2013 XMU-China iGEM

A SynBio Oscillation Signal Converter
Background

Digital Signal

Powerful

Precisely
Background
Oscillation in Synthetic biology

Background
**Mechanism**

**MAGIC circuit**

**Coupling among colonies**

**Oscillator**
- **luxI**
- **ndh**

**Reporter**
- **sfGFP**
- **luxR-AHL**

**Coupling**
- **AHL**
- **H$_2$O$_2$**

**Quorum Sensing**
- **ArcAB**
- **lux pR**

**Lux pl**
- **R0062**
- **B0034**

**LuxR**
- **C0062**
- **B0010**
- **B0012**
- **Lux pR**
- **R0062**
Welcome Conductor Catalase!

Mechanism

E. coli

A controllable promoter

catalase

H₂O₂

H₂O

Catalase

O₂
To realize this of *E. coli*, we constructed...

<table>
<thead>
<tr>
<th>No.</th>
<th>Plasmid</th>
<th>Backbone</th>
<th>Copy Origin</th>
<th>Copy number</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>pSB1C3-gfp-luxI</td>
<td>pSB1C3</td>
<td>High (100~300)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RBS B0034</td>
<td>pMB1</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>luxI-LVA C0061</td>
<td>p3H</td>
<td></td>
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<td></td>
<td></td>
<td>ColE1</td>
<td>Middle (18~22)</td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>pSB1C3-sfgfp-luxI</td>
<td>pSB1C3</td>
<td>High (100~300)</td>
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<tr>
<td></td>
<td>RBS B0034</td>
<td>pMB1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>sfgfp-LVA J04031</td>
<td>p3H</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ColE1</td>
<td>Middle (18~22)</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>pSB3T5-aiiA</td>
<td>pSB3T5</td>
<td>Middle (10~12)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RBS B0034</td>
<td>p15A</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>aiiA-LVA C0060</td>
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<tr>
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<td></td>
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</tr>
<tr>
<td>C</td>
<td>pSB4K5-ndh</td>
<td>pSB4K5</td>
<td>Low (5)</td>
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</tr>
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<td>RBS B0034</td>
<td>pSC101</td>
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<tr>
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<td>ndh-LVA</td>
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</table>
## Oscillator: A & B plasmids

<table>
<thead>
<tr>
<th>NO.</th>
<th>Plasmids</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>pSB1C3-gfp/sfgfp-luxI</td>
</tr>
<tr>
<td></td>
<td>pSB3T5-aiiA</td>
</tr>
</tbody>
</table>

### Circuit Construction

#### NO. | Parts Submitted
--- | ---
1 | BBa 1036002  
2 | BBa 1036003  

#### NO. | Parts Used
--- | ---
1 | BBa_K546000  
2 | BBa_I763020  
3 | BBa_F2621  
4 | BBa_K546001  

---

[Diagram of Oscillator: A & B plasmids]

- **Circuit Construction**
- **NO.**
- **Plasmids**
- **Parts Submitted**
- **Parts Used**
### Coupling: C plasmid

#### NO. | Plasmids
---|---
C | pSB4K5-ndh

![Diagram](image)

#### Parts Submitted

<table>
<thead>
<tr>
<th>NO.</th>
<th>Parts Submitted</th>
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</thead>
<tbody>
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<td>1</td>
<td>BBa_K1036000</td>
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<td>2</td>
<td>BBa_K1036001</td>
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</tbody>
</table>

#### Parts Used

<table>
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<th>NO.</th>
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<tbody>
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<td>3</td>
<td>BBa_K546001</td>
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</tbody>
</table>
Microfluidic

(A) Swimming Pool Model

Advantage:
High AHL concentration

Disadvantages:
• Hard to control
• Increasing RFU baseline
Microfluidic

(B) Passage Model
Oscillation in a single colony of BL21(DE3)

Circuit: pSB1C3-gfp-luxI & pSB3T5-aiiA plasmids

(A) Swimming-pool

(B) Passage

Results
## Host casting

<table>
<thead>
<tr>
<th>Strain</th>
<th>Type</th>
<th>Growing Rate</th>
<th>Other Reasons we choose it</th>
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<tbody>
<tr>
<td>BL21 (DE3)</td>
<td>B</td>
<td>Faster</td>
<td>1) Competent in transformation and protein expression; 2) <em>ndh</em> gene is originally expressed in BL21 (DE3)</td>
</tr>
<tr>
<td>DH5α</td>
<td>K</td>
<td>Slow</td>
<td>Belong to K strain as MG1655</td>
</tr>
<tr>
<td>BL21 (wild type)</td>
<td>B</td>
<td>Fastest</td>
<td>A BL21 strain with less artificial modification</td>
</tr>
</tbody>
</table>
Weakness of DH5α

