiologically important cell components (Chauvat et al., 1989; Bouyoub et al., 1993; Yoshihara et al., 2000; Bhaya et al., 2001; Kufryk et al., 2002), and to express the functional proteins involved in drugs and environments (Wang et al., 2000; Song et al., 2001). However, the transformation of targeted genes is usually the first step

in developing such projects.

Natural transformation of *Synechocystis* cells is more effective and popular than other methods such as electroporation and ultrasonic transformation (Zang et al., 2007). A typical procedure for the natural transformation of *Synechocystis* is depicted in Figure 1. Many factors have been proposed to affect the efficiency of transformation, including the concentration of exogenous DNA (Porter, 1986; Barten and Lill, 1995; Kufryk et al., 2002), use of linear or circular DNA (Barten and Lill, 1995; Kufryk et al., 2002), length of the homologous recombination fragments (Chauvat et al., 1986; Williams, 1988; Labarre et al., 1989; Barten and Lill, 1995; Zang et al., 2007), physiological condition of the cyanobacterium (Grigorieva and Shestakov, 1982; Zang et al., 2007), detailed procedure for transformation (Grigorieva and Shestakov, 1982; Williams, 1988; Kufryk et al., 2002; Zang et al., 2007), and genetic modification of the host strain (Kufryk et al., 2002). However, little is known

\*Corresponding author. E-mail: [wma@shnu.edu.cn](mailto:wma@shnu.edu.cn). Tel: +86 21 64321617. Fax: +86 21 64322931.



**Figure 1.** Typical procedure for the natural transformation of *Synechocystis*. This procedure consists of at least 6 steps.

regarding the effects of changes in the spectral quality and intensity of light on the natural transformation of cyanobacterial cells and the possible mechanism underlying such effects.

The aim of this study was to investigate the effects of changes in the spectral quality and intensity of light during steps 4 and 5 of the transformation procedure (Figure 1) on the efficiency of transformation and appearance time of transformants on the plates. Comparison of the frequency of transformation, appearance time of transformants, and changes in the permeability of the cell membranes under the treatments with various

light conditions indicated that low light (LL) illumination is the most efficient for enhancing the transformation efficiency and high light (HL) illumination shortens the lengths of the appearance time of transformants on the plates. Further, the possible working mechanisms and applications are discussed.

## MATERIALS AND METHODS

### Culture conditions

Cells of *Synechocystis*, a glucose-tolerant strain, were cultured at

**Table 1.** Effects of the light intensities on the transformation efficiency of *Synechocystis*.

No	Incubation <sup>1</sup>	Inducement <sup>2</sup>	Transformation efficiency × 10 <sup>-4</sup> (%) <sup>3</sup>	Time (day)
1		LL	22.3±4.1 (370.9±67.4)	12
2	LL	GL	10.8±2.2 (178.6±37.2)	6
3		HL	6.6±1.2 (110.2±19.5)	3
4		LL	13.3±1.8 (221.6±29.5)	12
5	GL	GL	6.0±1.2 (100.0±19.2)	6
6		HL	3.4±0.6 (55.6±9.5)	3
7		LL	10.9±1.5 (180.3±25.4)	12
8	HL	GL	4.4±0.6 (73.5±10.0)	6
9		HL	3.2±0.4 (52.6±7.1)	3

<sup>1</sup>Incubation of the cell suspension and DNA before plating (step 4 in Figure 1). <sup>2</sup>Incubation of cells with DNA after plating together with the inducement of colonies of transformed cells on the plates (step 5 in Figure 1). <sup>3</sup>The transformation efficiency obtained under the GL incubation and succedent GL inducement (GL–GL) was assumed to be 100.

30°C in liquid BG-11 medium (Allen, 1968) and buffered with Tris-HCl (5 mM, pH 8.0) bubbled with 2% (v/v) CO<sub>2</sub> in air under continuous illumination by using fluorescent lamps (40 μmol photons/m<sup>2</sup>/s). The transformants were grown on BG-11 solid medium containing 5 μg/ml kanamycin (Kam<sup>R</sup>) in the presence of 2% (v/v) CO<sub>2</sub>.

#### Construction of the pUC- *ndhO* plasmid

The construction of the pUC- *ndhO* plasmid is schematically described in Appendix - Supplementary Figure 1 and is explained as follows:

(1) The upstream DNA region containing the partial *ssI1690* (*ndhO*) coding sequence (1 to 114) was amplified by PCR using primers a and b (Appendix - Supplementary Table 1). The PCR product (Up) was digested with *Sall/Bam*HI and unidirectionally inserted into vector pUC19 to generate the pUC-Up plasmid.

