Xiamen University

Intelligent Control of Cell Density in Bacteria

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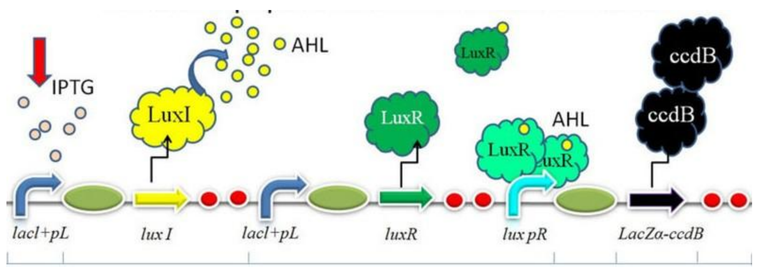
Project Description

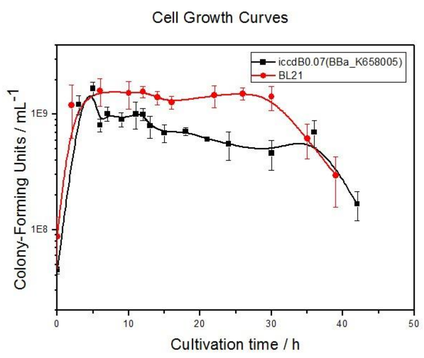
We have developed a series of devices which program a bacteria population to maintain at different cell densities. We have designed and characterized the genetic circuit to establish a bacterial 'population-control' device in *E. coli* based on the well-known quorum-sensing system from *Vibrio fischeri*, which autonomously regulates the density of an *E. coli* population. The cell density however is influenced by the expression levels of a killer gene (*ccdB*) in our device. As such, we have successfully controlled the expression levels of *ccdB* by using RBS of different strength and site-directed mutagenesis of a promoter (*lux pR).*This work can serve as a foundation for future advances involving fermentation industry.

iccdB



Proposed reaction mechanism of the iccdB

The synthesis of LuxR protein and the signalling molecule N-acyl-homoserine lactone (AHL) is induced by adding Isopropyl β-D-1-thiogala-ctopyranoside (IPTG). The AHL accumulates in the experimental medium and inside the cells as the cell density increases. At sufficiently high concentrations, it binds and activates the LuxR transcriptional regulator, which in turn induces the expression of a killer gene *ccdB* under the control of a promoter *lux pR*. Sufficiently high levels of the killer protein cause cell death. This circuit programs a bacterial population to maintain a cell density that is lower than the limits imposed by the environment.



The cell growth curves showed the bacteria population-control device successfully maintained the cell density at a lower value at the steady state compared with BL21's cells without this circuit.

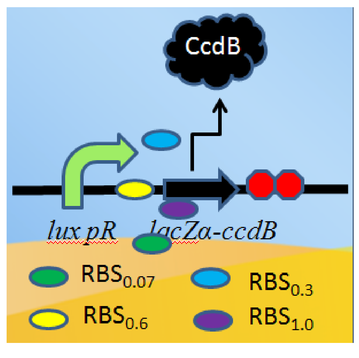
Besides, circuit-regulated cell growth (black dots) has a relatively longer steady state than cells without this circuit (red dots). For iccdB0.07 regulated cell growth, the viable cell density first start to decline around 8h and then reached a steady state after two minor oscillations. Compared with bacterium without this circuit, the iccdB0.07 regulated bacterium has a lower media-consuming rate due to its lower cell density. The iccdB0.07 regulated cell growth might be explained by "ON-OFF" mechanism based on the quorum - sensing system.

iccdB with different RBSes

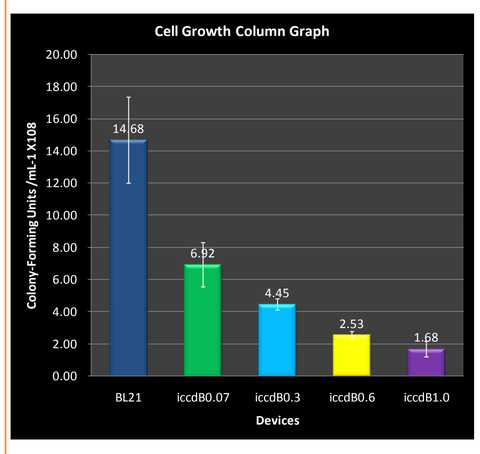
From the mechanism that for circuit-regulated growth, we know that the cell death rate is regarded proportional to the intracellular concentration of the killer protein.