Results

<table>
<thead>
<tr>
<th></th>
<th>gfp-aiiA-ndh</th>
<th>gfp-aiiA</th>
<th>pSB1C3-gfp</th>
<th>DH5α</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>S</td>
<td>P</td>
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<tr>
<td>25</td>
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</tr>
</tbody>
</table>

P-Precipitate
S-Supernatant

Ndh  AiiA  GFP  LuxI
Results

Oscillation in Microfluidic of BL21(WT)

High Copy Numbers

\[
\text{(A) } pSB1C3\text{-gfp-luxI } \& \text{ pSB3T5-aiiA}
\]

Middle Copy Numbers

\[
\text{(B) } p3H\text{-gfp-luxI } \& \text{ pSB3T5-aiiA}
\]
Oscillation Phenomenon of BL21(WT)

(A) BL21, p3H-gfp-luxI / BL21, pSB1C3-gfp-luxI / BL21

(B) BL21, p3H-gfp-aiiA/BL21, pSB1C3-gfp-aiiA/BL21
Confirm most suitable microfluidic parameters for oscillation.

Amplify the effect of different copy numbers

Complete the comparison of different strains’ effect on oscillation

Confirm that every part is functioning as expected (i.e. LVA-tag).
Figure 1. Delay differential equations (DDE)

What is DDE?

DDE is short for delay differential equation.

\[
\frac{\partial A}{\partial t} = C_A [1 - (d / d_0)^4] P(\alpha, \tau) - \frac{\lambda_A A}{1 + f(A + I)}
\]

\[
\frac{\partial I}{\partial t} = C_I [1 - (d / d_0)^4] P(\alpha, \tau) - \frac{\gamma_A A}{1 + f(A + I)}
\]

\[
\delta + \alpha \left( H_i(t - \tau) \right)^2 \quad \frac{1}{1 + k_1 \left( H_i(t - \tau) \right)^2}
\]

MATLAB

DDE can perfectly simulate our circuit.

Figure 2. Hill function
Figure 3. The concentration change of the four main proteins

Figure 4. The fluorescence change of each trap
So, modeling is finished Isn’t it?

NO!

Help me

So easy

OK...
I will make a GUI user interface
Figure 5. The main user interface of Gene OS

Modeling & Software

Optimize the experiment conditions

Noisy system

Help message is here
Figure 6. Gene Oscillate Simulator

Gene OS
An own-designed Image process software

Humanized & intelligent

Quick & efficient

ImageMe
Figure 7. The main user interface of ImageMe

Simpler and easier.
Figure 8. One example image of microfluidic

Figure 9. The software output the image after edge processing

Edge Detection

Figure 10. The fluorescence change in the same position calculated by ImageMe using gray level integration

Figure 11. The fluorescence change

Gray Level integration

Figure 12. The results of test images given by ImageMe
Modeling & Software

Gene OS

ImageMe

Designed only for the experiments and solve the problems we met.
Applications

Traditional methods

Physical method

Chemical method

Cancer Cell
How to combine diagnosis and treatment together?

Synthetic Biology?
Applications

Periodical protein synthesis

• What if we change our GFP to a certain toxalbumin?

May be we can realize periodical toxalbumin synthesis which will be more gentle for cancerous person.

1. Lecture
2. Campus Fair
3. Collaboration
Outreach

Games
Outreach

Bio bang

A change of the nucleotide sequence of the genome.

In his turn, he draws 2 cards...

Biosafety: Every biological equipment should be autoclaved before reused.
Outreach

fresco in Furong Tunnel
Outreach
Acknowledgement

Prof. Hasty and his student Arthur Prindle, Department of Bioengineering, University of California, San Diego, La Jolla, California. Graduates in Lab 571, Lab 580 and Lab 119, College of Chemistry and Chemical Engineering.
Prof. Ningshao Xia and his graduates, School of Life Sciences, Xiamen University.
Prof. Shoufa Han and his graduates Institute of Urban Environment, Chinese Academy of Sciences. Fujian Chemical Society and Prof. Yihui Chen.
High school students from all over Fujian Province taking part in our Human Practice.
Chinese University of HK
Peking iGEM 2013
XMU-Software
Thanks for your attention!