(2) The kanamycin gene coding sequence was amplified by PCR using primers c and d (Appendix - Supplementary Table 1). The PCR product (Kam<sup>R</sup>) was cleaved with *Bam*HI/*Kpn*I and unidirectionally cloned into pUC-Up to yield the pUC-Up-Kam<sup>R</sup> plasmid.

(3) The downstream DNA region including the remanent nucleotides of the *ndhO* coding sequence (115 to 219) was also amplified by PCR using primers e and f (Appendix - Supplementary Table 1).

The PCR product (Down) was digested with *Kpn*I/*Sac*I and unidirectionally inserted into pUC-Up-Kam<sup>R</sup> to generate the pUC-Up-Kam<sup>R</sup>-Down plasmid, or the pUC- *ndhO* plasmid, which was used for the plasmid DNA of natural transformation (step 1 in Figure 1). The general methods and protocols used for molecular cloning were as described by Sambrook et al. (1989).

#### Natural transformation of *Synechocystis*

The procedure for the natural transformation of *Synechocystis* was performed as described previously (Williams, 1988) with some modifications (Figure 1). In brief, *Synechocystis* cells in the exponential growth phase ( $A_{730} = 0.8$ ; Ma and Mi, 2005; Zang et al., 2007) were harvested by centrifugation at 5,000 × g for 5 min at room temperature. The cell pellet was suspended in fresh BG-11 medium at a density of 7.0 × 10<sup>8</sup> cells/ml and used immediately for transformation (step 2 in Figure 1). One hundred microliters of the cell suspension was mixed with 2 μl of pUC- *ndhO* DNA, and the

final concentration of DNA was 50 μg/ml (step 3 in Figure 1). The mixture of cells and DNA was incubated for 6 h in a sterile test tube at 30°C under various light conditions (step 4 in Figure 1). The mixture was spread onto BG-11-agar plates supplemented with kanamycin at 5 μg/ml, and these plates were maintained at 30°C under 2% (v/v) CO<sub>2</sub> in air and various light conditions (step 5 in Figure 1). After several days of inducement of the transformants, colonies of transformed cells could be seen on the plates, and no colonies appeared in the control experiment from which pUC- *ndhO* DNA was omitted (step 6 in Figure 1).

#### Treatments with various light conditions during the transformation procedure

During steps 4 and 5 of the transformation procedure (Figure 1), *Synechocystis* cells were illuminated with low light (LL; 5 μmol photons/m<sup>2</sup>/s, white light), low red light (LRL; 5 μmol photons/m<sup>2</sup>/s, 620 nm), and low blue light (LBL; 5 μmol photons/m<sup>2</sup>/s, 460 nm); growth light (GL; 40 μmol photons/m<sup>2</sup>/s, white light); and high light (HL; 200 μmol photons/m<sup>2</sup>/s, white light) for several time points. The detailed combinations of the light treatments are described in Tables 1 and 2.

#### Analysis of the transformants

The lengths of the appearance time of transformants were recorded as the time of the first transformant of *Synechocystis*. After the colonies of transformed cells grew on the plates for three days under HL illumination, six days under GL illumination, and twelve days under LL illumination, the number of transformants was counted. Subsequently, the transformation efficiency (%) was calculated as the number of transformants per total number of cells before transformation multiplied by 100.

#### Measurements of cell membrane permeability

Permeability of the cell membranes was measured by detecting electrolyte leakage during the HL or LL treatments. *Synechocystis* cells in the exponential growth phase ( $A_{730} = 0.8$ ; Ma and Mi, 2005; Zang et al., 2007) were harvested by centrifugation at 5,000 × g for 5 min at room temperature and then suspended in fresh BG-11 medium at a density of 7.0 × 10<sup>8</sup> cells/ml. After the cells were illuminated with HL or LL for several time points, the electric conductivities of the cell suspension were recorded at 25°C by

**Table 2.** Effects of the light qualities under low intensities on the transformation efficiency of *Synechocystis*

No	Incubation <sup>1</sup>	Inducement <sup>2</sup>	Transformation efficiency $\times 10^{-3}$ (%) <sup>3</sup>	Time (day)
1	LRL	LRL	2.0 $\pm$ 0.3 (92.5 $\pm$ 7.5)	13
2	LBL	LBL	2.1 $\pm$ 0.3 (94.6 $\pm$ 7.9)	14
3	LL	LL	2.2 $\pm$ 0.4 (100.0 $\pm$ 18.2)	12

