Bank *E. coli* as an Educational Tool
Integrated Project

Policy & Practices

Outline of our activities

Realized our lack of knowledge on economics from general public

Received Advice from entrepreneurs

Modeling
Wet Lab
Modeling
Wet Lab
Modeling

Evaluated by biology students
Important questions beyond the bench

**Policy & Practices**

Interaction with general public on preliminary ideas

Isn’t this lack of knowledge common to many iGEMers?

An educational tool for economics using *E. coli* will be perfect for iGEMers!
The aim of our educational tool

Bank *E. coli*
emulate economic system
by using quorum sensing of AHL

An educational tool
for economics using *E. coli*
will be perfect for iGEMers!
Our educational tool contains 3 characters

Bank E. coli

Company

Customer
Overview of our Science and Engineering

Mutualism between Company and Customer

Regulation of money supply by Bank

Introduction of an economic wave to our model
First Section (Mutualism)

- Genetic circuit design of Company and Customer
- Improvement of Prhl promoter
- The experimental result of mutualism
What do Company and Customer do?
In the presence of C4, Company produces C12
Company cannot survive without C4
In the presence of C12, Customer produces C4.
Without C12, Customer cannot survive.
Company and Customer are mutually dependent.
Achievement (Mutualism)

Mutualism between Company and Customer

- Genetic circuit design of Company and Customer
- Improvement of Prhl promoter
- The experimental result of mutualism
Modeling our Company and Customer

\[
\frac{d}{dt} P_{co} = k \left( 1 - \frac{P_b + P_{co} + P_{cu}}{P_{max}} \right) P_{co} - \gamma P_{co} ([Cm] - \eta_{cm} [CmR_{co}])
\]

\[
\frac{d}{dt} [LasI_{co}] = \text{leak} + \frac{\alpha [C4]^n}{K^n + [C4]^n} - d[LasI_{co}]
\]

\[
\frac{d}{dt} [CmR_{co}] = \text{leak} + \frac{\alpha [C4]^n}{K^n + [C4]^n} - d[CmR_{co}]
\]

\[
\frac{d}{dt} P_{cu} = k \left( 1 - \frac{P_b + P_{co} + P_{cu}}{P_{max}} \right) P_{cu} - \gamma P_{cu} ([Cm] - \eta_{cm} [CmR_{cu}])
\]

\[
\frac{d}{dt} [RhlI_{cu}] = \text{leak} + \frac{\alpha [C12]^n}{K^n + [C12]^n} - d[RhlI_{cu}]
\]

\[
\frac{d}{dt} [CmR_{cu}] = \text{leak} + \frac{\alpha [C12]^n}{K^n + [C12]^n} - d[CmR_{cu}]
\]

\[
\frac{d}{dt} [C4] = k_1 [RhlI_b] P_b + k_2 [RhlI_{cu}] P_{cu} - \frac{\beta [AiiA_b][C6]}{K + [C6]} P_b - d[C4]
\]

\[
\frac{d}{dt} [C12] = k [LasI_{co}] P_{co} - \frac{\beta [AiiA_b][C4]}{K + [C4]} P_b - d[C4]
\]

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Hu et al., 2010, PLoS ONE

Modeling by Takuya

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Improvement of Prhl promoter was required

- **Modeling**
  - Done by Takuya
  - Mutualism
  - No Mutualism

- **Ideal strength**
  - \[ \text{Prhl strength} = \text{Plux} \]
Improvement of Prhl promoter was required

### Modeling
Done by Takuya

**Mutualism**

**No Mutualism**

---

Plux, Prhl reporter assay

**Wet Lab**

---

**Ideal strength**

**Prhl strength**

**Prhl** = **Plux**
Improvement of Prhl promoter was required

Modeling

Done by Takuya

Mutualism
No Mutualism

Prhl strength
Plux strength

Ideal strength

Prhl = Prhl

Plux

Prhl

Plux

Fluorescence Intensity (a.u.)

Result

Low

Ideal strength

Prhl

Plux

591

C4 - +

C12 - +

Promoter

Prhl (R0071)

Prhl (R0062)

Done by Naoto, Keina and Ayaka
Improvement of Prhl promoter was required

Modeling

Done by Takuya

Mutualism

No Mutualism

Ideal strength

Prhl strength

Prhl = Plux

Result

Improved !!

Prhl(RR)

Prhl(LR)

Prhl(RL)

Low
Design of Prhl promoters we improved

We changed **LuxR** binding sites of $P_{\text{lux}}$ to **RhlR** binding sites

![Diagram showing design of Prhl promoters with BBa_K1529320, BBa_K1529310, and BBa_K1529300 with LuxR and RhlR binding sites and their promoters Prhl(RR), Prhl(LR), and Prhl(RL).]

*Grey et al., 1994, Journal of Bacteriology*

*Chuang et al., 2009, SCIENCE*
Prhl(RL) : Higher expression with few leaks

Relative fluorescence intensity

Fluorescence intensity (a.u.)

