Flower Fairy E.coli
Magical Power
Florigen

∥

FT-protein
What is FT protein?

1. Plant hormone.
2. Expressed in plants’ leaves.
3. Moves to shoot apex and blooms flowers.

Plants Produce FT protein
Our goal is...

“Just by spreading E.coli on the surface of the leaves, you can make flowers bloom!”
Project overview

1. EXPRESSION
2. SECRETION
3. PENETRATION
4. ACTIVATION
Project overview

1. EXPRESSION

2. SECRETION

3. PENETRATION

4. ACTIVATION
1. *E.coli* doesn’t have *FT* gene.

2. We had to make new BioBrick including *FT* gene.

3. We introduced *FT* gene into *E.coli*.
Construction of FT

1. Using a plant’s gene
   - FT gene
     - Arabidopsis thaliana

2. Necessity of mutagenesis
   - EcoRI & PstI cut this sequence
   - Improper BioBrick in iGEM

3. Making new BioBrick about FT gene
   - Removing the restriction sites
   - We performed mutation PCR
   - pSB1C3

1. EXPRESSION
We have to confirm the expression of FT protein!
Expression of *FT*

Western Blotting assay

T7 polymerase is regulated by IPTG.

Western Blotting with anti-FT antibody

1. EXPRESSION
Expression of FT

We confirmed the expression of FT!!

Florigen here!
Expression-Achievement

- Making new BioBrick of FT gene
- FT expression

1. EXPRESSION
Project overview

1. EXPRESSION

2. SECRETION

3. PENETRATION

4. ACTIVATION
Cell Lysis... ?

it’s not cute
Cell Lysis... ?

Lysis

Temporary

Secretion!

Continuously

2. SECRETION
Cell Lysis...?

WE CHOSE SECRETION!!

Secretion!

Continuously

2.SECRETION
Two hurdles

To Secrete Proteins...
1. Wild E. coli has this transporter.

2. TorA signal is required to pass this transporter.

- Proteins without torA
- Proteins with TorA
1. Wild E. coli has this transporter.

2. TorA signal is required to pass this transporter.

=Tat transporter=

= Proteins without torA

= Proteins with TorA
Modified torA signal

Old torA part

New torA part

Every iGEMer can use it!
Modified torA signal

When we could modify torA signal....

GFP should be expressed!!
Our part worked well

torA-GFP was expressed!!

2. SECRETION
Our part worked well

We modified the torA signal!!

torA-GFP was expressed!!
2. Derived from λ phage.
Pass through the outer membrane

kil makes holes

=Kil protein=

1. Derived from \(\lambda\) phage.
Before using kil gene...

Is making holes harmful?
Is making holes harmful?

Check me!!
Is kil gene harmful?

We made this construction

and checked whether kil gene is harmful or not.
Is kil gene harmful?

No difference between kil(+) and kil(-)!

OD600

IPTG
- 0
- 0.001mM
- 0.01mM
- 0.1mM
- 1mM

TIME

0.5hr 1hr 1.5hr 2hr 2.5hr 3hr 3.5hr 4hr 4.5hr 5hr 5.5hr 6hr

2. SECRETION
Is kil gene harmful?

No difference between kil(+) and kil(-)!

we figured out kil gene is not harmful
For further improvement

=Tat pathway generator=
BBa_K797004
2. SECRETION

- Modifying torA signal parts
- Confirming that kil gene is not harmful
- Improving tat pathway generator
Project overview

1. EXPRESSION

2. SECRETION

3. PENETRATION

4. ACTIVATION
Penetration of FT

Normally proteins cannot get inside cells.
The way R9 peptide works

1: R9 peptides adhere to cell membrane
2,3: Plant cells respond to this stimulus and cause endocytosis
4: FT proteins near R9 are taken in the cells
5: FT proteins are get out of the endoplasm
Proteins with R9 can get inside cells.
How to verify R9 function

1. Cut!!

2. Scratch!! Remove cuticles!

3. Penetration

4. Wash away!
R9 enables penetration?

Negative control (R9-)

Sample (R9+)

Plant cell (R9-)

Plant cell (R9+)

3. PENETRATION
R9 enables penetration?

R9 enabled proteins to get inside the cell!!
Confirming that R9 peptides enable FT proteins to penetrate membranes of plant cells.
Project overview

1. EXPRESSION

2. SECRETION

3. PENETRATION

4. ACTIVATION
A variety of proteins are up-regulated by FT.
Experiment for verification of FT

control

FT injection

**4.ACTIVATION**
Check the function of FT

The relative RNA expression corrected by TUBULIN

4.ACTIVATION
Check the function of FT

The relative RNA expression corrected by TUBULIN

What makes these differences?
Improvement

RNA extraction

4.ACTIVATION
Improvement

RNA extraction

There is a room in improving the RNA extraction method!!
Improvement

RNA extraction

We improved the RNA extraction method!!
Future Plan

Separated cells
Improving the experimental method for verifying FT function.
Future Work
Cooperation
Science agora
Open campus
Science training
Symposium
Academic day
Education
School festival
Cooperation

Human Practice
Japanese Attitudes toward Genetic Engineering

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Abstract: Whether or not the general public supports biotechnology and genetic engineering is an important current problem. In this paper, we report that people’s attitudes toward the terms “biotechnology” and “genetic engineering” are highly dependent upon their knowledge of the field. For this reason, it is necessary to promote activities that provide the general public with information on the current state of biotechnology and genetic engineering so that they can form educated opinions.

Key Words: biotechnology, GM (genetically modified) foods

1. Introduction

Many people starve to death because they are unable to grow enough crops in their impoverished countries. Food shortage has become one of the most serious problems in the world. Some people expect that genetic engineering can solve this problem because genetically modified plants can grow more easily in barren land (1). However, some people worry that genetically modified foods may do harm to our health and the environment. It is often reported that Japanese people tend to avoid genetically modified foods. Sure enough, previous surveys of attitudes toward genetic engineering showed that, in Japan, more people had “negative” or “neutral” opinions regarding genetically modified foods than people in other nations.

These findings piqued our interest in the Japanese public’s views on genetic engineering and made clear to us the importance of active discussion on the subject of genetic engineering. In conjunction with August 28 and 29 at Masukata Shopping Street and on September 11 and 12 at the Coop Shimogamo. All sites are located in Kyoto city, Japan.

2.2. Questionnaires

We prepared two questionnaires, “Attitude Survey of Genetic Engineering” and “Attitude Survey of Biotechnology.” We interchanged the terms “genetic engineering” and “biotechnology” in order to assess subjects’ different associations with these two terms.

2.3. Search of “Biotechnology” and “Genetic Engineering” in Japanese in Google

On October 31, 2010 we performed a Google search of these terms in Japanese.
Human Practice

i.Coli
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