Colorful *E. coli* Weave Time and Space

**Our Project**

![Image of DNA structure]

**Oscillator: Design**

In the time weaving section, we designed a gene circuit with which the *E. coli* can work as an oscillator. The key players are *Lsr*R and *Lsr*K. Based on their interaction, we assume the process follows: At the beginning, the accumulation of *Lsr*R inhibits the expression of *Lsr*K; after a while, *Lsr*R drops due to its self-induction and degradation and thus giving *Lsr*K a chance to express. With the gradually mounting *Lsr*R, it promotes itself as well as *Lsr*K by offsetting *Lsr*K’s inhibition. In this way, the two players’ concentrations wave alternatively in a regular way.

**Oscillator: Models**

![Graph of oscillation model]

Derived from the gene circuit, we can simplify the two players’ relationship as shown above: *Lsr*K sheds positive regulation on *Lsr*R and itself and *Lsr*R inhibits *Lsr*K. This is a typical two-node system and we generate an Ordinary Differential Equation to describe it. In the equation, *X* and *X* denote the two key players. We assume that the basal production rate of *K* is *a* and the degradation rate of *R* is *b*. And the positive auto-regulation of *K* and the negative regulation of *R on K* are described by Hill functions.

The following simulation diagrams are derived from the equation and we can see that both the deterministic and stochastic analysis proves the efficiency and stability of our oscillator.

**Color Film: Introduction**

Light tricolor theory: Red, green and blue can compose any color with different proportion. Their complementary colors are cyan, magenta and yellow that also can form any color with different proportion. The relation between blue and yellow is that yellow is the complementary color of blue and the other two complementary colors: magenta and cyan yield blue. When mixed together:

- Traditional Film
- *E. coli* Color Film

Disadvantages: Conventional, Harmful, Pollution, Expensive, Waste

Advantages: Convenient, Harmless, Environmental friendly, Costless, Save silver, High resolution

**New Standards**

The diagram shows the assembly process of standard biobricks. Brilliant as it is, we have encountered problems with it in our experiment. The 1st one is its high consumption of time and money when assembling many biobricks in a single complicated gene circuit. And the 2nd one is that we are not able to extract the standard biobrick back once it is assembled into a device. To solve these two problems, we have proposed two new standards respectively.

**Golden Gate Method**

Golden gate cloning is a method of assembling multiple DNA fragments in an ordered fashion in a single reaction. The classic method takes at least a week to assemble ten biobricks. In contrast, golden gate cloning can shorten this process to five days. This advantage is due to the use of a unique Type IIS restriction endonuclease. (1) This kind of endonuclease can recognize a specific site, but cleave at another position downstream of the recognition site, creating a 4bp overhang, so they can create different sticky ends long as the downstream sequences are not identical. (2) Besides, cloning can be expedite by digesting and ligating in the same reaction mixture because correct assembly eliminates the enzyme recognition sites.

**Reverse Assembly Design**

As to the other problem that we had no way to get a single biobrick from a composite device, except by designing specific PCR reactions. So we hope to modify the standard backbone to make it more easily disassembled. While keeping all the conformal standards of the backbone plasmids, we add the recognition site of HindIII both in the prefix and suffix of the backbones. Biobricks with this kind of backbone can be assembled as the former standard. However, part of the composite product will be flanked by two HindIII sites. Therefore, if we want to get certain component of a composite device, we cannot cut this product with the HindIII restriction endonuclease. After the cloning, we will get each part and also the vector. Then we just need to link the part we want to the vector, and we will get a standard biobrick again.

**Color Film: Results**

*Figure 1* Blue light induced bacteria with part BBA K500000, blue light sensing and reporting system. The result shows that bacteria under blue light exposed little RFP and the colony did not turn red. Bacterial colony in the dark yield more RFP than the experiment group.

*Figure 2* Blue Light Sensor effect shown by plate streak. The two lines of TopR(C) E. coli were streaked on the same plate. Similar to the colonies, without inhibition of blue light, the streak on the right section yielded more RFP than the left.

*Figure 3* Control experiment show by plate streak. The TopR(C) E. coli which only contains the reporting system were streaked on the plate. Without sensor, the reporter system yield red pigment both in the presence and absence of blue light.

**Color Film: Gene Circuit**

This part was designed to build the complete blue light sensing and reporting system in a single plasmid. Promoter BBA J23117 is a constitutive promoter but not a strong one. It will keep concentration of *LovTAp* in bacteria on a certain level. When exposed to blue light, conformational rearrangements in *LovTAp* will occur due to the absorption of a photon. The *LovTAp* can then bind to DNA and repress the transcription of genes downstream. Here we use RFP as the reporter.