C4HSL

Promoter

Prhl
Prhl(RR)
Prhl(LR)
Prhl(RL)

Positive Control
Negative Control

Prhl(RL)

Wet Lab

Done by Naoto, Keina and Ayaka
Achievement (Mutualism)

Mutualism between Company and Customer

- Genetic circuit design of Company and Customer
- Improvement of Prhl promoter
  
  Designed Prhl(RL) promoter

- The experimental results of mutualism

$BBa\_K1529300$
1: C4HSL-dependent Company growth

**Wet Lab**
Done by Shoko, Naoto and Keina

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**Company**

- **C4**
  - pSB6A1
  - **gfp**
  - **P_{tet}**
  - **rhlR**

- **New part**
  - **BBa_K1529302**
  - pSB3K3
  - **Prhl(RL)**
  - **cmR**
  - **lasI**

---

**OD_{590}**

- **C4HSL(+)**
  - **Prhl(RL)-CmR-LasI**
  - Positive Control
  - Negative Control

- **C4HSL(-)**
  - **Prhl(RL)-CmR-LasI**
  - Positive Control
  - Negative Control

---

**Time (min)**

- 0
- 200
- 400
- 600

---

**Antibiotics**

- **Cm**
  - 100 µg / mL
2: C4HSL-dependent C12HSL Production – Reporter Assay

- **C4HSL**
  - **Sender**: Prhl(RL)_CmR_LasI
  - **Authentic 3OC12HSL**: 265
  - **DMSO**: 7

- **C12**
  - **Company**
    - **pSB6A1**
      - **P_{tet}**
        - **gfp**
        - **rhIR**
  - **Reporter**
    - **pSB6A1**
      - **P_{trc}**
        - **lasR**
    - **pSB3K3**
      - **P_{las}**
        - **gfp**

Done by Shoko, Naoto and Keina
3: 3OC12HSL-dependent Customer growth

3OC12HSL(+)  
- Plux-CmR-RhlI  
- Positive Control

3OC12HSL(−)  
- Plux-CmR-RhlI  
- Positive Control

Wet Lab  
Done by Shoko, Naoto and Keina

Wet Lab Experiment

100 µg / mL

Cm Antibiotics

Customer

New part  
BBa_K1529797
pSB3K3

Plux  
cmR  
rhlI

Plac  
rfp

P_{tet}  
luxR

P_{lux}
4: C12HSL-dependent C4HSL Production – Reporter Assay

- **Sender**: Plux_CmR_RhII
- **Authentic C4HSL**: 304
- **DMSO**: 4

**Fluorescence Intensity of reporter (a.u.)**

- C4HSL
- **C12**

**Reporter**
- pSB6A1
- P_{tet} rhlR
- P_{lux} gfp

**Customer**
- pSB6A1
- P_{tet} luxR
- Plac rfp
- New part BBa_K1529797
- P_{lux} cmR
- P_{lux} rhlII

**Wet Lab Experiment**
Done by Shoko, Naoto and Keina
5: Co-culture experiment confirmed mutualism

Done by Yuya and Gyomon

---

**Company**

- BBa_K1529302
  - pSB6A1
  - P_{tet} → gfp
  - P_{tet} → rhlR

**Customer**

- BBa_K1529797
  - pSB3K3
  - P_{lux} → cmR
  - rhII

---

Initial OD<sub>590</sub>:
- Company: 0.05
- Customer: 0.02

Final OD<sub>590</sub>:
- Company: 0.24
- Customer: 0.53

Cell Count:
- FL1-H

---

**Wet Lab Experiment**

- Initial OD<sub>590</sub>:
  - 0.06 → 0.02

---

**5: Co-culture experiment confirmed mutualism**

**Done by Yuya and Gyomon**

**Company**

- BBa_K1529302
  - pSB6A1
  - P_{tet} → gfp
  - P_{tet} → rhlR

**Customer**

- BBa_K1529797
  - pSB3K3
  - P_{lux} → cmR
  - rhII
Achievement (Mutualism)

Mutualism between Company and Customer

- Genetic circuit design of Company and Customer
- Improvement of Prhl promoter
- The experimental result of mutualism

Designed Prhl(RL) promoter \((\text{BBa\_K1529300})\)

Two new parts \((\text{BBa\_K1529302,K1529797})\)
Next Section (Addition of Bank)

Mutualism between Company and Customer

Regulation of money supply by Bank

Introduction of an economic wave to our model
Making mutualism feasible on a small scale
Making mutualism feasible on a small scale
Problem: The economy is enviable on small scale.

Low initial cell concentration

High initial cell concentration

Decrease the initial cell concentration
Co-culturing with various initial cell concentrations

**Low** initial cell concentration

- Population (OD$_{590}$)
  - Time (min)

**High** initial cell concentration

- Population (OD$_{590}$)
  - Time (min)

**Amplification of**

**OD(co-culture) / OD(mono culture)**

**Initial amount (total) (µL)**

- Low
- High

**Wet Lab**
Done by Yuya and Gyomon

**Modeling**

Done by Takuya
Bank regulations the money supply

Bank

Company

Customer

CmR

LasI

C4HSL

3OC12HSL

AiiA

TetR

LacI

RhlI

RhIR

CmR

LuxR

RhlII
Bank regulates the money supply

Gardner et al., 2000, Nature
Sekine et al., 2011, PNAS
Bank regulates the money supply

