AegiSafe O-Key
A construction of the orthogonal transcription-translation network for biosafety and regulation
Tianjin University iGEM Team, 2012

1. Abstract

With the development of the synthetic biology, one of the most important questions is how to express the targeted gene in chassis organism. However, if external gene is not compatible with the cell to some extent, the gene could not be well expressed. Now, we try to solve this problem by the orthogonal system. Since this system is independent of the original system, the targeted gene could be well expressed. And we also utilize the system to adjust the pathway of metabolism. For the part of Human practice, we propagated the knowledge of synthetic biology and iGEM competition to NanJing middle school and NanKai University. In addition, we communicated with Peking University and exchanged ideas on each other's project, and helped NanJing University and SUST complete their survey and develop the software.

2. Orthogonal System

The Glossary: 
Deg: the orthogonal systems - the orthogonal system contains a pair of orthogonal bases. 
E: the O-Lock, the orthogonal ribosome, which serves as a key to translate the orthogonal mRNAs. 
A: the O-Box, the orthogonal mRNAs, which can only be deciphered by the O-Key.

In terms of reprogramming translation, o-ribosomes are especially powerful as they enable one to partially decouple translation from the native protein synthesis machinery. In particular, o-ribosomes can translate genes with altered Shine-Dalgarno (SD) sequences recognized by host ribosomes. Therefore, o-ribosomes can be used to explore gene expression dynamics.

Before the wet lab, we had established a model to verify the orthogonality of the two protein expression systems.

We have predicted the relative protein expression amount to verify the orthogonality. Prediction simulated from our model is shown in Figure 2.8, Figure 2.9.

We can see from the file that the protein expression amount of n-RBS: n-166 and that of the o-RBS: o-166 is significantly larger than other mismatched conditions. Consequently, the orthogonality of the system is perfect.

After the perfect prediction of orthogonality, we also conducted the wet lab. In order to characterize orthogonality, we designed the interaction in the above picture. We constructed pathway 1, 2, 4 in the wet lab. See Figure 2.6

We constructed the O-RBS by mutating the 5D sequence of RFP, and altered the 5D sequence on the 16S, as shown in Figure 2.6. The results are shown in Figure 2.7, and the numbers of the tube correspond with the numbers in Table 2.1. Number 1 shows our O-Lock system works, Number 2 and 3 shows great orthogonality of our system. Pathway 4 is the control. We can see Pathway 1 and 4 expressed almost equal number of protein.

3. Logic Metabolism Regulation

Figure 3.1 Orthogonal system which plays roles in metabolic regulation can be described as key and lock. They work together to form an AND gate. (From TJJ iGEM Team 2012)

Figure 3.2 The orthogonal system could adjust metabolism. We chose the metabolic pathway of Violsaceae. We put the O-RBS before the Viola gene, and N-RBS with the genes of Viola, Viola, Viola, Viola. Then we assembled the whole ViolaT pathway in Yeast Assembler. The pathway and the O-Key gene with pSil promoter were finally co-transformed into the cells. As the cell itself doesn’t have the O-Key, the gene is strictly shut down. Then the pathway could mainly produce deoxyviolaxin. When we added arabinose, the O-Key was produced to open the O-Lock, so the cell could produce violaxin.

4. Technology


Figure 4.1 How to introduce the O-Lock to a plasmid? As the figure shows, we just use two primers to make PCR. Those two primers have about 20bp overhands in their 3’ end, which contain the O-Lock or other mutations. Then we directly transform those PCR products into E.coli by electroporation-mediated method. E.coli could cyclize those PCR products and form the complete plasmid.

Figure 4.2 In our project, we have to construct violaxin biochemical pathways which contain 5 individual operons and 11 DNA constructs in total. If we used restriction sites to joint those 11 DNA constructs step by step, it may cost at least 2 months. But we successfully used the "DNA assembler" method to synthesize the 10kb pathways on pRS426 in no more than two weeks. Assembling DNA constructs by yeast is a highly efficient method in solving synthetic problems.

5. Future Work

First, the prevention of gene spread from the laboratory. Since our orthogonal system works as Lock and Key, it could be used to prevent gene pollution from the laboratory. To verify the system’s feasibility, we imitated the protection process by preventing Amp resistance gene diffusion.

Second, the prospect of constructing a fully orthogonal creature. If we could change all native RBS to O-RBS in a cell, we could really construct a creature with bran-new translation system. To achieve such a goal, we design a project to mutate all RBS of pRS426 to O-RBS.

Third, to apply O-key system in regulating metabolism. Our orthogonal system consists of two parts: O-Key and O-Lock. These two parts could serve as the two inputs for our new AND gate. We use the O-RBS to "lock" the targeted sequence, and make it decipherable only under o-ribosome.

6. Modeling

As mentioned before, we have constructed a model to predict the orthogonality of our system.

In the process of translation, the initiation rate is the rate-determining step, and the protein expression level is directly proportional to the initial translation rate. Furthermore, the initial translation speed is strongly related to the G0. In our model, we calculated the G0 from the Formula 7.1.

The formula used to describe the protein expression amount is shown in Formula 7.2. The orthogonality predicted by our model before the wet lab is shown in Figure 7.1, which is similar to our following wet lab data (Figure 7.2). This prediction corresponds with the experiment data.

7. Human Practice

This year, inter-university collaboration has become our emphasis, thus we worked with several universities including Peking and NanJing. In addition, we gave lectures to NanKai High School students to propagate synthetic biology, and compiled biosafety handbooks, regulation summary for other teams to use. We even made a short movie to alert the public of potential biohazard issue.

8. Team

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9. Reference