Ehime-Japan
2012 iGEM Team
This is a bow lingual.
This is a miaow lingual.
What is this?

E. coli
E. coli ingestual !!
It is funny, if we can know the feeling of *E. coli*. 
How to catch the feeling of \textit{E coli}?

E.colingual is composed by three components.

1. Sensing
2. Connection
3. Screen
1. Sensing
Sensing is composed by heat and cold shock systems.
The heat shock system functions by combination of two types of *E. coli*.
Heat shock system utilizes two plasmids.

Sensor *E. coli*

- Heat shock promoter + LuxI

Speaker *E. coli*

- p(tetR) + luxR
- mCherry + luxpR
Response of speaker *E. coli* to signaling molecule (3OC6HSL) was checked.

Light intensity was changed due to the concentrations of signaling molecule.

Image of the microtiter plate with E.coli.
We confirmed the induction of mCherry in the speaker *E. coli* by signaling molecule.
Effective work of heat shock system requires excess Sensor \textit{E. coli}.

After 4 hours from heat shock at 50°C
Thus, we successfully constructed the heat shock system as “Sensing”.
Cold shock system

Sensor *E. coli*

Speaker *E. coli*
Cold shock system

**Sensor E. coli**

Cold shock promoter + LasI

**Speaker E. coli**

constitutive promoter + lasR

lasR+PAI + GFP
The sensor plasmid is a new BioBrick part, BBa_K794000

This band is plasmid BBa_K794000 cut by EcoR1
Now, we are testing the cold shock system.
Conclusions

1. Sensing system consists of heat shock system and cold shock system.

2. Our Heat shock system works.
Connection
Optical fiber transmits the green light from GFP and to another tube.

Transmitted green light could be caught by green light sensor in another tube.
lacZ was used for the original red and green light sensors

When the green light is caught by the E. coli, lacZ is expressed.

Tabor et al., J. Mol. Biol. (2011)
So, we replaced the lacZ gene in the light sensor plasmid with the GFP gene.

$pJT122(-lacZ+GFP)$
Apparatus for optical fiber assay

- Optical fiber
- GFP in a test tube
- E. coli wrapped with Aluminum foil
GFP light passed through an optical fiber

**Graph:**
- **UV:** 350 nm
- **Green light:** 510 nm
The transmitted light can induce expression of GFP.

The green light sensor E. coli that catches the optical fiber light glows more than the control E. coli.
How to establish two-way communication?
In the two-way communication system, GFP should be degraded by red light, enabling repeated transmission of light.
Initially, we considered that ClpXP • LVA induced by red light could degrade GFP • LVA and ClpXP • LVA.
However, the system may have a demerit, suicide reaction of ClpXP • LVA.
To prevent the suicide reaction, we employed *Mesoplasma florum* ssrA and Lon protease.

![Diagram](http://bacmap.wishartlab.com/organisms/194)

*M. Florum* (mf)-Lon

Specific degradation

*M. florum* (mf)-ssrA

AANKNEENTNEVPTFMLNAGQANYAFA
ClpXP does not degrade the GFP \cdot mf-ssrA.
The mf-Lon \cdot LVA degrades the GFP \cdot mf-ssrA.
ClpXP degrades the mf-Lon•LVA.
Thus, the mf- ssrA and Lon enable us to regulate the expression level of GFP strictly by green and red lights.
To realize the mf-ssrA and Lon system, the lacZ genes of the light sensor systems were substituted.
Degradation system

\[ E. \ coli \]
- pJT122
- pJT106b
- pPLBCB(S)

Green light

mf-ssrA

GFP

Red light

LVA

mf-Lon

ClpXP

mf-Lon
The effects of Mf-Lon on the degradation of mf-ssrA-tagged GFP was small.
The effect of the ssrA on the light of GFP in the presence of Mf-Lon was moderate.
The LVA tag on Mf-Lon likely prevents degradation of ssrA-tagged GFP.
3. Screen
When E. coli receives the light through an optical fiber, the E. coli expresses RFP. This RFP induces RFP expression in the neighbor well. This process is repeated, the character is appeared. For example, when the E. coli feels hot, Screen shows “H”.
The plasmids for Screen

• pCph8 + pJT106b + pPLPCB(S)
⇒ The red light sensor
The lacZ in pJT106b was replaced with RFP. RFP is expressed by red light.
After cultivation at $37^\circ$C and for 42 hours, RFP was expressed.
A simple model of Screen was tested.
The assay of simple model

- JT2 strain having pCph8, pJT106b, and pPLPCB(S)

Shaking at 37°C, 125 rpm for 16 hours under the white light.

↓

Gather and suspend the cell.

↓

Irradiate UV (364 nm) the cell.
Red light is contained in the white light.
One of four cells is red, another is not red

RFP is expressed

RFP is not expressed
Experiment equipment
① RFP+UV

RFP was expressed after 4 hours

After 4 hours
UV only
RFP was not expressed after 4 hours

After 4 hours
The RFP expression was increased under the control of RFP+UV
In the dark, RFP was not expressed in the domino.

0 hour

Domino system

Constitutive RFP expression

4 hour

RFP was not expressed without UV, if RFP was expressed in the neighbor well.
The screen system looks dominoes!
When those three parts are combined...
E.colingual !!
How about having E.coligual in your lab?
Human Practice
Our Human Practice

• Questionnaire
• Lecture and recreation for high school students
Questionnaire
Have you ever heard the word, “Synthetic biology”?  

85 % of the responders said “No”.

85%(686)

15%(125)
About 70% of the responders were teen age.
Therefore, we performed our human practice for high school students.
We used “E.create” as a recreation tool.

Educational card game for learning of synthetic biology
How to play E.create?

Players choose and combine the parts to construct a plasmid for complete of missions from *E. coli*.

There are several missions according to the player’s level “E.colevel”.
Let’s try it!
For example, E. coelevis 0

Mission

- He always makes GFP.
- He has Km resistance.
Choose cards and build a plasmid!
Like this, the mission is completed!
Another missions

E.colevel 1 Ao

E.colevel 2 Hana chan

E.colevel 3 Postman X
For details...

Please visit our poster.

“E.create” will be presented.
Evaluation of our educational activities
Q1: Do you know gene recombination before our lecture?

The majority did not know gene recombination.
Q3: Did you change your opinion to Q1 after our lecture?

- Yes, I understood better than before. 98% (63)
- No, I did not.
- Yes, but I was more complexed. 2% (1)

Their opinion was dramatically improved by our human practice.
Q5. Did you change your interest in gene recombination after the lecture and playing with E.create?

- Yes, I have got more interested.
- No change.
- No, I have got less interested.

94% (60)

6% (4)
Our activities were very successful!!
Our sponsors

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