

## **Supplemental Material**

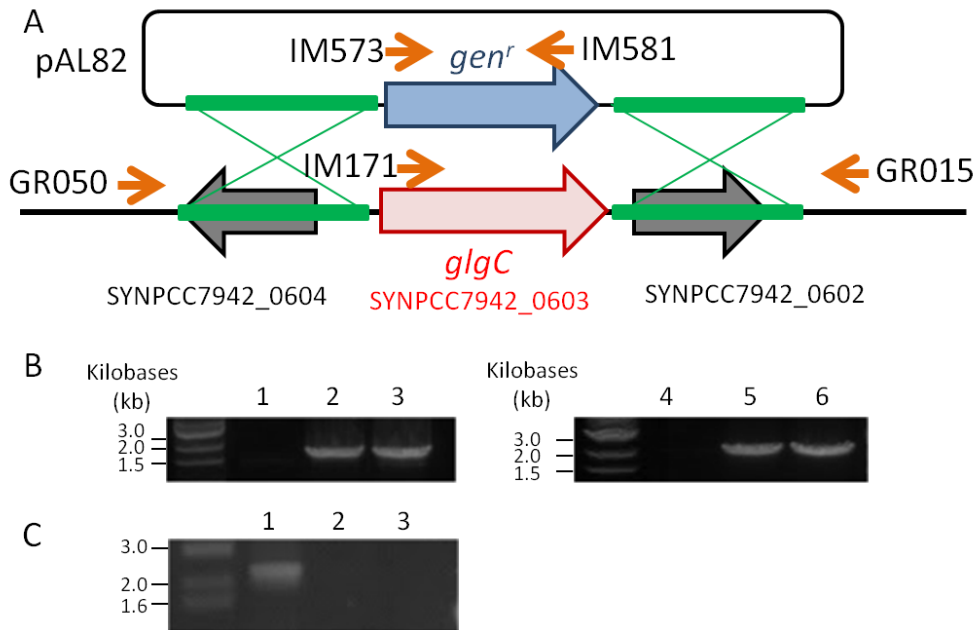
### **Engineering *Synechococcus elongatus* PCC7942 to grow continuously in diurnal conditions**

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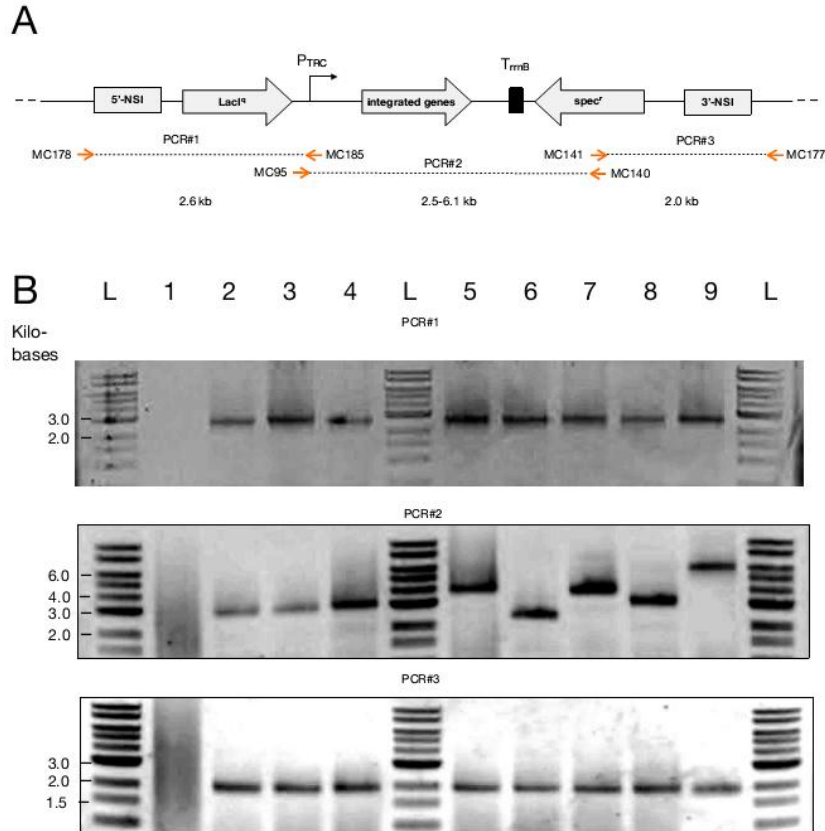
I. Figure S1. Construction and verification of the deletion of *glgC*

II. Figure S2. PCR confirmation of the integration of the target pathways into the *S. elongatus* chromosome.



**Fig. S1 Construction and verification of the deletion of *glgC***

**A.** Schematic representation of recombination to delete *glgC* with pAL82. Green bars indicate homologous regions for recombination. Orange arrowheads indicate primers used for the verification of the recombination. **B.** PCR confirmation of correct recombinants. PCR was performed with primers GR050 and IM581 (Lane 1-3, product size: 1.7 kb) and IM573 and GR015 (Lane 4-6, product size: 2.2 kb), using genomic DNA of *S. elongatus* strains, wild type (lane 1 & 4), AL535 (lane 2 & 5) and AL536 (lane 3 & 6) as templates. **C.** PCR confirmation of segregation. PCR was performed with primers IM171 and GR015 (Lane 1 product size: 2.2 kb), using genomic DNA of *S. elongatus* strains, wild type (lane 1), AL535 (lane 2) and AL536 (lane 3) as templates.



**Figure S2. PCR confirmation of the integration of the target pathways into the *S. elongatus* chromosome.**

A. Schematic representation of integration of selected genes into *S. elongatus* genome. Orange arrowheads indicate primers used for PCR verification of chromosome recombination. NSI indicates neutral site 1. B. PCR confirmation of correct recombinants. Genomic DNA was used for all PCR templates as follows: Lane 1, AL257 (wild-type), Lane 2, AL361 (*galP*), Lane 3, AL360 (*GLUT1*), Lane 4 AL358 (*glcP*), Lane 5, AL505 (*galP-gfp*), Lane 6 AL504 (*gfp*), Lane 7 AL1030 (*cscKB*), Lane 8, AL1067 (*xylE*), Lane 9, AL434 (*xylEAB*). PCR#1 was performed with primers MC178 and MC185 with an expected product size of 2.6 kb for all reactions except Lane 1. PCR#2 was performed with primers MC95 and MC140 with the following expected product sizes: Lane 2, 3.2 kb, Lane 3, 3.3 kb, Lane 4, 3.2 kb, Lane 5, 3.9 kb, Lane 6, 2.5 kb, Lane 7, 4.0 kb, Lane 8, 3.3 kb, Lane 9, 6.1 kb. PCR#3 was performed with primers MC141 and MC177 with an expected product of 2.0 kb for all reactions except Lane 1.