The expression of the killer gene is regulated by the strength of its upstream RBS. Therefore, a RBS with higher strength promises more killer protein *in vivo*, which leads to a higher death rate of the bacteria population.

In order to control the expression of *ccdB* at different levels thus maintaining cells at different densities, we constructed a series of population-control devices with RBSes of different strength.



Performance



Fluorescence of four IR-GFP devices at 20h

The mechanism of the population-control devices is the same as iccdB0.07. they are different from the upstream RBS of *ccdB* gene are of different efficiency .

It illustrates that by using RBS of different strength in the population-control device, we were able to control the steady-state cell density of a bacteria population at different levels. And a population-control device with RBS of high strength results in a low steady-state cell density.

Modeling of Different RBSes

$$\frac{dN}{dt} = k_N N\left(1-\frac{N}{N_m}\right) - d_N E N \quad \cdots\cdots (1)$$

$$\frac{dE}{dt} = k_E A - d_E E \quad \cdots\cdots (2)$$

$$\frac{dA}{dt} = k_A N - d_A A \quad \cdots\cdots (3)$$

[Basic model from You L, Cox RS, Weiss R, Arnold FH. Programmed population control by cell-cell communication and regulated killing[J]. Nature, 2004, 428(6985): 868-871.]

$$\frac{1}{N_S} = \frac{d_N k_E k_A}{k_N d_A d_E} + \frac{1}{N_m}$$

N_S represents for steady-state cell density. Generally in circuit regulated growth

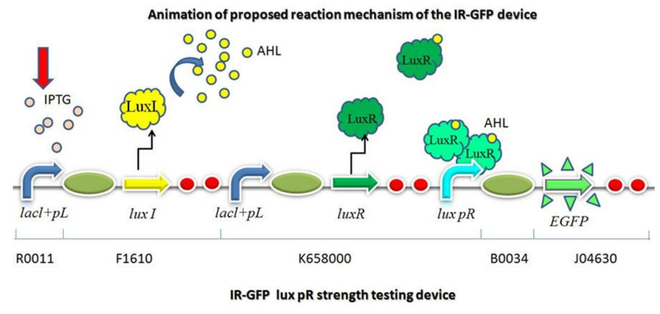
The experimentally measured value of $\frac{d_N k_E k_A}{k_N d_A d_E}$ from four population-control circuit is proportional to the strength of RBSes used in them respectively as shown below.

Table 1 the relationship between RBS efficiency and $\frac{d_N k_E k_A}{k_N d_A d_E}$

Name	RBS efficiency	$\frac{d_N k_E k_A}{k_N d_A d_E}$
BBa_B0031	0.07	0.0806C
BBa_B0032	0.3	0.336C
BBa_B0030	0.6	0.594C
BBa_B0034	1.0	C

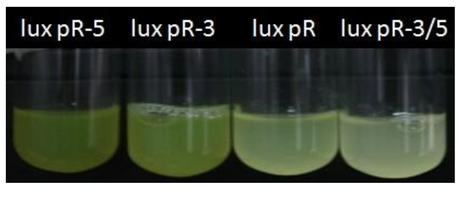
Mutations of *lux pR*

***lux pR* is another factor influencing expression of *ccdB* gene. Therefore, we used the method of site-directed mutagenesis to modify the promoter *lux pR*. we generated three point mutants by site-directed mutagenesis, designated *lux pR3*, *lux pR5* and *lux pR3/5*. In these mutants the C residue at position 3 was changed to a T, the G residue at position 5 was changed to a C ,position 5 and 3 were both changed.**



IR-GFP lux pR strength testing device

In order to test the strength of promoter *lux pR* and its mutants, we designed four devices (IR-GFP,IR-3-GFP,IR-5-GFP,IR-3/5-GFP).The mechanism is shown above.