<sup>1</sup>Incubation of the cell suspension and DNA before plating (step 4 in Figure 1). <sup>2</sup>Incubation of cells with DNA after plating together with the inducement of colonies of transformed cells on the plates (step 5 in Figure 1). <sup>3</sup>The transformation efficiency obtained under the LL incubation and succedent LL inducement (LL–LL) was assumed to be 100.

using a conductivity meter (CyberScan CON1500; EUTECH, USA).

plates.

## RESULTS

### Effects of light intensities on the natural transformation of *Synechocystis*

Figure 1 shows that at least 6 steps are involved in the natural transformation of *Synechocystis*. Among these steps, the transformation efficiency can be regulated by light illumination during steps 4 and 5, namely incubation of the cell suspension and DNA before plating (hereafter referred to as incubation) and incubation of cells with DNA after plating together with the inducement of colonies of transformed cells on the plates (hereafter referred to as inducement), respectively.

On illumination of the incubation of 6 h (that is., step 4) with low light (LL; 5  $\mu\text{mol photons/m}^2/\text{s}$ , white light), growth light (GL; 40  $\mu\text{mol photons/m}^2/\text{s}$ , white light), or high light (HL; 200  $\mu\text{mol photons/m}^2/\text{s}$ , white light) and succedent inducement (that is., step 5) under the same GL, a high and low frequency of transformation was observed under LL and HL incubation, respectively, in comparison with that under GL incubation (Table 1). In contrast, on illumination of the incubation of 6 h with the same GL and succedent inducement with LL, GL, or HL, a high and low efficiency of transformation was observed under LL and HL inducement, respectively, relative to that under GL inducement (Table 1). Further, the LL incubation and succedent LL inducement (LL–LL) exhibited the highest frequency of transformation whereas the HL incubation and succedent HL inducement (HL–HL) showed the lowest efficiency of transformation (Table 1). Thus, these results indicated that the light intensities significantly influenced the transformation efficiency of *Synechocystis*.

Further, the lengths of the appearance time of transformants depended on the light intensities of inducement but not those of incubation (Table 1). Specifically, the transformants appeared on the third day under HL inducement, sixth day under GL inducement, and 12th day under LL inducement (Table 1), indicating that HL is the most efficient light condition for inducing the appearance of colonies of transformed cells on the

### Effects of light qualities on the natural transformation of *Synechocystis*

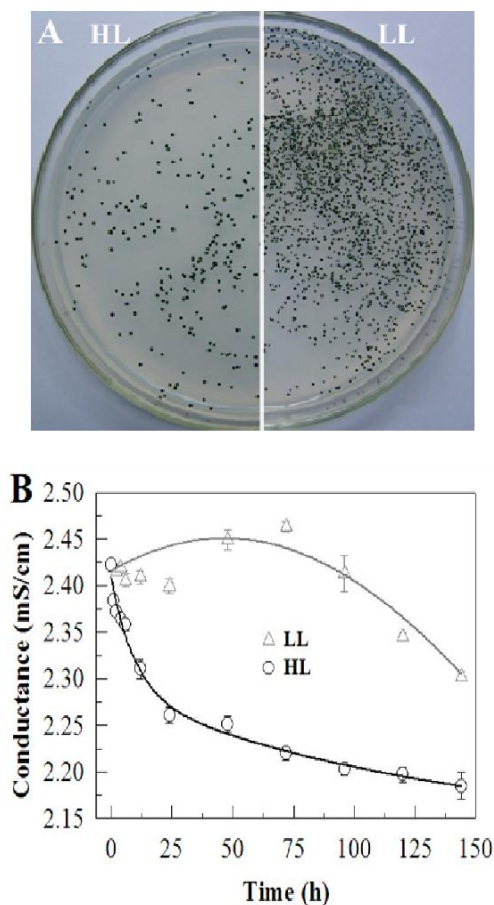
To examine whether the light quality also affects the transformation efficiency of *Synechocystis*, the effects of changes in the spectral quality on the frequency of transformation were examined. As the LL–LL treatment exhibited the highest frequency of transformation, low red light (LRL; 5  $\mu\text{mol photons/m}^2/\text{s}$ , 620 nm) or low blue light (LBL; 5  $\mu\text{mol photons/m}^2/\text{s}$ , 460 nm) was applied for incubation and succedent inducement (that is., steps 4 and 5 in Figure 1). Table 2 shows that the frequency of transformation under the LRL incubation and succedent LRL inducement (LRL–LRL) was similar to that under the LBL incubation and succedent LBL inducement (LBL–LBL), indicating that the light quality did not affect the transformation efficiency of *Synechocystis*.