Collection State

Distribution State

P_{con} \rightarrow rhIR

aiiA \rightarrow tetR \rightarrow P_{lux/lac} \rightarrow P_{tet} \rightarrow lacI \rightarrow rhII
Distribution: RhII produces C4

Collection State

Excess money supply

Distribution State

P_{con} \rightarrow rhIR

P_{lux/lac} \rightarrow aiiA, tetR

C4

P_{tet} \rightarrow lacI, rhII

$\rightarrow$
Change to Collection State

Collection State

Distribution State

$\text{P}_{\text{con}}$ \text{rhlR} \text{P}_{\text{lux/lac}}$

$\text{aiiA}$ \text{tetR} \text{P}_{\text{tet}}$

$\text{C}4$ \text{lacI rhlII}$

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Collection State

- $P_{\text{con}}$
- rhlR
- P
- aiIA
- tetR
- $P_{\text{lux/lac}}$

Distribution State

- C4
- $P_{\text{tet}}$
- lacI
- rhII
Collection : AiiA degrades C4

Insufficient money supply

Collection State

Distribution State

\[ \text{P}_{\text{cor}} \rightarrow \text{rhIR} \rightarrow \text{aiiA} \rightarrow \text{P}_{\text{lux/lac}} \]

\[ \text{C4} \rightarrow \text{P}_{\text{tet}} \]

\[ \text{P}_{\text{tet}} \rightarrow \text{lacI} \rightarrow \text{rhII} \]
Change to Distribution State

Collection State

- $P_{con}$
- $rhIR$
- $aIIA$
- $tetR$
- $P_{lux/lac}$

Distribution State

- $C4$
- $P_{tet}$
- $lacI$
- $rhlII$

Change to Distribution State
Modeling including Bank

Modeling

Done by Takuya

Hu et al., 2010, PLoS ONE
Sekine et al., 2011, PNAS

Bank

- AiIA
- TetR
- LacI
- RhlI

Company

- RhlR
- CmR
- LasI

Customer

- CmR
- RhlI

Modeling done by Takuya Hu et al., 2010, PLoS ONE
Sekine et al., 2011, PNAS
C4 concentration decides the state of Bank

Modeling

Done by Takuya

C4 concentration low to high
C4 concentration high to low

Mono

Bi

Mono

C4 concentration (nM)

LacI concentration (nM)
Bank rescued Company and Customer from dying

High initial cell concentration

Low initial cell concentration

Decrease the initial cell concentration

Modeling

Done by Takuya
Introducing Bank Low initial cell concentration

High initial cell concentration

Decrease the initial cell concentration

No Bank

Bank rescued Company and Customer from dying

Modeling Done by Takuya

Introducing Bank
The economy is enviable on a small scale

Regulation of money supply by Bank

Enable mutualism on a small scale
Next Section (Economic wave)

Mutualism between Company and Customer

Regulation of money supply by Bank

Introduction of an economic wave to our model
Entrepreneurs introduced a new approach

Important question beyond the bench

How can we make our economic system more realistic?
We introduced economic wave into our scenario

Money supply in external market is always changing

Hokusai, 1831
Economic wave destroys the mutualism

Introducing Wave

Modeling
Done by Takuya
Introducing Wave

Introducing Bank
In some conditions, Bank turns to dark side

Modeling

Done by Takuya

In some conditions, Bank turns to dark side

Introducing Wave

Introducing Bank
Evaluation of our educational tool

Evaluated by undergraduate colleagues majoring in biology

Do you know the function of a central bank?

I don’t know the system of central bank

I realized the system of central bank

Very well ▢ ▢ ▢ ▢ ▢ Moderately ▢ ▢ ▢ ▢ Slightly ▢ ▢ ▢ Little ▢ ▢ ▢ Not at all

Important question beyond the bench

Is educational tool using synthetic biology effective to biology students?

The answer is Yes!
Integrated Project

Policy & Practices

Realized our lack of knowledge on economics from general public

Received Advice from entrepreneurs

Evaluated by biology students

Completed making education tool !!!
Achievements (Science and Engineering)

✓ Emulation of the economic system with *E. coli*

→ Mutualism between **Company** and **Customer**

→ Money supply regulation by Bank (and disoperation of Bank)

✓ Promoter with increased strength and less leaky
Our big dream is to allow *E. coli* to obtain "sociability".

Future Work: adaptation to other education

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Cysteine

Kerner et al., 2012, PLoS ONE
Gaitonde, 1967, Biochem.J.
Wiriyathanawudhiwong et al., 2009, Appl Microbiol Biotechnol
Other Achievements

We upgraded an iGEM card game

Introduced the Fun of Bioscience to schoolchildren

Article of our team interview in a scientific journal
Thank you
Supplement
Genetic circuit design not avoiding Crosstalk

- AiiA
- TetR
- LacI
- RhlI
- RhlR
- C4HSL
- LuxI
- CmR
- LuxR
- 3OC6HSL
- Bank
- Company
- Customer

Money flow and molecular interactions:
- $\rightarrow$ C4HSL
- C4HSL \rightarrow 3OC6HSL
- 3OC6HSL \rightarrow$
Genetic circuit design avoiding Crosstalk

Company

- RhIR
- CmR
- LasI

Bank

- AiIA
- TetR
- LacI
- RhlI
- RhIR

Customer

- CmR
- RhlI
- LuxR

C4HSL

3OC12HSL
Mechanism of action of Chloramphenicol

Daniel N. W.  Nature Reviews Microbiology 2014; 12: 35–48
Strength levels equality of Prhl and Plux derivation
Modeling with more realistic parameters

Demand for Prhl promter increase
Final population dependency for Prhl and Plux

Company

Customer

Prhl strength

Plux strength

Prhl strength

log10(a_{Plux})

log10(a_{P})
Difference between LuxR and RhlR

The upper structure is different, so LuxR and RhlR bind to different autoinducer.

