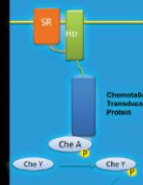
THE PHOTOTACTIC SYSTEM



SRI (orange light sensitive), SRII (blue light sensitive) [1] were put into use in *Escherichia coli* as a series of wide spectrum light sensor by fusing with their native transducer proteins to the *E. coli* counterparts (Tsr and Tar) [2]. Once the fusion proteins were triggered, the histidine kinase (CheA) will be regulated in order to control flagella movement [3, 4].

ABSTRACT & MOTIVATION

Although the sensory technology has been deeply explored and implemented in various means, most of the developed sensors are chemically-dependent promoters which regulate downstream gene expression. We exploited the use of halobacterial sensors, the sensory rhodopsins which are sensitive to a wide spectrum of readily available light source and build a series of sensing systems to control cellular movement and gene regulation. This system can be executed as a fundamental part for further applications, such as cell targeting and refining. Furthermore, to counter the safety issues caused by the leakage of bioengineered cells, this sensing method altogether with the CRISPR/Cas sytem can target and achieve the cleavage of the transformed plasmid under the stimulation of natural light sources.

LIGHT OF NO RETURN

CUHK iGEM 2012

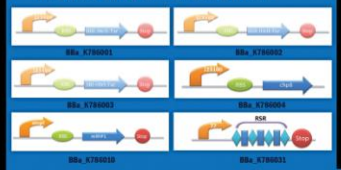
THE CHINESE UNIVERSITY OF HONG KONG

TRACK : Foundational Advance

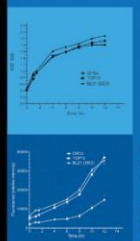
CHARACTERIZATIONS

A. Biobrick Constructions

We have successfully constructed six biobricks BBa_K786001, BBa_K786002, BBa_K786003, BBa_K786004, BBa_K786010 and BBa_K786031.



B. Promoter Efficiency For BBa_K786001, BBa_K786002, BBa_K786003

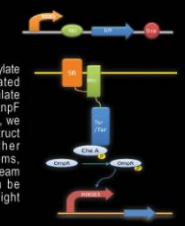


Constitutive promotor BBa_J23100 has been included in our biobricks, the promotor efficiency in various strains of bacteria was tested by using inserting red fluorescence protein as a reporter and examined the fluorescence output.

No significant difference was observed on the growth curves, but for the protein expression, the results showed that the fluorescence intensity in DH5α was significantly lower compared with TOP10 and BL21(DE3).

DH5α was found to be not an optimal strain to utilize promoter BBa_J23100.

THE SENSORY RHODOPSINS INDUCED GENE EXPRESSION SYSTEM (BBa_K786010)



CheA is able to phosphorylate OmpR and phosphorylated OmpR can in turn stimulate transcription from the otnpF promoter (part R0083) [5], we therefore built the construct BBa_K786010. Together with SR sensory systems, gene expression downstream of otnpF promoter can be controlled by different light source.

C. Western blot analysis was performed to verify whether BBa_K786001 was expressed in *E. coli*.

The fusion protein was expressed in TOP10, BL21 and Rossetta, while there is no expression in DH5α

D. Positive Phototactic Effect Of BBa_K786002 Under Blue Light Exposure

Cells transformed with BBa_K786002 were transferred to 0.5% soft agar under blue light exposure overnight for 12 h at 25°C. Diameter of colonies between the plates with or without blue light exposure were compared. It is found that the average diameters of three clones exposed under blue light are significantly bigger than the counterparts in dark.

The photo of soft agar plates on the left showed the cells transformed with biobricks moved toward blue and orange light sources placed in opposite respectively.

FUTURE PERSPECTIVE

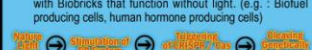
1. Improving the HR desalination system

Sensory rhodopsins system and the halorodopsin system [7] are sensitive to long wavelength visible light source. With our Biobrick, *E.coli* move toward light source and gain a stronger light intensity for the halorhodopsin system to absorb Cl⁻ more efficiently.

The phototactic device that is sensitive to near-UV light and can be integrated to direct the cells/Cl⁻ ions to another place, so the remaining solution is being desalinated.