Further, the transformation efficiency under the LRL–LRL or LBL–LBL illumination was also similar to that under the LL–LL condition (Tables 1 and 2), suggesting that the transformation efficiency of *Synechocystis* depended on the intensity and not the spectral quality of light.

On the other hand, the appearance time of transformants under the LRL or LBL inducement was slightly retarded in comparison with that under the LL inducement (Table 2), implying that the white light was more efficient than that monochromatic light for inducing the appearance of transformants on the plates.

### Changes in the permeability of *Synechocystis* cell membranes caused by the treatment with high or low light

As shown in Table 1 and Figure 2A, the efficiency of transformation under the LL–LL condition was over 7 times than that under the HL–HL treatment. To determine the relationship between the permeability of the cell membranes and the frequency of transformation of *Synechocystis*, the permeability of the cell membranes under the HL or LL treatment, as determined by the



**Figure 2.** Effects of high or low light treatment during both the incubation (step 4) and the succedent inducement (step 5) on the frequency of transformation (A) and permeability of the cell membranes (B). The permeability of the cell membranes was determined from the conductance. The intensities of high light (HL) and low light (LL) were 5 and 200  $\mu\text{mol}$  photos/ $\text{m}^2/\text{s}$ , respectively. The vertical bars represent the mean  $\pm$  SE from at least five independent experiments.

conductance, was measured at several time points. During the LL illumination, the permeability slowly rose until about 72 h and then gradually declined (Figure 2B). In contrast, the permeability always decreased during the HL treatment (Figure 2B). Therefore, the results suggested that the transformation efficiency of *Synechocystis* was closely associated with the permeability of the cells membranes.

## DISCUSSION

The efficiency of transformation is reportedly affected by many factors, including the length, form, and concentration of the incorporated DNA (Chauvat et al.,

1986; Porter, 1986; Williams, 1988; Labarre et al., 1989; Barten and Lill, 1995; Kufryk et al., 2002; Zang et al., 2007); length of the incubation time of the cell suspension with DNA before plating (Grigorieva and Shestakov, 1982; Chauvat et al., 1986; Williams, 1988; Kufryk et al., 2002; Zang et al., 2007); growth phase of the strain (Grigorieva and Shestakov, 1982; Zang et al., 2007); and genetic modification of the host strain (Kufryk et al., 2002). The results of this study indicate that the intensity and not the quality of light during the incubation (step 4 in Figure 1) and succedent inducement (step 5 in Figure 1) influences the efficiency of transformation of *Synechocystis* (Tables 1 and 2). Although it has been proposed that the plasmid DNA uptake is a light-dependent process (Chauvat et al., 1983; Golden and Sherman, 1984; Zang et al., 2007), to our knowledge, this is the first study to reveal the highest efficiency of transformation under the LL–LL treatment and fastest appearance of transformants on the plates under the HL inducement.

Previous studies using the factor of light intensity mainly focused on the effects of light or dark conditions during the incubation (step 4 in Figure 1) but not the inducement (step 5 in Figure 1) on the frequency of transformation (Chauvat et al., 1983; Golden and Sherman, 1984; Zang et al., 2007). The findings of this study not only indicate that the highest efficiency of transformation occurs under the LL incubation but also demonstrate that the intensities of light during the inducement also significantly affect the frequency of transformation in *Synechocystis* (Table 1). This result indicates that the inducement of transformants on the plates (step 5 in Figure 1) can also regulate the transformation efficiency of cyanobacterial cells.