The bottom structure is similar, so LuxR and RhlR can both bind to Lux Box.

<table>
<thead>
<tr>
<th></th>
<th>C_4</th>
<th>C_6</th>
<th>C_12</th>
</tr>
</thead>
<tbody>
<tr>
<td>LuxR</td>
<td>×</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>RhlR</td>
<td>○</td>
<td>○</td>
<td>×</td>
</tr>
</tbody>
</table>
Design of Customer and Company

Company

<table>
<thead>
<tr>
<th>Promoter</th>
<th>Rhl(RL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptor</td>
<td>Lux(C₆)</td>
</tr>
<tr>
<td>Inducer</td>
<td>Rhl(C₄)</td>
</tr>
</tbody>
</table>

Customer

<table>
<thead>
<tr>
<th>Promoter</th>
<th>Lux</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptor</td>
<td>Rhl(C₄)</td>
</tr>
<tr>
<td>Inducer</td>
<td>Las(C₁₂)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>bindable</th>
<th>unbindable</th>
</tr>
</thead>
<tbody>
<tr>
<td>binding capacity</td>
<td></td>
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<table>
<thead>
<tr>
<th></th>
<th>C₄</th>
<th>C₆</th>
<th>C₁₂</th>
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<td>○</td>
</tr>
<tr>
<td>RhlR</td>
<td>○</td>
<td>○</td>
<td>×</td>
</tr>
</tbody>
</table>
How to improve rhl promoter

Chuang et al., 2009, SCIENCE
The reason we made Prhl(RR)

**A**

- **Lux Box1**
  - acctgtagga

- **Lux Box2**
  - tcgtacaggt

- **-35**
  - ttacgcaagaaatggttggtatagtcgaataaa

- **-10**

**P_{lux}**

- **Chuang et al., 2009, SCIENCE**

- **Rhl Box1**
  - tcctgtgaaa

- **Rhl Box2**
  - tctggcagtt

- **-35**
  - ttacgcaagaaatggttggtatagtcgaataaa

- **-10**

**P_{rhl(RR)}**

**B**

**Fluorescence Intensity (a.u.)**

- **C4HSL**
  - **Prhl**
    - -
    - +
  - **Prhl(RR)**
    - -
    - +

- **Prhl** is a low strength promoter.
- **Prhl** is a low strength promoter.
- We improved **Prhl** as shown in A.
- As shown in B, **Prhl(RL)** became more stronger promoter than **Prhl**.
- However **Prhl(RL)** had more leak than **Prhl**.
The reason we made Prhl(RL) and Prhl(LR)

We need a new promoter fulfilling the following two conditions:
1. More stronger promoter than Prhl
2. Keep low leak like Prhl

As shown in A, Prhl(LR) fulfilled the two condition.
Overview of Assay

1. Plux and Prhl Reporter Assay
2. Improved Prhl Reporter Assay
3. C4-dependent CmR Expression Assay
4. C4-dependent C12 Production Assay
5. C12-dependent CmR Expression Assay
6. C12-dependent C4 Production Assay
7. Mutualism Confirmation by Co-culture Assay
1. Plux and Prhl Reporter Assay

**Construction**

- Ptet-LuxR(6A1), Plux-GFP(3K3)
- Ptet-RhlR(6A1), PlacIq-GFP(3K3) …positive control
- Ptet-RhlR(6A1), ΔP-GFP(3K3) …negative control
- Ptet-RhlR(6A1), Prhl-GFP(3K3)

**Strain**

JM2.300
1. Plux and Prhl Reporter Assay

Fresh Culture → Induction → FACS

37°C Incubation
Until OD=0.3

37°C Incubation
240 minutes

*Overnight cultures in LB

Measure OD590

Sample 0.03 mL
+ 
LB 3 mL

Measure OD590
(around 0.01)
1. Plux and Prhl Reporter Assay

Fresh Culture → Induction → FACS

37°C Incubation

Until OD=0.3

240 minutes

Measure OD590

A. Plux

P_{tet} → pSB6A1 → luxR → P_{lux} → pSB3K3

B. Prhl

P_{tet} → pSB6A1 → rhlR → P_{rhl} → pSB3K3

C. Placlq

P_{tet} → pSB6A1 → rhlR → Placlq → gfp

D. Placlq

P_{tet} → pSB6A1 → rhlR → Placlq → gfp

+ (1) 500μM 3O12HSL 30μL
+(2) DMSO 30μL

+ (1) 500μM C4HSL 30μL
+(2) DMSO 30μL

+ DMSO 30μL

+ DMSO 30μL
1. Plux and Prhl Reporter Assay

- **Fresh Culture**
  - 37°C Incubation
- **Induction**
  - Until OD=0.3
- **FACS**
  - 37°C Incubation
  - 240 minutes

