**Design**

**Site-Specific Recombination**

Site-specific recombination system (SSRs) is a type of genetic recombination. It is widely used to carry out excisions, insertions, translocations and inversions in the DNA of cells. We used 2 SSRs, Cre-loxP and Bab1 system in our project to accomplish the inversions in the DNA.

For example, in the Cre-loxP system, when the external stimulus comes, the Cre recombinase will be expressed and then flip the sequence between the two LoxP sites.

**A Simple Example**

In this simple example, under the initial condition, the promoter is able to induce the Output. But once the external signal comes, the expression of the Cre recombinase will be activated and it will lead to the flip between the two LoxP sites. Consequently, the promoter cannot bring out the output, that is, the logic changes. Though it is simple, this example shows the basic principle of our design.

**Core Design**

We construct our design as follows. Between the LoxP sites, there are two promoters. In the left state, only when both promoter A and B are activated can we get the expression of the Output. This module acts as an AND gate.

In response to the external stimulus we’ve given, the Cre recombinase comes out and starts the flip from the left state to the right. Now either promoter A or B can activate the expression of both signal X and Y. It works as an OR gate.

**Scalability**

Our project shows strong scalability. Based on the design mentioned above, we can also realize the switch between any two of the 2-input logical relations and even N-input logical relations. The three modules serve different functions. Module 1 is used to decide what the input is, A or NOT A. The second module is the key module of our project, which fulfills the switch between the AND gate and the OR gate. Module 3 is built to get the XOR gate and XNOR gate.

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**Modeling**

Our design has its biological foundations and engineering backgrounds. To make it valid and provide some evidence for our wetlab experiments, we use mathematical models to simulate the whole procedure.

We divide our modeling into two parts: the first is the process of the generation of Cre protein and Cre protein binding to LoxP sites, the second is the inversion of DNA strands. In addition, we make some sensitivity analysis; and a feed forward pathway is added to make our design get better efficiency.

First we use ODE equations to describe the process of the generation of Cre and binding reaction between Cre and LoxP. And we can get the result showed in the right figure.

Then we simulate the trajectory of every single molecule to get the result of the inversion through Gillespie algorithm and Markov chain. The figures below shows numbers of two states of cells (flipped or not flipped). The only difference between these two figures is the number of DNA molecules, 1, 10, 100, 1000 respectively.

**Sensitivity Analysis**

We analyzed three parameters and found that the degradation rate of Cre protein (opcra) have an obvious effect on the final percent of the genes in different state.

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**Abstract**

The ambitious Tsinghua-A iGEMers are still dedicated to a beautiful combination of biology and engineering, and this year, the realization of a programmable logic device (PLD) on the gene sequence becomes the focus of our attention. A series of symmetric logic-togglers, for example, AND-OR switching gates, are designed to act as the basic parts of this Cell PLD.

The idea comes from PLD which is widely used in electronics engineering. Hopefully, the construction of these modules in the cell will be achieved, with the help of the site-specific recombination systems. Feed forward control theory is introduced into the module and mechanism on the behavior has long been under our analysis, all aiming at a better performance of the logic gate. Modeling as well as computer simulation will help to evaluate and improve the robustness in this process.

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**iGEM 2012 Tsinghua-A Cell Programmable Logic Device**

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**Application**

Our system can be integrated for a complicated logic further, and can also be used in back tracking algorithm. Programmable logic in cells may make bio-logics more flexible.

Cells show different responses to various extra-cellular signals in spite of the same genetic information in nucleus. Our system mimicks a simplified situation occurred in both prokaryotic and eukaryotic cells. We hope this system can facilitate the transformation from theory to engineering in the future.

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**Human Practice**

Tsinghua-A 2012 had a deep collaboration with a school students who are interested in synthetic biology, with the help of ITCC, a Chinese organization committed to promoting science and technology competitions for youth. We had a heated communication with many high school students in Beijing. We talked about some basic information, concrete of synthetic biology, the status of iGEM and some interesting ideas. They showed great enthusiasm in synthetic biology and iGEM. The vice director of ITCC had several communications with us on aspects such as Chinese high school students taking part in iGEM HS Division and the foundation of a bio-lab. We offered our experience and discussed the way of undergraduate iGEMers’ participation in HS Division as mentors. We also connected with Tsinghua and Tsinghua-D. We all together held a lecture on synthetic biology for students from various departments in Tsinghua University.

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**Wetlab**

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**Construction in Prokaryotic Cells**

In prokaryotic cells, the system consists of three circuits. One induces flip, one flips and the other expresses. First we use arabinose as an inducer to generate the recombinase Cre.

After the concentration of Cre increasing, loxP sites flip, causing the expression of suCD RNA and T7tag acting as an AND gate which can activate T7 promoter.

**Construction in Eukaryotic Cells**

Life process is much more complex in eukaryotic cells. We use micro-RNA and Bab1 system to realize our flipping system.

Using micro-RNA can not only simplify the design of the system, making sure the process is efficient and precise, but also ensure the dependency with other life process due to the orthogonality of micro-RNA and other signals. Micro-FNA has great scalability as well.

Compared to Cre-loxP recombination system, Bab1 requires different enzyme to flip back and forth, which makes the whole system more controllable.

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**Team**

**Members**

Bao Tianpeng | Chen Kejie | Feng Zhiche | Guo Mingzhou | Liu Xi | Huang Xi | Feng Zhi | Wu Zhaoming | Wu Hang | Xu Huaing | Ye Hanjie | Zhou Zheng | Zhu Zhigang | Tang Zhao | Liao Weike | Wei Lei

**Instructors**

Xie Zhen | Wang Xiaowen | Chen Yang | Liu Honglei

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