Outline

The Project
1. Design & Construction of the O-Key System
2. Regulation of the metabolism networks by O-Key System
3. BioBricks submitted to the Registry
4. Potential Combination of O-Key System with other SynBio technologies

Human Practice
1. Collaboration
2. SynBio Museum & Lectures
3. Biosafety Handbook
4. HP Modeling
Part 1
Design & Construction of the O-Key system
Design & Construction of the O-Key system

Project

mRNA

50S subunit

30S subunit

Shine-Dalgarino (SD), RBS

AGGAGG

UCCUCC

Anti-Shine-Dalgarino (ASD)

ΔG↑
O-Key System

Modified SD

AUGCGGG

UACGCC

Modified ASD

O-Lock

mRNA

AUG

50S subunit

30S subunit

O-Key
O-Key: an orthogonal system

N-RBS

N-16S

O-Lock (O-RBS)

O-Key (O-16S)
Design & Construction of the O-Key system

Project

N-RBS

1

N-16S

O-Lock
(O-RBS)

O-Key
(O-16S)

50s subunit

Native RBS

A G G A G G

U C C U C C

Native ASD Sequence

A U G

30s subunit
Design & Construction of the O-Key system

Project

Native RBS

AUG

AGG

AUG

AGG

30s subunit

CAGGGC

O-Key

N-RBS

O-Lock (O-RBS)

N-16S

O-Key (O-16S)

O-16S

N-RBS

O-Lock (O-RBS)
Orthogonal RBS

N-RBS

O-Lock (O-RBS)

3

N-16S

O-Key (O-16S)

Native ASD Sequence

U C C U C C C

30s subunit

A U G

G U U C C G

Project

Design & Construction of the O-Key system
Design & Construction of the O-Key system

- N-RBS
- O-Lock (O-RBS)
- N-16S

Orthogonal RBS

G U U C C G

30s subunit
C A A G G C

Orthogonal ASD Sequence

A U G

50s subunit

Project

- O-Key (O-16S)
Theoretical basis of Model: RBS calculator

\[ \Delta G_{\text{tot}} = \Delta G_{\text{mRNA:rRNA}} + \Delta G_{\text{start}} + \Delta G_{\text{spacing}} - \Delta G_{\text{standby}} - \Delta G_{\text{mRNA}} \]

\[ E = k \cdot m \cdot R \cdot \exp(-\beta \Delta G_{\text{tot}}) \]

- k: system parameter
- m: the number of mRNA transcript
- R: the number of ribosomes
- \( \beta \): the apparent Boltzmann constant
- \( \Delta G \): the Gibbs free energy change

Design & Construction of the O-Key system

Prediction of single O-Key system by modeling

- **Project Design & Construction of the O-Key system**
  - N-16S~N-RBS
  - N-16S~O-RBS
  - O-16S~N-RBS
  - O-16S~O-RBS

**Graph:**
- **Relative Fluorescence/OD (600nm)**
- **Bars:**
  - N-16S
  - O-16S
  - N-RBS
  - O-RBS

**Legend:**
- N-16S~N-RBS
- N-16S~O-RBS
- O-16S~N-RBS
- O-16S~O-RBS
Gene-circuit of the O-Key System

BBa_J04450

BBa_K82001

BBa_K821012
## Design & Construction of the O-Key system

### Project

### Table

<table>
<thead>
<tr>
<th>Group</th>
<th>RFP-Plasmid</th>
<th>O-16S-Plasmid</th>
<th>Result</th>
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<td>1</td>
<td>N-RBS</td>
<td>N/A</td>
<td>Expression</td>
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<tr>
<td>3</td>
<td>O-RBS</td>
<td>Pbad+O-16S</td>
<td>N/A</td>
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<tr>
<td></td>
<td></td>
<td>(No induced)</td>
<td></td>
</tr>
<tr>
<td>3’</td>
<td>O-RBS</td>
<td>N/A</td>
<td>N/A</td>
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<tr>
<td>4</td>
<td>O-RBS</td>
<td>Pbad+O-16S</td>
<td>Expression</td>
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<td></td>
<td></td>
<td>(induced)</td>
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### Diagram

- **N-RBS**
- **O-16S**
- **O-Lock (O-RBS)**
- **O-Key (O-16S)**

### Part:
- **BBa_K821001**
- **BBa_K821012**
Result of Experiment

- **N-RBS**
- **O-Lock (O-RBS)**
- **N-16S**
- **O-Key (O-16S)**

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<th>Experiment</th>
<th>Relative Fluorescence/OD(600nm)</th>
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<td>3</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>120</td>
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Design & Construction of the O-Key system

Mutate

BBa_K8210012
### Design & Construction of the O-Key system

#### Project

![Image of petri dishes showing bacterial colonies](image)