- Measure OD590
- Sample + PBS (depend on OD)
- FACS
1. Plux and Prhl Reporter Assay

![Fluorescence Intensity](chart)

**Regulator**
- LuxR(C0062)
- RhlR(C0071)
- RhlR(C0071)
- RhlR(C0071)

**Promoter**
- Plux(R0062)
- Prhl(R0071)
- Positive Control (PlacUV5)
- Negative Control (ΔP)

**C4HSL**
- 
- 
- 
- +

**C12HSL**
- 
+ 
- 
-
1. Plux and Prhl Reporter Assay

Construction

- Ptet-RhlR(6A1), Prhl-GFP(3K3)
- Ptet-RhlR(6A1), Prhl(RR)-GFP(3K3)
- Ptet-RhlR(6A1), Prhl(LR)-GFP(3K3)
- Ptet-RhlR(6A1), Prhl(RL)-GFP(3K3)
- Ptet-RhlR(6A1), Placlq-GFP(3K3) …positive control
- Ptet-RhlR(6A1), ΔP-GFP(3K3) …negative control

Strain  JM2.300
2. Improved Prhl Reporter Assay

Fresh Culture → Induction → FACS

37°C Incubation Until OD=0.3
37°C Incubation 240 minutes

*Overnight cultures in LB

Measure OD590

Sample 0.03 mL + LB 3 mL

Measure OD590 (around 0.01)
2. Improved Prhl Reporter Assay

- Fresh Culture
  - 37°C Incubation
  - Until OD=0.3

- Induction
  - 37°C Incubation
  - 240 minutes

- FACS
  - Measure OD590
  - Sample
    - +
      - 500μM C4HSL 30μL or DMSO 30μL
  - Measure OD590 (around 0.01)
2. Improved Prhl Reporter Assay

Fresh Culture → Induction → FACS

37°C Incubation

Until OD=0.3

37°C Incubation

240 minutes

Measure OD590

→

Sample + PBS (depend on OD)

→

FACS
2. Improved Prhl Reporter Assay

In the diagram, the fluorescence intensity (a.u.) is measured under different conditions of C4HSL and various promoters. The promoters are:

1. Prhl R0071
2. Prhl(RR) K1529320
3. Prhl(LR) K1529310
4. Prhl(RL) K1529300
5. Positive Control
6. Negative Control

The fluorescence intensity is indicated as:
- Prhl R0071: 12-fold
- Prhl(RR) K1529320: 17-fold
- Prhl(LR) K1529310: 82-fold
- Prhl(RL) K1529300: 22-fold

The diagram shows a comparison between the control (−) and treated (+) conditions for each promoter.
3. C4-dependent CmR Expression Assay

Strain
All the samples were JM2.300 strain

Plasmids

Positive control….Constitutive CmR expression cell

Negative control….CmR non produce cell
3. C4-dependent CmR Expression Assay

- Fresh Culture
- Add Inducer and Cm
  - Until OD=0.5
  - OD590 = 0.5
- Measure OD
  - For 8 hours

Sample 0.03 mL + LB 3 mL

Dilution

Measure OD590

Prepare LB medium (3mL)
- Cm concentration
  - 0µg/ml ← 500mM C4HSL 30µl ← DMSO 30µl
  - 50 µg/ml ← 500mM C4HSL 30µl ← DMSO 30µl
  - 100 µg/ml ← 500mM C4HSL 30µl ← DMSO 30µl

Add 0.03mL sample to the LB medium
3. C4-dependent CmR Expression Assay

**Cm(-)**

C4HSL (+)
- Prhl(RL)-CmR-LasI
- Positive_Control
- Negative_Control

C4HSL (-)
- Prhl(RL)-CmR-LasI
- Positive_Control
- Negative_Control
3. C4-dependent CmR Expression Assay

Cm(50 microg / mL)

OD$_{590}$

Time (min)

C4HSL (+)
- Prhl(RL)-CmR-LasI
- Positive_Control
- Negative_Control

C4HSL (-)
- C4HSL (+)
- Positive_Control
- Negative_Control
3. C4-dependent CmR Expression Assay

Cm(100 microg / L)

![Graph showing OD590 over time for different conditions: C4HSL (+) and C4HSL (-)]

- **C4HSL (+)**
  - Green line: Prhl(RL)-CmR-LasI
  - Orange line: Positive_Control
  - Blue line: Negative_Control

- **C4HSL (-)**
  - Green line: Prhl(RL)-CmR-LasI
  - Orange line: Positive_Control
  - Blue line: Negative_Control
3. C4-dependent CmR Expression Assay

Measured for a long time

Cm(0 microg/mL)

OD

Time (min)

C4HSL (+)
- Prhl(RL)-CmR-LasI
- Positive_Control
- Negative_Control

C4HSL (-)
- Prhl(RL)-CmR-LasI
- Positive_Control
- Negative_Control
3. C4-dependent CmR Expression Assay

Measured for a long time

Cm(50 microg/mL)

**OD**

**Time (min)**

C4HSL (+)
- Prhl(RL)-CmR-LasI
- Positive_Control
- Negative_Control

C4HSL (-)
- Prhl(RL)-CmR-LasI
- Positive_Control
- Negative_Control
3. C4-dependent CmR Expression Assay

Cm(100 microg/mL)

Measured for a long time

OD590

Time (min)

C4HSL (+)
- Prhl(RL)-CmR-LasI
- Positive_Control
- Negative_Control

C4HSL (-)
- Prhl(RL)-CmR-LasI
- Positive_Control
- Negative_Control

Measured for a long time
4. C4-dependent C12 Production Assay

Strain

All the samples were JM2.300 strain

Plasmids

Sender cell

Reporter cell

Positive control...

