iGEM Ehime-Japan team

Ehime University

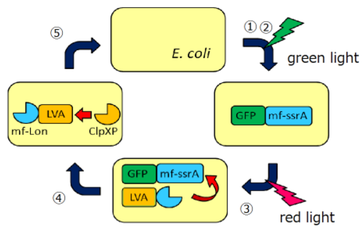
Introduction

We aim to create E. colingual which is a tool to know E. coli feelings. To realize our project, the light sensor genes from *Synechocystis* sp. PCC6803 were used. Also, we constructed new degradation system by using the Lon protease and ssrA tag from *Mesoplasma florum*. E.colingual consists of three parts; Sensing, Connection, and Screen. First, Sensor E. coli senses the change of temperature and produces GFP or RFP. Second, those lights are transmitted through an optical fiber to Screen by UV (364 nm). Finally, Screen E. coli expresses GFP or RFP activated by light from GFP or RFP under UV lamp, and display messages on a plate.

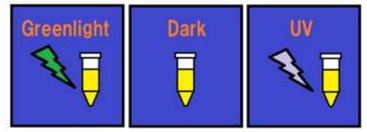
E.co-mail

E. co-mail

"E. co-mail" is a communication tool with E. coli and an optical fiber. We would be able to transfer digital information depicted in E. coli through a distance, and probably could send e-mails with this tool.



①One of the two test tubes with E. coli (pJT122-GFP+mf. ssrA tag, pJT106b-mf.Lon+LVA tag, and pPLPCB(S)) is exposed to green light. The E. coli expresses GFP＋mf. ssrA tag.
②GFP emits its green light by activation with UV (364 nm) and the light is transferred to the other test tube through an optical fiber. The other E. coli receives the green light and expresses GFP too.
③When the E. coli is exposed to red light, mf.Lon+LVA tag is expressed and GFP is degraded by the Lon protease.
④mf.Lon+LVA tag is degraded by ClpXP preexisting in E. coli.
⑤Return to No. 1.



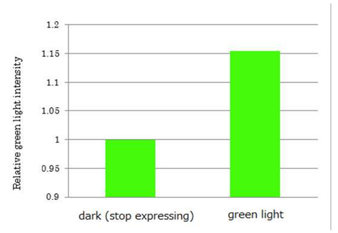
Degradation system

In most bacteria, ssrA-tagged proteins are degraded by protease. In E.coli, the ssrA tag has 11-amino acid sequence that is recognized mainly by the ClpXP protease. The tag has been minimized into the three amino acid LVA tag, and this minimum tag is utilized widely in synthetic biology and in iGEM for temporal expression and degradation of stable protein, such as GFP and repressors.
Mycoplasamas, however, do not have the ClpXP protease. The ssrA tag sequences are quite different from those of the other bacteria. It was found that the tag is degraded mainly by the Lon protease in Mesoplasma florum, a Mycoplasma species [1].So, we call the tag "Lon-tag".

[1] Gur, E. and Suer, R. T. (2008) Proc. Natl. Acad. Sci. USA, 105, 16113-16118. Evolution of the ssrA degradation tag in Mycoplasma: Specificity switch to a different protease.

An optical fiber assay

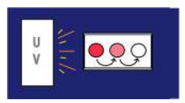
We tested if the red/green sensor system could catch green light transmitted through an optical fiber from a distance. The source green light was generated by irradiation of a GFP solution by UV light. One end of the optical fiber was dipped in this light-emitting solution, and the other end was dipped in a suspension of the light sensor E. coli. This sample was placed in the dark.The light intensity for this sample was higher than that for the control sample, which was the light sensor E. coli simply placed in the dark without the optical fiber connection.



E.co-domino

E.co-Domino

We tried to reconstruct toppling dominoes with E. coli, and this part would work as the Screen part of E. colingual. For the purpose, we used the red light sensor plasmids. The lacZ in pJT106b was replaced by RFP, and we have succeeded the construction of the E.co-domino system, in which irradiation of red light induces RFP expression. The expressed RFP induces next RFP expression in the neighbor well of a micro-titer plate.



