Fudan 2011

E.Tree, Neon Lights and Dinner Service

Introduction

What is the first thing that comes to your mind when you see these three words: tree, neon lights and dinner service? **Christmas!** Well, exactly, and that is what our project is all about. Our synthetic biology project will modify several E.coli to perform different jobs:

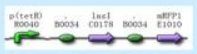
- **E.tree** The tree includes the leaf part and the trunk part. The "leaves" will change color according to the nutrients in the "soil": if the soil is rich in nitrates, the "leaves" are green and healthy; otherwise, the leaves will turn yellow.
- **Neon Lights** Each engineered E.coli can emit one light at first (such as red); after a while, the red light fades and another light is emitted. The different combination of such E.coli could therefore achieve the effect of neon lights.
- **Dinner service** The genetically modified bacteria involve a certain self-feedback system. When the "customer" is starving, it orders dinner from the "chef"; and the chef serves meals. While the "customer" is full, it releases a signal molecule and tells the "chef" that no more food is wanted, so the "chef" stops cooking.

Parts

BBa_K604005
lasR activator under Promoter lux pL

Constitutively expressed promoter with a LasR coding device and a strong RBS to create one part of the quorum sensing device. When LasI is present, the AHL autoinducer produced will bind to LasR, thus activate quorum sensing circuit.

BBa_K604006
LasI and RFP under Promoter tetR



TetR repressible promoter with a LasI coding device and a strong RBS, together with a RFP reporter device. This pathway is constitutively expressed unless TetR is present.

BBa_K604007
autoinducer synthetase rhlI and GFP under PyeaR

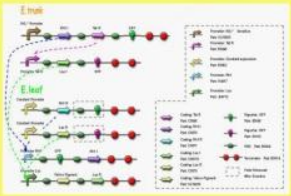
The pathway detects nitrates in the medium; if KNO3 is present, the circuit is activated, RhlI produces the AHL (C4-homoserine lactone) and TetR is synthesized to block another pathway. GFP is emitted to show the start of transcription.

Projects

Project 1

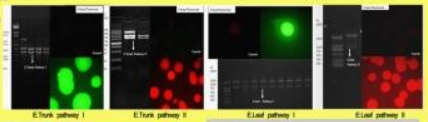
E.trunk bacteria detect nitrates in the medium and release either LasI or RhlI directed signals. If the medium contains nitrates, the entire pathway will be on and GFP will be produced. If the medium contains no nitrates, the first pathway is repressed while the second one is unblocked,and RFP will be synthesized.

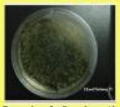
E.leaf bacteria change color according to the signal molecules released byE.trunk. LasR and RhlR are constitutive synthesized in E.leaf; they bind to the LasI and RhlI directed signals, respectively.



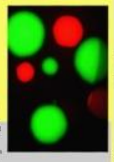
Results

Examination of our parts:



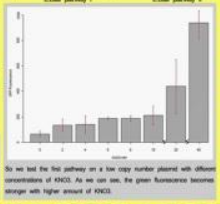


Expression of yellow pigment in Pathway IV of E.Leaf



Examination of E. Trunk's circuits :

E. trunk is linked to pSB1C3 and is spread on LB plate. Though the medium contains no nitrates, the cell emits GFP. We suppose that it is caused by the high copy number of the plasmids.



So we test the first pathway on a low copy number plasmid with different concentrations of KNO3. As we can see, the green fluorescence becomes stronger with higher amount of KNO3.

Overall effect If the medium contains nitrates, signal molecules released byE.trunk enter E.leaf and green fluorescence is emitted. RhlI molecules further are synthesized in E.leaf bacteria, so not only bacteria grow along the border but all E.leaf bacteria on the plate will turn green. Similarly, if the medium lacks nitrates, E.trunk bacteria release 3OC12-homoserine lactone *and "leaves" turn yellow.*

Project 2

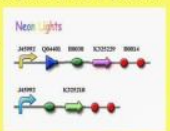
The "customer" E.coli is consisted of two pathways. The first pathway codes lsrR, a siganl that activates the second pathway, and aiiA, which degrades lsrR. The promoter for lsrR is stronger than that of aiiA. In that case, part of the lsrR will be degraded, but still some will be able to activate the second pathway. When the second pathway is activated, LuxI is produced and CFP emitted.

The "chef" E.coli also includes two pathways. The first synthesize LuxR, in the presence of AHL(the product of LuxI), it binds to the promoter LuxR and LuxS(the meal) is served, YFP is emited to show this process. In sum, the hungry customer orders the meal by giving out signal "luxI". The "chef" containing luxR, senses the "order" signal and prepares for the meal-LuxS. The dinner service is accompanied with yellow fluorescent. When the customer is full, lsrR synthesis is inhibited, the aiiA degrades the remaining lsrR in the cell, thus the production of LuxI is stopped. And meal is no longer served.

Project 3

In this project, we intended to have E.coli engineered for emitting one color of luminescence during exponential phase and another color during stationary phase, so that it is convenient to identify the growth phase and may also be utilizedfor other purposes.

To achieve this goal, we chose BBa_J45992, a stationary phase promoter, and BBa_J45996, a promoterthat is only active during exponential phase and assemble different luciferase under the control of the promoters. Parts of various luciferases have been obtained from the registry submitted by the team of Cambridge in 2010, for example, BBa_K325210, BBa_K325229, etc. System of degradation, such as ClpXP, can be used to optimize the project by degrading the previous luciferase when the latter is being produced, to make the latter color purer and the process of color changing faster. In addition, the fluctuation of the lusiferase activity due to the growth should be taken into consideration.



Human Practice

Part I: Team collaboration

(1)Survey: Sent out 50 questionnaires to Zhejiang University to survey their opinions about safety and ethical issues in synthetic biology, which helps us better investigate undergraduates' understanding about synthetic biology with comparison to those in Fudan University.

(2)Postcards: Received well-designed postcards from Shanghai Jiao Tong University and the friendship between the two closely related universities in one city are further consolidated by two iGEM teams .

Part II: Survey and discussion

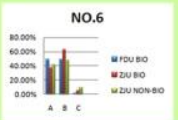
200 valid questionnaires were collected, including 150 from biology students in Fudan University (FDU), 19 from biology students in Zhejiang University (ZJU) and 31 from non-biology students in ZJU. The survey results are summarized as histograms, showing the variance between different schools and different majors.

Q6.What impact you think synthetic organisms would bring to the environment?

A. Synthetic organisms might have unpredictable interactions with natural life or environment,which probablyturns out to be a huge threat to the ecological balance.

B. Synthetic organisms have potential risks to the environment, but it could be avoided by technical means and effective oversight and restrictions

C. There would be no impact and we needn't worry



Part III: Meeting with academician Zhao Guoping in Chinese Academy of Sciences (CAS)

Attributions

All work presented on this poster is the sole work of the Fudan iGEM team members. We are also grateful for the guidances of our advisors.

Team members: Ruojun Wang, Ying Huang, Yang Zhou, Zhenchao Ran, Jingcheng Huang, Bohan Shao, Yi Zhang, Li Wang, Chao Zhang, Xiaoting Zhu, Wenjie Sheng, Yuantong Ding, Jingwei Sun, Ji Zhong, Cui Cui, Chen Dan, Yuancheng Lv

Instructors: Xiaoming Ding, Guoping Zhao, Ruoyu Luo

School of Life Science