Negative control...

Negative control...
4. C4-dependent C12 Production Assay

Induction of Sender cell → Induction of reporter cell → FACS

**Induction of Sender cell**
- Sample 0.03 mL + LB 3 mL
- Dilution
- Measure OD590

**Induction of reporter cell**
- OD590 = 0.5
- Prepare LB medium
  - 1: LB 3mL + 10mM C4HSL 15μL (Final concentration is 50μM)
  - 2: LB 3mL + DMSO 15μL

**FACS**
- Fresh Culture Sender cell
- Prepare LB medium
- Add 0.03mL sample to the LB medium (1 and 2)
4. C4-dependent C12 Production Assay

Induction of Sender cell

Induction of reporter cell

FACS

For 8 hours

For 4 hours

Fresh Culture Reporter cell

Sample 0.03 mL + LB 3 mL

Dilution

Incuvate until OD590=0.5

Collect supernatant of the culture of the sender cell

Centrifuge 5000×g

Filter sterilize supernatant. (Pore size is 0.22 μm.)

Induction of reporter cell

Prepare medium

The supernatant 2.7mL +LB 0.3 mL

LB 3mL +5μM 3OC12HSL 3μL

LB 3mL +DMSO 3μL

Add 30μL reporter cell
4. C4-dependent C12 Production Assay

- Induction of Sender cell for 8 hours
- Induction of reporter cell for 4 hours
- Sample + PBS (depend on OD)
  - Measure OD590
  - FACS
4. C4-dependent C12 Production Assay

<table>
<thead>
<tr>
<th>Sample</th>
<th>C4HSL</th>
<th>Sender</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prhl(RL)_CmR_LasI_BBa_K1529302</td>
<td>Plux_CmR</td>
<td>Authentic 3OC12HSL + DMSO</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Plas-GFP</th>
<th>Placlq-GFP</th>
<th>Promoter-less-GFP</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>11</td>
<td>88</td>
<td>2</td>
</tr>
<tr>
<td>(2)</td>
<td>12</td>
<td>67</td>
<td>1</td>
</tr>
<tr>
<td>(3)</td>
<td>11</td>
<td>58</td>
<td>2</td>
</tr>
<tr>
<td>(4)</td>
<td>7</td>
<td>58</td>
<td>1</td>
</tr>
<tr>
<td>(5)</td>
<td></td>
<td>43</td>
<td>2</td>
</tr>
<tr>
<td>(6)</td>
<td></td>
<td>44</td>
<td>2</td>
</tr>
</tbody>
</table>

Fluorescence Intensity (a.u.)
Strain
All the samples were JM2.300 strain

Plasmids

Positive control....Constitutive CmR expression cell
5. C12-dependent CmR Expression Assay

Fresh Culture → Add Inducer and Cm → Measure OD590

Until OD=0.5

OD590 = 0.5

Prepare LB medium (3mL)

Cm concentration

- 0μg/ml ← 500mM C4HSL 30μl
  ← DMSO 30μl
- 50 μg/ml ← 500mM C4HSL 30μl
  ← DMSO 30μl
- 100 μg/ml ← 500mM C4HSL 30μl
  ← DMSO 30μl

Add 0.03mL sample to the LB medium

Sample 0.03 mL + LB 3 mL

Dilution

Measure OD590

For 8 hours
5. C12-dependent CmR Expression Assay

Cm(-)

Time (min)

OD$_{590}$

3OC12HSL(+)
- Plux-CmR-RhlI
- Positive Control

3OC12HSL(-)
- Plux-CmR-RhlI
- Positive Control
5. C12-dependent CmR Expression Assay

Cm(50 microg/mL)

[Graph showing OD590 over time with different conditions: 3OC12HSL(+) and 3OC12HSL(-) with Plux-CmR-RhlII and Positive Control]
5. C12-dependent CmR Expression Assay

Cm(100microg/L)

![Graph showing the CmR expression over time for different conditions.](image-url)
6. C12-dependent C4 Production Assay

Strain
All the samples were JM2.300 strain

Plasmids

Sender cell

Reporter cell

Positive control…

Negative control…

Negative control…
6. C12-dependent C4 Production Assay

Induction of Sender cell

For 8 hours

Induction of reporter cell

For 4 hours

FACS

Fresh Culture Sender cell

Sample 0.03 mL + LB 3 mL

Dilution

Measure OD590

OD590 = 0.5

Prepare LB medium

1: LB 3 mL + 500 μM 3OC12HSL 30 μL
(Final concentration is 50 μM)

