Project At-a-Glance

To make the cellular memory come true, we designed a system with two different strains of *E.coli* to be our “signal recorder” and “data saver”, two different plasmids to control the process of “write-in” and “erase”, and one plasmid to connect these processes.

-Background

What is memory? What kind of process can be called “remember”? As far as we’re concerned, the memory is to change a transient presence into a long-term existence. Maybe they will have different existing forms, but there will be strict correspondence between them. For example, the persistent expression of a fluorescent protein caused by a transient light stimulus can be regarded as a “memory”.

But there is also a problem that we can't ignore. As we all know, life can be regarded as a highly efficient machine. There is always a principle to guide its behavior: whether such an approach would make it better to live in this world. If yes, this action will survive, if not, it will be eliminated by the pressure of evolution. In most of the time, the response of the organism to the outside environment is transient. So we can’t to record this brief existence of external factors. Efficient living bodies do not allow themselves to express a certain substance in the condition of no need. This is precisely the key that we need to overcome.

Many scientists who are working on this area have their own unique insights on how to solve this problem. But there is a point of agreement whether using the recombinant enzyme or the reverse transcriptase or something else, they all use the DNA, a kind of material which is almost permanent stability in the life body.

So in our project, we also use DNA to storage information, but in an unprecedented way: we use the conjugation to realize the cellular memory.

-A Short Description

Bacterial conjugation is the transfer of genetic material between bacterial cells by direct cell-to-cell contact or by a bridge-like connection between two cells. Discovered in 1946 by Joshua Lederberg and Edward Tatum, conjugation is a mechanism of horizontal gene transfer as the transformation and transduction although these two other mechanisms do not involve cell-to-cell contact.

We use the Escherichia coli K-12(HfrH) strain to be our “Recorder”, if it feels a specific stimulation (the blue light in our experiment), it can encode the TraI (a necessary protein to the conjugation which has been knocked out in the Bacterial genome), then the *oriT* (transfer initiation sites, also be knocked out) can lead the reporter gene (GFP in our experiment) transfer from the “Recorder” to the “Saver” through the sex fimbria. The reporter gene has a T7 promoter, which cannot be promoted transcription in the “Recorder” because of the lock of T7 RNA pol. But when it is in the “Saver” strain BL21, it will become a constitutive expression, displayed as a bacterial group of green fluorescence. And if we want to rewrite the record, we can use a specific artificial signal (the red light in our experiment), to start the CRISPR_Cas9 system in the “Saver”, which can eliminate the reporter gene efficiently.

-System operation stage

First stage: the initial stage, the “write-in” plasmid and the shuttle plasmid are both in the “donor” cell, the “erase” plasmid is in the recipient cell, the conjugation doesn’t begin and there is no green fluorescence.

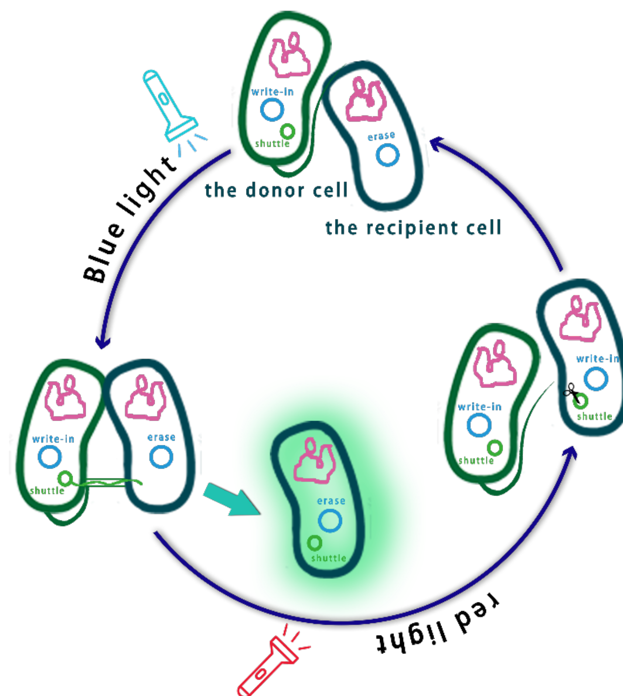
Second stage: signal reception stage, when there is blue light in the environment, the blue light sensor is excited then encode the TraI protein. The conjugation begins and the shuttle plasmid is transported to the recipient cell.

