Bioclock: Temporal Control in *E.Coli*

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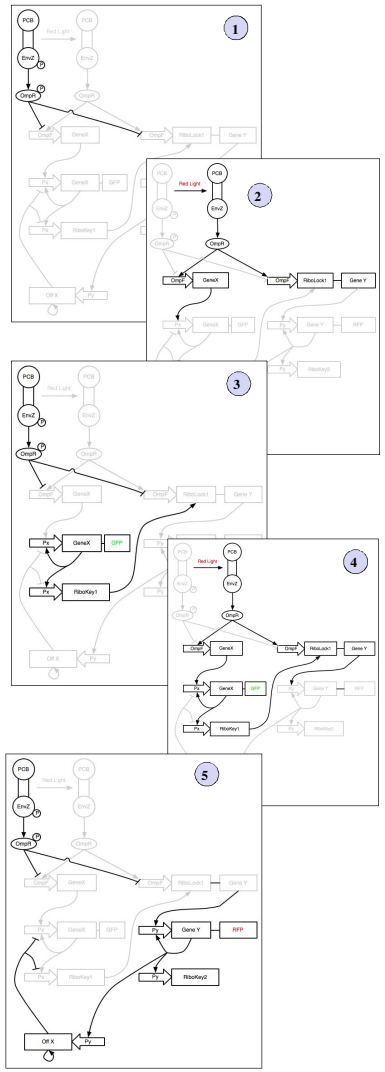
Abstract

This year Melbourne iGEM team seeks to build a temporal controller in E. coli. The idea is to build a system which expresses gene(s) at a specific time in a sequential manner. By utilizing red-light as the stimulating signal, the system can be altered between multiple states at a flick of the light switch. At each state, a different gene is expressed. This system allows the users to carry out multiple reactions in a sequence, while being able to resist one reaction from occurring until the previous one reaches its optimization. In this study, we show the design, modelling and some experimental results towards a proof of principle of the system. The design uses the leverage of existing biobricks of red light bacterial photography system, positive feedback loops and riboswitches. We propose that the architecture presented should scale well with increasing number of genes to be temporally regulated. It is anticipated that such system will be useful in metabolic engineering because enzymes can be turn on and off in a sequential manner.

Mechanism



1. Initially in the dark, therefore OmpR phosphorylated and OmpF repressed

2. First Pulse: Red light results in OmpR beingdephosphorylated, therefore transcription initiated at OmpF promoters.
Gene Y transcript is locked off by RiboLock1 therefore no Gene Y is translated .
Gene X **is** translated, which activates P_x .

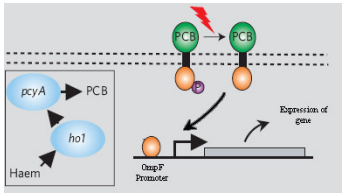
3. Red light is switched off, therefore OmpF is repressed again. The activated P_x produces more Gene X, the RiboKey1 and GFP.
The system is in state 1 with state 2 prepared for activation on the next pulse of red light.

4. Second Pulse: Red light results in OmpR being dephosphorylated therefore transcription initiated at OmpF promoters .
RiboLock1 is produced again. Since RiboKey 1 is already present, RiboLock1 is opened and Gene Y can be translated .
Gene Y activates P_y which kick-starts the activation of part 2 and repression of part 1.

5. Red light is switched off, therefore OmpF is repressed again. The activated P_y produces more Gene Y, RiboKey2, RFP and Off X. Off X repressed P_x, switching off part 1.
RiboKey 2 will open RiboLock 2 in part 3 (not shown) .
RFP indicates that the system is in state 2 with state 3 (not shown), ready for activation on the next pulse of red light

Components

• Red light sensing



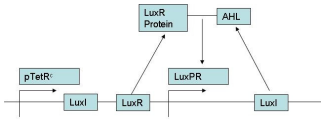
Red light induces the dephosphorylation of EnvZ (orange circle) which activates OmpF promoter, allowing downstream gene expression. (Levskaya A. *et al* (2005) Nature, **438** 441)

• Riboswitches

Biobrick Constructed: lacI regulated ribokey synthesis.

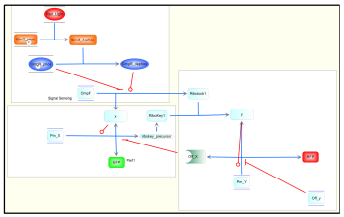
Secondarily structured RNAs allowing post-transcriptional regulation of gene expression. Ribolock (black fragment) upstream to protein-coding gene conceals the RBS. Ribokey with complementary sequence to the ribolock bind and unlock the RBS, allowing downstream gene expression. (Altuvia S. *et al* (2000) PNAS, **97** 9825)

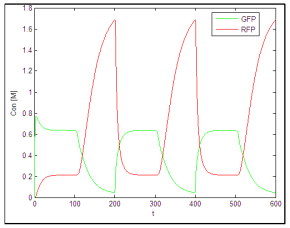
• Positive Feedback



Constitutively expressed LuxR associates with AHL to induce LuxPR, which results in more AHL produced. By connecting desired gene (such as GFP) downstream to luxI, a positive feedback loop can be constructed.

Modeling





Utilizing JDesigner2 (System Biology Workbench) and Matlab (Mathswork), a computer model was constructed. The alternating states of the system is shown.

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