2: LB 3 mL + DMSO 30 μL

Add 0.03 mL sample to the LB medium (1 and 2)
6. C12-dependent C4 Production Assay

**Induction of Sender cell**
- Sample 0.03 mL + LB 3 mL
- Incubate until OD590 = 0.5
- Dilution

**Induction of reporter cell**
- Collect supernatant of the culture of the sender cell
  - Centrifuge 5000x g
  - Filter sterilize supernatant.
    (Pore size is 0.22 μm.)
- Add 30μL reporter cell

**FACS**

**Induction of reporter cell**
- Prepare medium
  - The supernatant 2.7mL + LB 0.3 mL
  - LB 3mL + 500μM C4HSL 30μL
  - LB 3mL + DMSO 30μL

For 8 hours
For 4 hours
6. C12-dependent C4 Production Assay

Induction of Sender cell → Induction of reporter cell → FACS

For 8 hours → For 4 hours

Sample + PBS (depend on OD) → FACS

Measure OD590
6. C12-dependent C4 Production Assay

![Graph showing fluorescence intensity](image)

- **Sample**: 3OC12HSL
- **Sender**: Plux_CmR_RhlI, BBa_K1529797
- **Reporters**: Prhl(RL)-GFP BBa_K1529301, PlacUV5-GFP, Promoter-less-GFP

<table>
<thead>
<tr>
<th>Sample</th>
<th>(1)</th>
<th>(2)</th>
<th>(3)</th>
<th>(4)</th>
<th>(5)</th>
<th>(6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3OC12HSL</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Sender</td>
<td>Plux_CmR_RhlI, BBa_K1529797</td>
<td>Plux_CmR</td>
<td>Authentic C4HSL</td>
<td>DMSO</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fluorescence Intensity (a.u.)

- 662
- 304
- 4
- 64
- 73
- 66
- 77
- 55
- 47
- 1
- 2
- 2
- 1
- 1
7. Mutualism Confirmation by Co-culture Assay

Construction

**Company**

- P_{tet}-GFP-P_{tet}-RhIR(6A1), P_{rhl}(RL)-CmR-LasI(3K3)

**Customer**

- P_{tet}-LuxR-P_{lac}-RFP(6A1), P_{lux}-CmR-RhII(3K3)
7. Mutualism Confirmation by Co-culture Assay

**Fresh Culture**

- Add the culture to LB medium

- Incubate at 37°C until OD = 0.5

- Incubate for 360 minutes at 37°C

**FACS**

*Overnight cultures in LB*

- Measure OD590

- Sample 0.03 mL

- Add LB 3 mL

- Measure OD590 (around 0.01)
7. Mutualism Confirmation by Co-culture Assay

Add the culture to LB medium

360 minutes
37°C Incubation

Fresh Culture

Until OD=0.5
37°C Incubation

FACS

LB medium contains 50 microg / mL ampicillin, 30 microg / mL kanamycin and 100 microg / mL chloramphenicol.

- **Company** 300 microL + **Customer** 130 microL + LB medium 2.57 mL
- **Company** 300 microL + LB medium 2.7 mL
- **Customer** 130 microL + LB medium 2.87 mL
7. Mutualism Confirmation by Co-culture Assay

Fresh Culture

Until OD=0.5
37°C Incubation

Add the culture to LB medium

360 minutes
37°C Incubation

FACS

Measure OD590

Sample + PBS (depend on OD)

FACS
We confirmed that the co-cultured samples were able to grow better than the samples single cultured.
But there is a question “Why is Company 300 microL and Customer 130 microL ?”

- Company 300 microL + Customer 130 microL + LB medium 2.57 mL
- Company 300 microL + LB medium 2.7 mL
- Customer 130 microL + LB medium 2.87 mL

Before this experiment, we did the other experiment…
7. Mutualism Confirmation by Co-culture Assay

1st experiment

2nd experiment

3rd experiment
7. Mutualism Confirmation by Co-culture Assay

1\textsuperscript{st} experiment

<table>
<thead>
<tr>
<th>Initial OD\textsubscript{590}</th>
<th>Company</th>
<th>Customer</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.005</td>
<td>0</td>
</tr>
<tr>
<td>0.005</td>
<td>0</td>
<td>0.005</td>
</tr>
<tr>
<td>0.015</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td>0.015</td>
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<tr>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

OD\textsubscript{590}

- Company: 0, 0.005, 0.01, 0.06, 0.17, 1.16, 1.36, 1.94, 1.77, 0.00
- Customer: 0, 0.005, 0.005, 0.015, 0.015, 0.05, 0.05, 0.15, 0.15, 2.30

1st experiment
We changed the rate at which the mix of companies and customers 
2nd experiment

7. Mutualism Confirmation by Co-culture Assay

<table>
<thead>
<tr>
<th>Initial OD590</th>
<th>Company</th>
<th>Customer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Company</td>
<td>0.05</td>
<td>0</td>
</tr>
<tr>
<td>Customer</td>
<td>0</td>
<td>0.01</td>
</tr>
<tr>
<td>Company</td>
<td>0.05</td>
<td>0</td>
</tr>
<tr>
<td>Customer</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Company</td>
<td>0.05</td>
<td>0.015</td>
</tr>
<tr>
<td>Customer</td>
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<td>0.015</td>
</tr>
<tr>
<td>Company</td>
<td>0.05</td>
<td>0.02</td>
</tr>
<tr>
<td>Customer</td>
<td>0.02</td>
<td>0.02</td>
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<tr>
<td>Company</td>
<td>0.05</td>
<td>0.03</td>
</tr>
<tr>
<td>Customer</td>
<td>0.03</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Final OD590