2. Incorporating with as a new safety approach

Our Biobricks could tackle the safety concerns of synthetic biology. When the gene expression system for sensory rhodopsin is incorporated with the CRISPR/Cas system, the cells cleave their own DNA into nucleotides when being exposed to natural light. This system could be combined with Biobricks that function without light. (e.g. : Biofuel producing cells, human hormone producing cells)

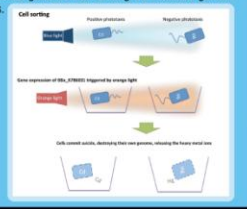


3. Cell Sorter

When the phototactic systems are integrated with different heavy metals collection devices [8, 9], heavy metal sorting in sewage and heavy metal recycling can be done by the bacteria.

All together :

Cell sorting and metal ions collection in an environmental friendly way. If we combine all our systems together: ***sensory rhodopsin system, gene expression system and genome targeting system,*** the bacteria would be able to sort out and collect heavy metal ions while minimizing potential environmental hazards by preventing the horizontal gene transfer among the released genetic materials.



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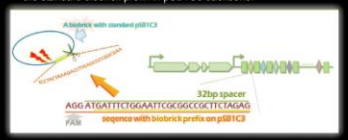
HUMAN PRACTICE

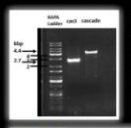
According to the 3 sets of survey analyses, iGEM and Synthetic Biology are not well-known in our community. To spread out the information effectively, exhibition should be organized asides from the educational programs. For better understanding of the high school students who can join iGEM HS, we need to modify the educational program to equip them to do wet labs and apply Synthetic Biology to create solutions. As for our fellow undergraduates, we may suggest CUHK to recruit students of different majors, so they are required to conduct active learning about Synthetic Biology. Moreover, the larger the data set the more accurate the result, we could continue the same investigation on undergraduates and high school students next year to validate today's result or even go beyond.

NEW SAFETY APPROACH

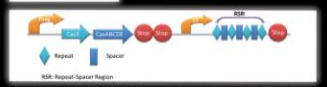
CRISPR/Cas system is an adaptive immune system of *E.coli*, which can target on specified plasmid DNA sequence[6]. By engineering the system to target on a segment of common sequence of standard biobrick and cleave the plasmid, we can develop a tool for providing higher safety level for synthetic biology.

In our design, the targeting sequence is a segment containing the standard biobrick prefix in pSB1C3 backbone.





To confirm the existence of CRISPR system in *E.coli* K12, we used primers to amplify the Cas3 and cascade from its genome DNA. Expected band size of cas3 2.7kbp and cascade 4.4kbp are shown.



For utilization of CRISPR system in other bacteria such as *Bacillus Subtilis*, we designed this biobrick including the essential proteins and also the targeting repeat-spacer region of the system, regulated by certain promoters and terminators.

ACKNOWLEDGEMENT & REFERENCES

[1] Hoff WD, Jung KH, Spudich JL (1997). Molecular mechanism of photosignaling by archaeal sensory rhodopsins. Annu Rev Biophys Biomol Struct. 26: 223-258.
[2] Trivedi VD, Spudich JL. (2002). Photostimulation of a sensory rhodopsin II•HtrII Tsr fusion chimera activates CheA-autophosphorylation and CheY-phosphotransfer in vitro. Biochemistry. 42: 13887-13892.
[3] Welsh M, Oosawa K, Aizawa S, Eisenbach M (1993). Phosphorylation-dependent binding of a signal molecule to the flagellar switch of bacteria. Proc Natl Acad Sci U S A. 90: 8787-8791.
[4] Barak R, Eisenbach M (1992). Correlation between phosphorylation of the chemotaxis protein CheY and its activity at the flagellar motor. Biochemistry. 31: 1821-1826.
[5] Igo MM, Ninfa AJ, Stock JB, Silhavy TJ (1989). Phosphorylation and dephosphorylation of a bacterial transcriptional activator by a transmembrane receptor. Genes Dev. 3: 1725-1734.
[6] Makarova KS, Haft DH, Barrangou R, et al. (2011). Evolution and classification of the CRISPR-Cas systems.Nat Rev Microbiol. 9: 467-477.
[7] http://2011.igem.org/Team:Hong_Kong-CUHK/Project/Halorhodopsin
[8] http://2010.igem.org/Team:Peking
[9] http://2011.igem.org/Team:Tokyo-NoKoGen