

## DEVELOPMENT OF ENGINEERED CHROMATIC ACCLIMATION SENSOR WITH STRICT AND REVERSE RESPONSE TO LIGHT SIGNAL, AND APPLICATION TO OPTOGENETIC CONTROL IN CYANOBACTERIA

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Genetic regulation and metabolic engineering enabled cyanobacteria to produce renewable chemical compounds from carbon dioxide via photosynthesis. Optogenetic control enables to precisely regulate the timing and level of gene expression without chemical inducer which is environment-hazardous. We recently developed a green-light regulated gene expression system in a model cyanobacterial strain *Synechocystis* sp. PCC6803 (hereafter PCC6803) [1] and a fast-growing marine cyanobacterial strain *Synechococcus* sp. NKBG15041c (hereafter NKBG15041c) [2] using a PCC6803-derived chromatic acclimation sensor, CcaS/CcaR two-component system [3]. However, the regulation of gene expression by CcaS is not strictly controllable and the background expression level under non-inductive condition is not negligible. Furthermore, altering the direction of gene expression, that is induction under red-light and repression under green-light, may expand its flexibility as one of the genetic tools.

To obtain stricter and versatile system, we fabricated engineered CcaSs focusing on its domain structure using *Escherichia coli* expression system. One of the engineered CcaSs, CcaS#11, showed reverse response to light signal, i.e. inducible under red-light and strictly repressible under green-light [4]. To investigate the potential application and versatility of CcaS#11 as the red-light regulated gene expression system in cyanobacteria, we next introduced CcaS#11/CcaR two-component system and GFP<sub>uv</sub> as a probe of gene expression into PCC6803 after knocking out genomic CcaS/CcaR two-component system to exclude the interference. In this strain, the gene expression was induced under red-light and strictly repressed under green-light as we expected. Then, we applied this system to NKBG15041c. Similarly, red-light inducible gene expression with 2-fold higher ON/OFF ratio compared with the original system was successfully observed in NKBG15041c. Remarkably, there was no leaky expression under green-light, indicating that this system enables strict regulation of gene expression by light signal.

In conclusion, we successfully constructed the engineered CcaS, CcaS#11, with strict and reverse response to light signal. Then we also confirmed its versatility and applicability as the red-light regulated gene expression system with strict regulation in cyanobacteria. Further development of the light regulated bioprocess will be expected using cyanobacterial hosts with this system, as a cell factory for the renewable chemical compounds production.

[1] K. Abe et al., 'Engineering of a Green-light Inducible Gene Expression System in *Synechocystis* sp. PCC 6803', *Microb. Biotechnol.* 7 (2014) 177-183

[2] A. Badary et al., 'The Development and Characterization of an Exogenous Green-light-regulated Gene Expression System in Marine Cyanobacteria', *Mar. Biotechnol.* 17 (2015) 245-251

[3] Y. Hirose et al., 'Cyanobacteriochrome CcaS is the Green Light Receptor That Induces the Expression of Phycobilisome Linker Protein', *Proc. Natl. Acad. Sci. USA* 105 (2008) 9528-9533

[4] M. Nakajima et al., 'Construction of a Miniaturized Chromatic Acclimation Sensor from Cyanobacteria with Reversed Response to a Light Signal', *Sci. Rep.* 6 (2016) 37595