- Company: 0.06, 0.52, 0.62, 0.00, 0.15, 0.38, 0.00, 0.70, 0.24, 0.94, 0.94, 0.29
- Customer: 0.00, 0.05, 0.05, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.94, 0.94, 0.29

Mutualism Confirmation by Co-culture Assay

2nd experiment
7. Mutualism Confirmation by Co-culture Assay

3rd experiment

<table>
<thead>
<tr>
<th>Initial OD590</th>
<th>Company</th>
<th>Customer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.04</td>
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<tr>
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<tr>
<td></td>
<td>0.01024</td>
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<tr>
<td></td>
<td>0.008192</td>
<td>0.008192</td>
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</table>

Final OD590

<table>
<thead>
<tr>
<th>Initial amount (total) (µL)</th>
<th>0</th>
<th>140</th>
<th>270</th>
<th>400</th>
</tr>
</thead>
<tbody>
<tr>
<td>Company</td>
<td>0.14</td>
<td>0.64</td>
<td>0.47</td>
<td>0.53</td>
</tr>
<tr>
<td>Customer</td>
<td>0.02</td>
<td>0.47</td>
<td>0.53</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Amplification of Customer

OD(co-culture) / OD(mono culture)

<table>
<thead>
<tr>
<th>Initial amount (total) (µL)</th>
<th>0</th>
<th>140</th>
<th>270</th>
<th>400</th>
</tr>
</thead>
<tbody>
<tr>
<td>Company</td>
<td>1.00</td>
<td>1.36</td>
<td>1.13</td>
<td>1.18</td>
</tr>
<tr>
<td>Customer</td>
<td>1.00</td>
<td>1.18</td>
<td>1.13</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Mutualism Confirmation by Co-culture Assay
(A) Mechanism of AHL-lactonase in inactivating OHHL. AHL-lactonase opened the homoserine lactone ring of OHHL in the presence of water to produce $N$-(3-oxohexanoyl)-L-homoserine.

(B) Electrospray ionization spectra of the AHL-lactonase hydrolysis product of OHHL (left column) and of the produced $N$-(3-oxohexanoyl)-L-homoserine (right column). We labelled the comparable peaks with their respective $m/z$. 

We labelled the comparable peaks with their respective $m/z$. 

\[ \text{(M-H } m/z = 212) \]

\[ \text{(M-H } m/z = 230) \]
We next analysed the enzyme-digested products of three other AHLs with differences in acyl-chain length and substitution at the C3 position, including N-(3-oxododecanoyl)-L-homoserine lactone (OdDHL), N-butanoyl-L-homoserine lactone (BHL), and N-(3-oxooctanoyl)-L-homoserine lactone (OOHL). Results showed again that each of their molecular masses had been increased by 18 after the enzyme reaction (data not shown).

(1) C4HSL (N-butanoyl-L-homoserine lactone, BHL)

\[
\text{C4HSL} + \text{H}_2\text{O} \xrightarrow{\text{AHL-lactonase}} \text{C4HSL}
\]

(2) 3OC12HSL (N-(3-oxododecanoyl)-L-homoserine lactone, OdDHL)

\[
\text{3OC12HSL} + \text{H}_2\text{O} \xrightarrow{\text{AHL-lactonase}} \text{3OC12HSL}
\]
Whether Bank can change its state depending on C4HSL concentration or not

Main circuit

### Equations

#### LacI

\[
\frac{d}{dt} [\text{LacI}] = \alpha_{\text{LacI}} \frac{K_{\text{TetR}}^2}{K_{\text{TetR}}^2 + [\text{TetR}]^2} - d_{\text{LacI}} [\text{LacI}]
\]

#### TetR

\[
\frac{d}{dt} [\text{TetR}] = \alpha_{\text{TetR}} \frac{[\text{AHL}]^2}{K_{\text{AHL}}^2 + [\text{AHL}]^2} \frac{K_{\text{LacI}}^2}{K_{\text{LacI}}^2 + [\text{LacI}]^2} - d_{\text{TetR}} [\text{TetR}]
\]

Allowed parameter area (striped area)
AiiA dilute AHL

Time course of AI inactivation by purified AiiA protein.

Dong Y et al. PNAS 2000;97:3526-3531
Integration of economic wave

Added perturbation to the C4 concentration externally

\[
\frac{d}{dt}[C4] = k_1[RhlI_b]P_b + k_2[RhlI_{cu}]P_{cu} - \frac{\beta[AiiA_b][C6]}{K + [C6]} P_b - d[C4] + EconomicWave(t, [C4])
\]

\[
EconomicWave(t, [C4]) = -0.25[C4] - 0.01[C4] \cos\left(\frac{2\pi}{1400} t\right)^2
\]

![Graph](image)