Extracellular appendages on the cell surface, such as type IV pili (Tfp), are essential for twitching or gliding mobility of a glucose-sensitive strain of *Synechocystis* (Wall and Kaiser, 1999; Yoshihara et al., 2000, 2001; Bhaya et al., 2001; Fiedler et al., 2005). In contrast, a glucose-tolerant strain of *Synechocystis*, used in this study, is incapable of motility because of a frameshift mutation in the *spkA* gene (Kamei et al., 2001), suggesting that Tfp are lost in this strain; this was confirmed by the results of scanning electron microscopy (SEM; Appendix - Supplementary Figure 2) and transmission electron microscopy (TEM; data not shown). Recently, studies using reverse genetics indicated that the frequency of transformation is closely associated with the presence and type of Tfp in the glucose-sensitive strain of *Synechocystis* (Yoshihara et al., 2001; Okamoto and Ohmori, 2002). Unfortunately, however, the strain in this study lost the Tfp. Thus, Tfp are not responsible for influencing the efficiency of transformation in the glucose-tolerant strain of *Synechocystis*.

The results of this study indicate that the frequency of transformation of *Synechocystis* is closely associated with the permeability of the cell membranes (Figure 2A

and B). The highest efficiency of transformation and permeability of membranes occurred under the LL–LL treatment (Table 1 and Figure 2). Under nutrient limitation, the capturing of food has been proposed as a selective force to increase the ability of cells for DNA uptake (Redfield, 1993, 2001). It seems likely that under the LL condition in this study, the hungry cells of *Synechocystis* increased their demand for capturing of food, including the DNA molecules, by slightly enlarging the permeability of the membranes (Figure 2), thereby enhancing the frequency of transformation (Table 1). This might be a major strategy for *Synechocystis* cells to enhance the efficiency of transformation under the environments with LL.

The activity of photosynthesis will significantly decrease under treatments with LL and monochromatic light. This is the reason why the appearance of transformants on the plates was retarded under the inducement in these light conditions (Tables 1 and 2).

These findings of this study can be extensively applied to future studies according to the specific demand of the transformation experiments of *Synechocystis*. For example, functional complementation analysis based on the known genomic sequence of *Synechocystis* depends considerably on a high transformation frequency at low DNA concentrations (Kufryk et al., 2002; Vermaas, 1998). In this case, the LL–LL condition is a preferential choice (Table 1). In contrast, to acquire the colonies of transformed cells on the plates rapidly and effectively, the application of the LL–HL condition is suitable (Table 1).

## Conclusions

Our results with a unicellular cyanobacterium increase the knowledge regarding the effects of changes in the spectral quality and intensity of light on the efficiency of transformation, indicating that (1) the low intensity and not the quality of light enhances the frequency of transformation of *Synechocystis* and (2) the HL inducement shortens the lengths of time for the appearance of transformants. Under these conditions, the (1) and (2) phenomena are most likely caused by increasing the demand for capturing of food (e.g. exogenous DNA molecules) and the activity of photosynthesis, respectively. Further, these findings can be extensively applied to future researches according to the specific demands of the cyanobacterial transformation experiments.

## ACKNOWLEDGEMENTS

This work was partially supported by the National Natural Science Foundation of China (No. 30770175), Key Project of Chinese Ministry of Education (No. 209045), National Basic Research Program of China (No. 2009CB118500), Leading Academic Discipline Project of

Shanghai Municipal Education Commission (No. J50401), Project of Shanghai Education Committee (Nos. 11YZ89, and 12ZZ132), and Leading Academic Discipline Project of Shanghai Normal University (No. DZL808).

