iGEM Chiba 2008
E.coli Time Manager
Chiba University, JAPAN
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Making Bacterial Timer

How can we apply a timer to bacteria?

Shouting to the match

Burn popcorn

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An easy way to measure time

\[ T = \frac{V}{v} \]

Threshold
Quorum Sensing as a threshold sensor

Sender Cell

Receiver Cell

AHL

LuxI

Pc

LuxI

luxR

luxR

Plux

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We want to measure different length of time
To change the time required to reach threshold

1. Change the AHL accumulation rate
   - Copy number of LuxI
   - Copy number of LuxR

2. Change the threshold
   - RBS - LuxI
   - AHL transmission interference (AiiA protein)
   - RBS - LuxR
   - Cross Talk

We succeeded in changing the time require to reach threshold!!

Cross Talk!!
What is Cross-talk?

Cross-talk with foreignization

Hello! こんにちは!
Hi! こんにちは?

Delayed

What is Cross-talk?
Time delay test using different senders

~Senders~

**LuxI** from *Vibrio fischeri*

- **3OC6HSL**
- Threshold: $10^{-9}$ M

**LasI** from *Pseudomonas aeruginosa*

- **3OC12HSL**
- Threshold: $10^{-6}$ M

~Receiver~

**BBa_T9002**

- **pLac**
- **luxR**
- **pLux**
- **GFP**
- **pMB1**
Experimental Method for measuring Time delay

AHL Sender (Stationary phase)

Mix sender with receiver add IPTG

AHL Receiver (Stationary phase)

Cultured in 30°C water bath

Measure fluorescence intensity

Time-lapse

T=0 T=t₁ T=t₂ T=tₓ Time
By visual judgement
LasI-LuxR pair exhibited time delayed action 2h after LuxI-LuxR pair.
What happens if we change the receivers?

~Sender~

BBa_S03623

pTet luxL

pMB1

~Receivers~

BBa_S03119

pLac luxR

P15A

BBa_J37032

pLux GFP

pMB1

BBa_K084005

pLac lasR

P15A

BBa_J37032

pLux GFP

pMB1

New part!
LuxI-LasR pair did not cross-talk

HIGH Selectivity
Threshold $3\text{OC}_{12}\text{HSL}$ (by LasI) for LasR \[ \cdots \cdots \cdots \cdot 10^{-10} \text{ M} \]
Threshold $3\text{OC}_{6}\text{HSL}$ (by LuxI) for LasR \[ \cdots \cdots \cdots \cdot 10^{-5} \text{ M} \]

Using LuxI-LuxR pair and LasI-Lux
384 well plate – dot painting R pair

Transfer bacterial mixtures to each of 384 wells
Dot painting one well by one well.

We found out the explicit effect of Cross-talk by this demonstration.

Irradiated by UV light.
Summary

1. We aimed at installing the timer function into Bacteria
2. On basis of **Quorum Sensing**, we tried to regulate time interval for reaching its threshold.
   - tuning senders
   - tuning receivers
   - interference by AiiA
   - **Cross-Talk**
3. Using Cross-talk Quorum Sensing, we have constructed an available time-delayed switch using the LasI-LuxR pair. The LasI-LuxR pair switches on 2 hours after the original LuxI–LuxR pair by visual judgement.
4. We demonstrated a **dot painting** by means of the sender-receiver mixture of LasI-LuxR and LuxI-LuxR as an E.coli painting.
Stimulus Accumulation Indicator

08Chiba Project

IPTG

Promoter

lasI

LuxI

AHL

Stimuli

UV

Promoter

luxR

LuxR

Plux

output

DNA repair

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Thank you for your attention.