

Pre-Proposal: Framework for Programmable Multistable Switch in *Escherichia coli*

Project Summary

In nature, biological regulatory pathways utilize complex decision-making mechanisms. Understanding simplified regulatory pathways such as multistable switches can help us understand the more complex natural decision-making mechanisms, and thus able to more readily replicate these biological processes in vitro. Following the mathematical model developed by Macia et. al, the proposed research would experimentally address the possibility of a multistable system.

Introduction

In synthetic biology, recent efforts have focused on constructing genetic circuits that mimic biological regulatory pathways in nature. Because many biological parts are modular, simple genetic circuits can be integrated in various ways to reengineer pre-existing biological systems to produce a desired response. Simple response systems such as positive and negative feedback, and autoregulation, have been wired into useful genetic regulatory networks such as the repressilator (Elowitz and Leibler) and the toggle switch (Gardner).

Another recurring theme in biology is producing different response states depending on input signals. Although many studies have focused on the behavior and construction of bistable systems, useful for switching graded input into an all-or-nothing response, not much is known about constructing a genetic circuit that displays multistability, having three or more stable states. Achieving multistability can be useful in manipulating cell fate in multicellular organisms, the regulation of cell-cycle oscillation during mitosis, and the maintenance of epigenetic traits in microbes (Ozbudak).

In our project, we aim to build a simple multistable switch for two-component signaling pathway. We use the mathematical model developed by Macia et al as our main design and employ a well-known genetic toggle switch circuit to change its bistable oscillation to multistable response.

Our idea

System Design:

In order to switch a graded external stimulus into multi-stable states, the input signal must be amplified within the system. Macia achieved signal amplification in his mathematical model by using mutual positive feedback loops between two genes as well as autoactivation of each gene (Figure 1). Autoregulation is especially critical in achieving multistable states because it either amplifies or compensates transient differences in gene expression (Guantes). This is an improvement from bistable systems which usually have (low,low) or (high,high) expression for mutual-activation and (low,high) or (high,low) expression for mutual-inhibition.

In our genetic circuit, protein A transcribed from gene A activates the constitutive promoter of gene B and auto-activates transcription of gene A (Figure 2). Likewise, when gene B is transcribed, it activates both gene A and B. Each gene has genetic markers that allow for gene transcription measurement.

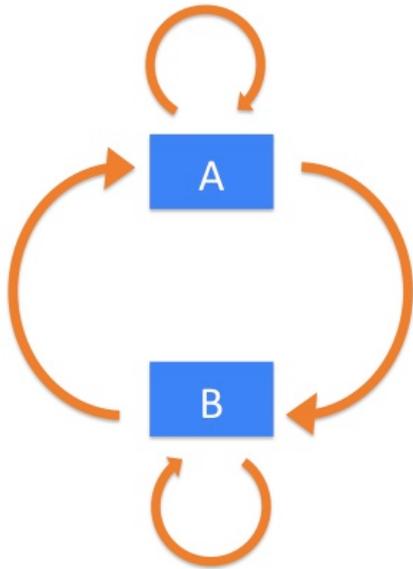


Figure 1

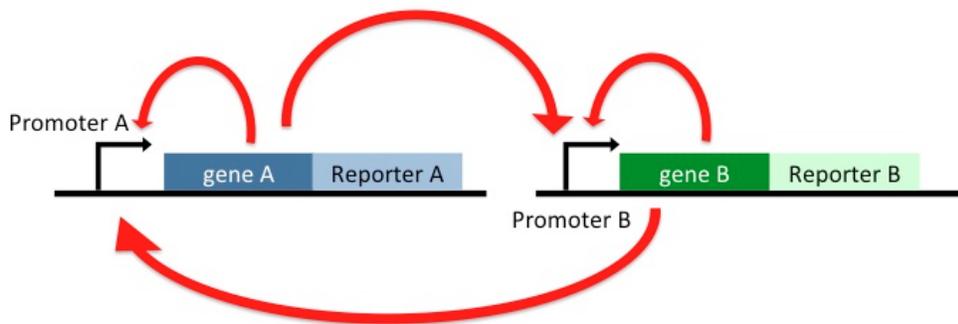


Figure 2

Plasmid construction & Testing multistability:

The genetic circuit can be implemented by constructing two different plasmids, one responsible for gene A and the other for gene B (Figure 3). We can use standard cloning techniques to make these plasmids. All genes, promoter, and reporters can be obtained from PCR amplification, and these components can be inserted into bacterial plasmids by digestion. Then both types of plasmids can be transformed into *E. coli*. The gene expression level can be tested by flow cytometry to detect fluorescence of each gene.

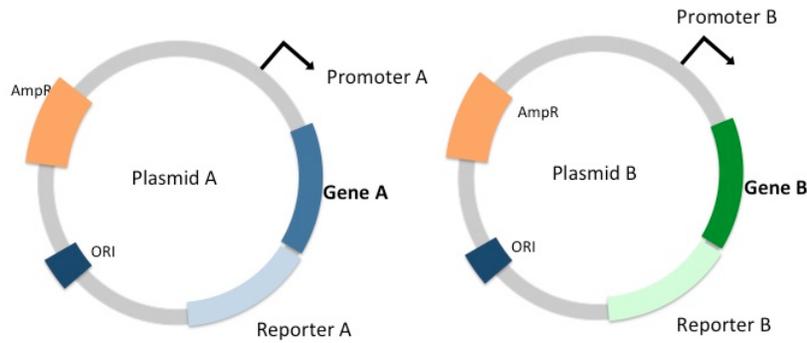


Figure 3

References

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