Third stage: signal storage stage, there is always T7 RNA Polymerase in the recipient cell and then GFP can be sustained existing by a large number of expression.

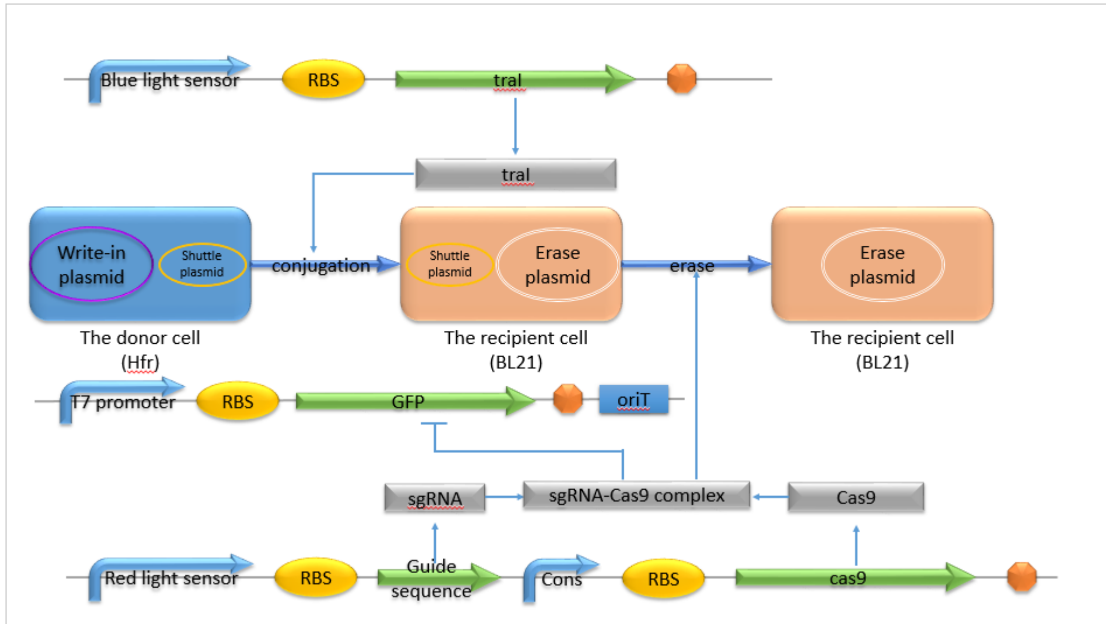
Fourth stage: signal erase stage, when there is red light in the environment, the CRISPR system is promoted in the recipient cell. The sgRNA is targeted to the GFP's gene sequence on the shuttle plasmid, so the shuttle plasmid in the recipient cell will be cut into pieces by Cas9 protein. Without the encoding sequence of GFP, the green fluorescence will fade away, and finally, the system will return to the first stage.