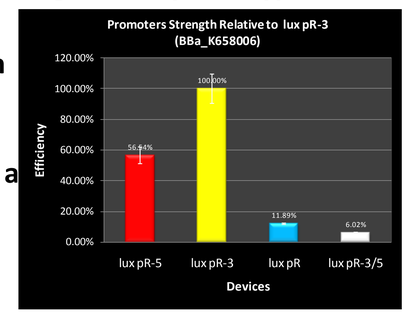
The strength of *lux pR* promoters were characterized by measuring the fluorescence of *E. coli* cells with IR-GFP

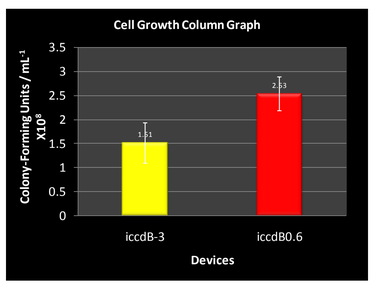
Acknowledgement

College of Chemistry & Chemical Engineering

Mutations of *lux pR*

As is shown below, promoter *lux pR-3* has the highest strength of the four. Mutation at position 3 might lower the threshold for the binding reaction between LuxR/AHL protein complex and promoter *lux pR*, which starts the quorum-sensing system at a relatively earlier period with a lower cell density compared with circuits regulated by wild type promoter *lux pR*. The earlier the QS system is started, the more GFP might be produced, leading to a higher fluorescence intensity at steady state.

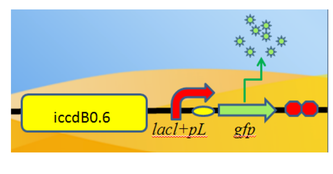




Average of experimentally measured cell densities of BL21's cells with iccdB0.6 and its mutant iccdB-3

The population-control device iccdB-3 programs a relatively lower steady-state cell density compared with iccdB0.6. This matched the result of the test on four *lux pR* promoters' strength.

Influence of iccdB on downstream genes

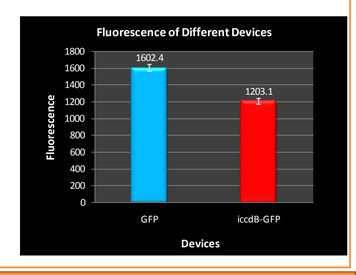


iccdB0.6-gfp device

We constructed the device iccdB-GFP to test the influence of iccdB on the expression of downstream gene. This part is made up of two subparts, a population-control device iccdB0.6 and an EGFP reporter.

The population-control device iccdB0.6 has the ability to maintain steady-state cell density at a relatively low value and extend the steady state in cell growth. The reporter EGFP's expression is regulated by the iccdB0.6 in upstream.

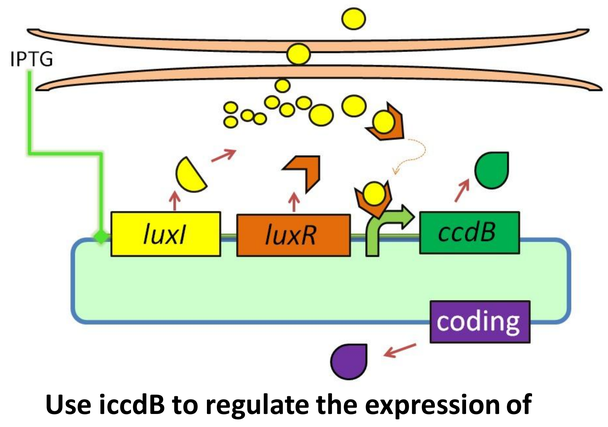
This device is an example of industrial use of population-control device.



Conclusion

- **Designed and characterized a series of bacteria population-control devices.**
- **Successfully improved the strength of *lux pR* by site-directed mutagenesis. Characterized the efficiency of promoters *lux pR* and its 3 mutants.**
- **Designed and characterized the device iccdB-GFP to test the influence of iccdB on downstream genes**

Future Plan



Use iccdB to regulate the expression of downstream genes and explore its industrial application, such as antibiotics and proteins fermentation.