<table>
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<tr>
<th>Group</th>
<th>PSB1A3-RFP</th>
<th>O-16S</th>
<th>Result</th>
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<td>1(left)</td>
<td>O-RBS + AmpR</td>
<td>N/A</td>
<td>No colony</td>
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<tr>
<td>2(right)</td>
<td>O-RBS + AmpR</td>
<td>O-16S</td>
<td>Red colony</td>
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**Part:** BBa_K821002
Prediction of Two O-Key system by modeling

Relative Fluorescence/OD(600nm) vs. n-16s, o1-16s, o2-16s, n-RBS, o1-RBS, o2-RBS
We have:

- Designed & constructed the O-Key System
- Predicted the orthogonality using modeling
- Characterized the orthogonality in the wet lab
- Installed the O-Lock in front of the Ampr resistance gene
- Constructed three orthogonal BioBricks and sent to the Registry
Part 2
Regulation of the Metabolism Network by O-Key System
Regulation of the Metabolism Network

Cell native control transcription

O-Key
O-Lock

AND

Protein
N-mRNA to O-mRNA

- Synthetize O-genomics or O-plasmids
  - RBS mutation in plasmid
  - Multiplex automated genome engineering (MAGE)

Project: Regulation of the Metabolism Network
Regulation of the Metabolism Network

Manual control

O-Key

O-Lock

Cell native control transcription

AND

Protein
Manual Control

- Constitutive promoter
- Chemical-inducible systems
- Temperature-inducible systems
- Quorum sensing systems
Single O-Key system

Open to Close
Single O-Key system

Close to Open
Double O-Key system

Switch flux to only product C
Double O-Key system

Switch flux only to right
Double O-Key system

Switch flux from right to left
The metabolic pathway of the violacein
Regulation of the Metabolism Network

Experiment 1
Normal metabolic pathway of violacein

The product is purple
Regulation of the Metabolism Network Project

Part: BBa_K821012

The Product is green
Regulation of the Metabolism Network

Part: BBa_K821012
We have:

- Successfully constructed the violacein pathway using yeast assembler
- Demonstrated the regulation role of O-Key System
- Designed various metabolism regulation modules
Part 3
BioBricks submitted to the Registry
We have:

**Tianjin 2012 iGEM Team Parts**

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<td>Jinlai Zhang, Dongchang Qin</td>
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**Tianjin 2012 iGEM Team Parts Sandbox**

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<td>BBA_K821009</td>
<td>RBS</td>
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<td>BBA_K821010</td>
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<td>PLacI+Native RBS+RFP+Native RBS+GFP+Terminator</td>
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<td>BBA_K821011</td>
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<td>Jinlai Zhang, Dongchang Qin</td>
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We have improved:

BBa_J04450: RFP Coding Device

BBa_K821001: RFP with O-Key
We have reconstructed:

BBa_K274004, BBa_K274003: violacein operon
We have constructed:

BBa_K821012: pBad+orthogonal 16S RBS,

BBa_K821002: orthogonal 16S DNA sequence from rrnB
Part 4
Potential Combination of O-Key System with other SynBio technologies
Replacement of RBS in plasmid

1. PCR
2. Transform E.coli
3. In vivo exonuclease digestion
4. Extract Plasmids
5. In vivo cyclizing
Assembling DNA constructs by Yeast

1. Transform Yeast
2. In vivo assemble
3. Extract Plasmids
4. Extract Plasmids
5. Transform E.coli
Global introduction of O-Lock by MAGE

Genomic

AATTTCACACATAGGAGGTACTAGATGGCTTCC
N-RBS

AATTTCACACATGTTCCGTAATAGATGGCTTCC
O-RBS

Continually evolving cell populations

Diverse genomes

Synthetic DNA

...ACNNNTCNNCTCNNNA...
Human Practice
Collaboration

**Peking University:** lab visiting, discussion session

**SUST:** Parts Registry software development

**Nanjing University:** iGEM survey and questionnaire
SynBio Museum of China
Human Practice
SynBio Lecture for Nankai Student
Biosafety Handbook & Summary

• Operation Part
• Social Part
• SynBio Part
• Transcriptional Regulation
• RNA Process
• Translational Control
• Stability of mRNA
• DNA Rearrangement
HP MODELING

Proposing
Risk Assessment
Environment Problem Evaluation

Analysing
Influences on Public’s Attitude to Genetic Pollution

Solution
iGEM Project Evaluation
Optimum Propagation
Advisor

Professor
Yingjin Yuan

Supported by:

The Key Laboratory of Systems Biomechanics
Tianjin University International Cooperation Office
Tianjin University Jiankun Foundation
Thank you for your time!