-System Schematic Diagram and Gene Pathway Map

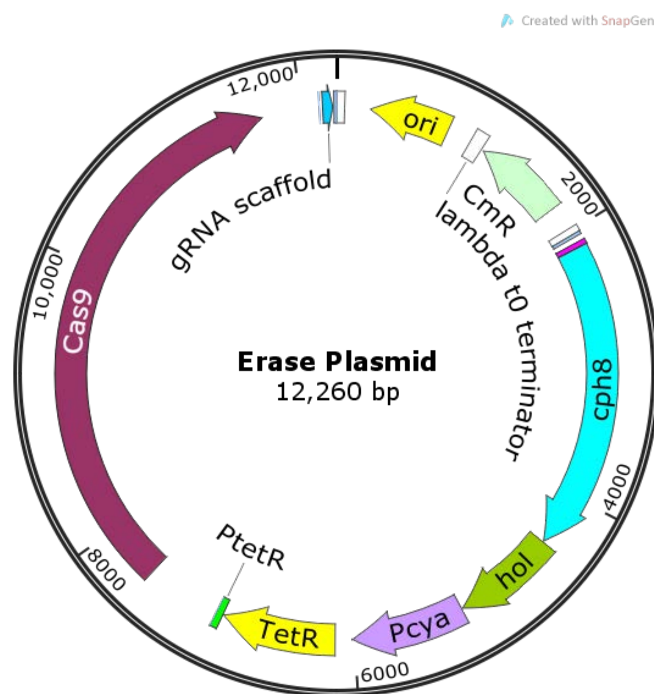
Here we use a unified genetic element to demonstrate our project at molecular and cellular levels. This is a simple system schematic diagram about our project.

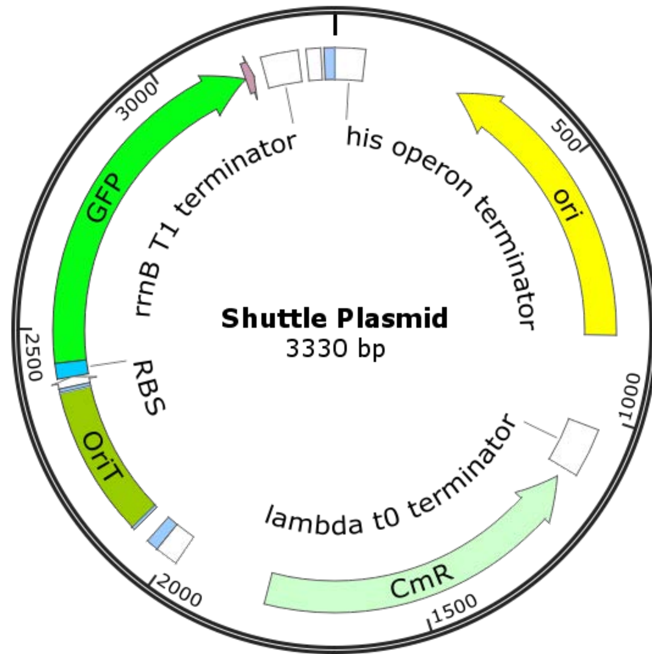


And then the gene pathway map of our project.



And the plasmid profile are as follows.

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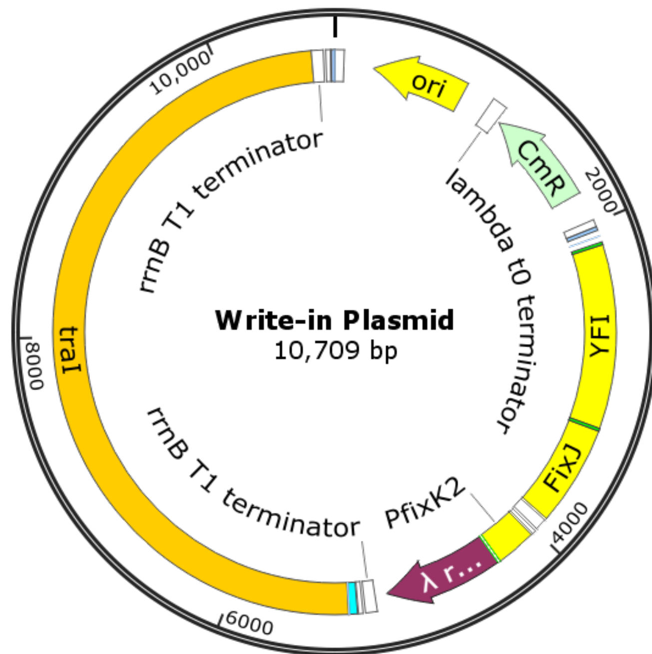
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