Abstract

Metabolic engineering has brought us handy tools to produce desired products. However, we have to induce genes to properly express them on time. We, KAIST 2012 iGEM team, suggest a module that automatically turns the transcription of genes on or off using dual-phase switching module. In our model, promoter orientation is designed to be reversed when sufficient quorum molecule is detected. Then, genes on the other side, indigo synthesizing enzyme bFGM lactonase, and excisionase are expressed. Indigo is meaningful in that it is the end-product tryptophan metabolism and, its color can be easily detected. Lactonase quenches quorum molecule (acyl-homoserine lactone or AHL), which will generate an oscillating fluctuation of AHL level. Excisionase acts on the recombinase site to set promoter to its initial orientation. In this way, we can regulate metabolic pathways. So we call it regulateE.coli, Reguli.

Exordium

Mykobacterium phage Bxb1 integrase is a DNA recombinase, more precisely a member of serine integrase family. As other DNA recombinases do, it recognizes specific sequences, called attB and attP, and then inserts, integrates, or excises double stranded DNA, depending on the orientation and location of recognition sites. We used this integrase to invert specific sequence in plasmid.

Another protein Bxb1 excisionase works with integrase to flip inverted DNA back into original state regenerating attB and attP sequences.

Prototype - Flip & Flop

The device, pFlipFlop, is a prototype to check whether phage Bxb1 DNA recombinase system and designed devices work in our host strain or not. B3A2_01 (refPP) and B3A2_06 (refP0) are attached to each end of the system. In its initial state, this device generates GFP in E.coli. When B3A2 integrase inverts the promoter orientation, it starts to generate mRFP. When B3A2 excisionase inverts the promoter orientation back into original state, it expresses GFP again.

Modeling

To predict the result, we constructed mathematical models of system using MATLAB. We considered single cell and whole cell culture using logistic cell growth equation, assuming it only follows simple DNA/enzyme kinetics and mass balances equations.

When inverted (at 100 min), GFP production stops, and RFP production takes place (Figure 1). Considering the whole cell culture, RFP levels continuously increasing after action of inverter, while GFP level does not (Figure 2).

Results

The pTrcHis2A vector containing Bxb1 Integrase controlled by Trb promoter, double transformed into pFlipFlopGFP and pFlipFlopRFP containing MG1655. The double transformed cells showed color different from the single transformants. Which means, our designed parts (B3A2 Integrase, pFlipFlopGFP and pFlipFlopRFP) are working as we expected.

Conclusion & Future Plan

WE KAIST iGEM TEAM, Suggest highly manageable biosystem that the ‘Reguli, E.coli equipped with auto-regulation device, using Bxb1 recombinase system, quorum sensing and degrading system.

In future work, We will optimize the regulatory network of quorum and integrase to make more tightly regulated device. And we will also build more applications such as ‘Bacteri-Guard’ which can be further applied to othersystems.

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3. All of our experiments were done in Systems and Synthetic Biology Laboratory in KAIST.
4. Confocal microscopy was done in Cancer and Neural Signaling Lab in KAIST.
5. GFP and RFP intensity measurement was done in Bio Molecular Engineering Laboratory in KAIST.

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