Intelligent Control of Cell Density in Bacteria

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 RBSs 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.

icdB

The synthesis of LuxI protein and the signalling molecule N-acyl-homoserine lactone (AHL) is induced by adding isopropyl β-D-1-thiogalactopyranoside (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 cell without this circuit.

Besides, circuit-regulated cell growth (black dots) has a relatively longer steady state than cells without this circuit (red dots). For iccdB0.7 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 bacteria without this circuit, the iccdB0.7 regulated bacterium has a lower media consuming rate due to its lower cell density. The iccdB0.7 regulated cell growth might be explained by "ON-OFF" mechanism based on the quorum-sensing system.

icdB 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 promotes 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 strengths.

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.

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, and 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

Performance

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 RBSs 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

\[ \frac{dN_s}{dt} = \frac{k_{SN_s}}{N_s} - \frac{k_{SN_s}N_a}{N_a + k_{SN_a}} \]


N_s represents for steady-state cell density. Generally in circuit regulated growth, the experimentally measured value of lux pR from four population-control circuit is proportional to the strength of RBSes used in them respectively as shown below.

<table>
<thead>
<tr>
<th>RBS</th>
<th>lux pR 600</th>
<th>lux pR 800</th>
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</thead>
<tbody>
<tr>
<td>lux pR0.07</td>
<td>0.07</td>
<td>0.10</td>
</tr>
<tr>
<td>lux pR0.3</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>lux pR0.5</td>
<td>1.0</td>
<td>1.5</td>
</tr>
<tr>
<td>lux pR0.7</td>
<td>3.5</td>
<td>5.0</td>
</tr>
</tbody>
</table>

LuxR-CCdB-GFP devices

In order to perform the experimental data on the ICDB0.6, we successfully improved the strength of lux pR and its mutants. Designed and characterized the device iccdB-GFP to test the influence of iccdB on the expression of downstream genes. This device is an example of industrial use of population-control device.

Future Plan

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