## REFERENCES

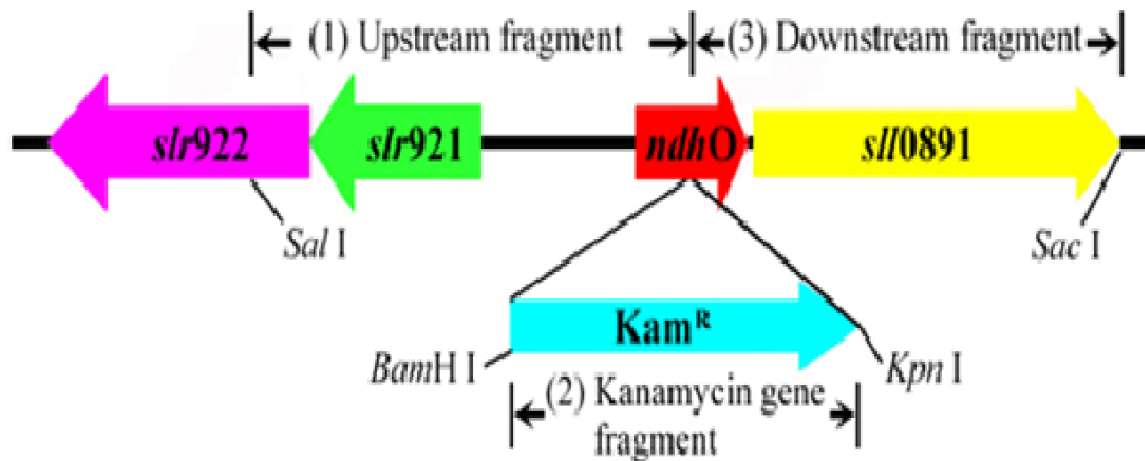
- Allen MM (1968). Simple conditions for growth of unicellular blue-green algae on plates. *J. Phycol.*, 4(1): 1–4.
- Barten R, Lill H (1995). DNA-uptake in the naturally competent cyanobacterium *Synechocystis* sp. PCC 6803. *FEMS Microbiol. Lett.*, 129(1): 83–87.
- Bhaya D, Takahashi A, Grossman AR (2001). Light regulation of type IV pilus-dependent motility by chemosensor-like elements in *Synechocystis* PCC6803. *Proc. Natl. Acad. Sci. USA*, 98(13): 7540–7545.
- Bouyoub A, Vernotte C, Astier C (1993). Functional analysis of the two homologous *psbA* gene copies in *Synechocystis* PCC 6714 and PCC 6803. *Plant Mol. Biol.*, 21(2): 249–258.
- Chauvat F, Astier C, Vedel F, Joset-Espardellier F (1983). Transformation in the cyanobacterium *Synechococcus* R2: improvement of efficiency: Role of the pUH24 plasmid. *Mol. Gen. Genet.*, 191(1): 39–45.
- Chauvat F, Rouet P, Bottin H, Boussac A (1989). Mutagenesis by random cloning of an *Escherichia coli* Kanamycin resistance gene into the genome of the cyanobacterium *Synechocystis* PCC 6803: Selection of mutants defective in photosynthesis. *Mol. Gen. Genet.*, 216(1): 51–59.
- Chauvat F, Vries L, Ende A, Arkel GA (1986). A host-vector system for gene cloning in the cyanobacterium *Synechocystis* PCC 6803. *Mol. Gen. Genet.*, 204(1): 185–191.
- Fiedler B, Börner T, Wilde A (2005). Phototaxis in the cyanobacterium *Synechocystis* sp. PCC 6803: Role of different photoreceptors. *Photochem. Photobiol.*, 81(6): 1481–1488.
- Golden SS, Sherman LA (1984). Optimal conditions for genetic transformation of the cyanobacterium *Anacystis nidulans* R2. *J. Bacteriol.*, 158(1): 36–42.
- Grigorieva G, Shestakov S (1982). Transformation in the cyanobacterium *Synechocystis* sp. PCC 6803. *FEMS Microbiol. Lett.*, 13(4): 367–370.
- Kamei A, Yuasa T, Orikawa K, Geng XX, Ikeuchi M (2001). A Eukaryotic-Type protein kinase, SpkA, is required for normal motility of the unicellular cyanobacterium *Synechocystis* sp. strain PCC 6803. *J. Bacteriol.*, 183(5): 1505–1510.
- Kufryk GI, Sachet M, Schmetterer G, Vermaas WFJ (2002). Transformation of the cyanobacterium *Synechocystis* sp. PCC 6803 as a tool for genetic mapping: Optimization of efficiency. *FEMS Microbiol. Lett.*, 206(2): 215–219.
- Labarre J, Chauvat F, Thuriaux P (1989). Insertional mutagenesis by random cloning of antibiotic resistance genes into the genome of the cyanobacterium *Synechocystis* strain PCC 6803. *J. Bacteriol.*, 171(6): 3449–3457.
- Ma W, Mi H (2005). Expression and activity of type-1 NAD(P)H dehydrogenase at different growth phases of cyanobacterium, *Synechocystis* PCC6803. *Physiol. Plant*, 125(1): 135–140.
- Okamoto S, Ohmori M (2002). The cyanobacterial PilT protein responsible for cell motility and transformation hydrolyzes ATP. *Plant Cell Physiol.*, 43(10): 1127–1136.
- Porter RD (1986). Transformation in cyanobacteria. *Crit. Rev. Microbiol.*, 13(2): 111–132.
- Redfield RJ (1993). Genes for Breakfast: The Have-Your-Cake and-Eat-It-Too of bacterial transformation. *J. Hered.*, 84(5): 400–404.
- Redfield RJ (2001). Do bacteria have sex? *Nat. Rev. Genet.*, 2(8): 634–639.
- Sambrook J, Fitch EF, Maniatis T (1989). *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, NY.
- Song LY, Shi DJ, Ning Y, Luo N, Shao N, Yu MM, Ru BG (2001). The integration and expression of  $\beta\beta$  mutant gene of human liver metallothionein in *Synechocystis* sp. PCC 6803 by homology