Domino mechanism

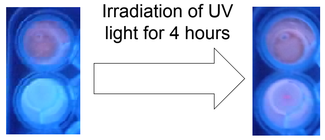
1. When the E. coli in the start well receives red light, RFP is produced and emits red light by UV irradiation.
2. The E. coli in the next well receives the red light from RFP in the start well, and RFP is synthesized as in the case of the start well.

If this process is repeated, the red light should be transferred continuously.

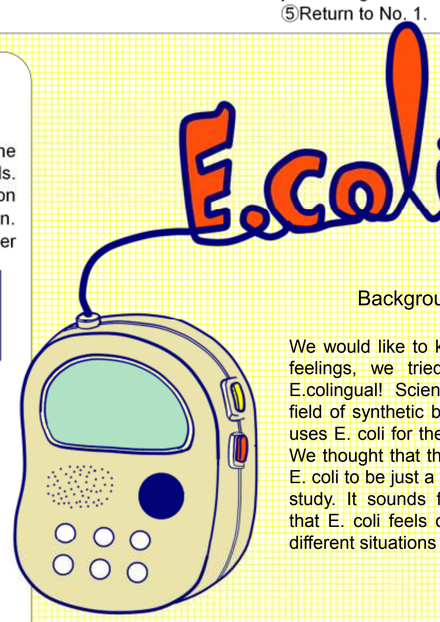
The E.co-domino system worked!

JT2 strain having pCph8, pJT106b-RFP, and pPLPCB(S)

Shaking at 37℃, 125 rpm for 16 hours under the white light.
↓
Collection and re-suspension of the cell.
↓
Irradiation of UV light (364 nm).



Irradiation of UV light for 4 hours



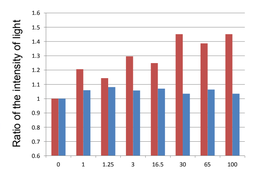
Background

We would like to know E. coli feelings, we tried to create E.colingual! Scientists in the field of synthetic biology often uses E. coli for their research. We thought that they consider E. coli to be just a tool for their study. It sounds fun to think that E. coli feels differently in different situations !

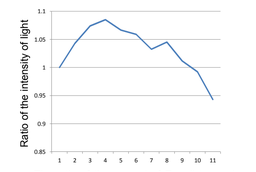
E.cold-heat sensing system

E.cold-heat sensing system

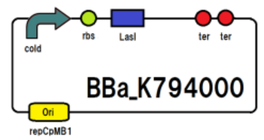
We designed the plasmids that enable E. coli to produce RFP or GFP when it is hot or cold.
The heat sensing plasmid has a heat shock promoter (HSP) and the downstream luxI. E. coli having this plasmid produces a signaling molecule, that could be caught by an E. coli that converts the signal into the red light of mCherry. In a similar way, the cold sensing plasmid has a cold shock promoter and the downstream lasI (BBa_K794000, Figure, right). The signal is caught by an LasR-expressing bacteria, and this expresses GFP.
We checked if the RFP-expressing plasmid really expresses RFP in the presence of different concentration of the signaling molecule (Figure, left). Then, we tested if the heat sensing E. coli works (Figure, middle), and found that it works if an appropreate ratio of the sensing and RFP-expressing cells are mixed together.



Concentration of signaling molecules



The ratio of the amount of E. coli to receive the signal molecules and E. coli issuing 3OC6HSL in hot weather



We submitted and registered this plasmid as a new BioBrick.

Future

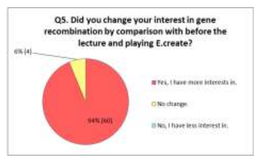
We hope to apply our system in the future to investigation of the environment: for example, under the ground, under water, and in the places where it is difficult for human to enter.

Conclusion

The main findings of this study are as follows. Firstly, circulative communication system of E.co-mail can be realized in theory. Secondly, the E.cold-heat sensing system can change the information that E. coli received into the form that can be transmitted. Then, E.co-domino could draw moving pictures that look like domino toppling. By combination of the above three parts, E.colingual is completed!

Human Practice

Three activities were performed in our university and a nearby high school.
1. Presentation about synthetic biology and iGEM.
2. Questionaaries among various ages on synthetic biology.
3. We designed a new puzzle game "E.create" and played with high school students !



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