recombination. *Acta Bot. Sin.*, 43(4): 399–404.  
 Vermaas WFJ (1998). Gene modifications and mutation mapping to study the function of photosystem II. *Methods Enzymol.*, 297: 293–310.  
 Wall D, Kaiser D (1999). Type IV pili and cell motility. *Mol. Microbiol.*, 32(1): 1–10.  
 Wang SF, Li YG, Li ZY, Shi DJ, Ru BG, Zhang SL (2000). Preliminary studies on the culture of transgenic *Synechocystis* sp. PCC 6803 with mouse metallothionein gene and *Synechocystis* sp. PCC 6803. *J. East China Univ. Sci. Technol.*, 26(1): 48–52.  
 Williams JGK (1988). Construction of specific mutations in photosystem II photosynthetic reaction center by genetic engineering method in *Synechocystis* 6803. *Methods Enzymol.*, 167: 766–778.

Yoshihara S, Geng X, Okamoto S, Yura K, Murata T, Go M, Ohmori M, Ikeuchi M (2001). Mutational analysis of genes involved in pilus structure, motility and transformation competency in the unicellular motile cyanobacterium *Synechocystis* sp. PCC 6803. *Plant Cell Physiol.*, 42(1): 63–73.  
 Yoshihara S, Suzuki F, Fujita H, Geng XX, Ikeuchi M (2000). Novel putative photoreceptor and regulatory genes required for the positive phototactic movement of the unicellular motile cyanobacterium *Synechocystis* sp. PCC 6803. *Plant Cell Physiol.*, 41(12): 1299–1304.  
 Zang X, Liu B, Liu S, Arunakumara KK, Zhang X (2007). Optimum conditions for transformation of *Synechocystis* sp. PCC 6803. *J. Microbiol.*, 45(3): 241–245.

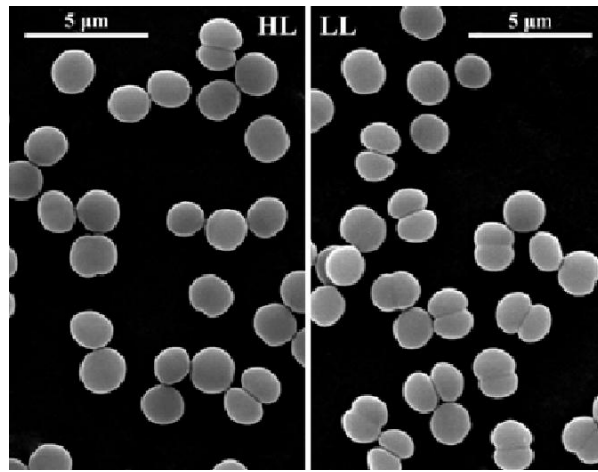
## APPENDIX



**Supplementary Figure 1.** Schematic describing the construction of the pUC- *ndhO* plasmid.

**Supplementary Table 1.** Primer sequences for the construction of the pUC- *ndhO* plasmid.

DNA fragment	No	Primer sequences (5'-3')
Upstream region	a	GCGTCGACAGCGCTTATGGTCATAC
	b	CGGGATCCGCTATGGAAAAGGTAG
Kanamycin gene	c	CGGGATCCGGGGGGGGGGGAAAG
	d	GGGGTACCGGGGGGGGGGGCG
Downstream region	e	GGGGTACCAAAGGAGAAGTGTG
	f	CGAGCTCTTAACCGTCGCTAAC



**Supplementary Figure 2.** Scanning electron microscopy (SEM) of a glucose-tolerant strain of *Synechocystis* after the treatments of high light (HL) or low light (LL). Under the HL or LL illuminations, the mixture of cells and DNA was incubated for 6 h and spread onto the BG-11-agar plates for 48 h; then, the cells on the plates was collected and used for the measurements by SEM. The intensities of HL and LL were 200 and 5  $\mu\text{mol photons/m}^2/\text{s}$ , respectively.