iGEM Kyoto

Time Bomb
~Gene Switch Depending on Duplication~

Cells Cells Cells

WE HAVE TWO PROJECTS
Time Bomb
~Gene Switch Depending on Duplication~
1. Introduction
Natural Environment
Chapter 1 Introduction

MUTATION
Chapter 1 Introduction

MUTATION
2. System Mechanism
Design strategy

The End Replication Problem
Replication origin
RNA primer
New DNA
shorter
Design strategy

The End Replication Problem

Replication origin

New DNA

RNA primer

shorter

Time Bomb Design

Timer Vector

Bomb Vector

LacI expression Vector

Chapter 2 System Mechanism
Controlling cell fate

Under the existence of LacI

While Timer vector exists, the cell is alive.

When Timer vector does not exist, the cell is killed.
As cell division is repeated, LacI\textsubscript{BS} becomes shorter and shorter. Finally, the one side of repetitive sequence is completely lost and, from that side, the DNA is degraded by exonuclease.
3. Simulation
Simulation Results

Cell Population

200bp

800bp

1400bp

Cell division Times

Repeat Length

Fluctuation of the number of cell division decreases as repeat length becomes longer.

Chapter 3 Simulation
4. Result
In order to evaluate the accuracy of Timer vector

1. Construction of repetitive sequences

2. Construction of Timer vector which contains GFP generator

and

Construction of LacI expression vector

3. Functional analysis of the Timer vector
1. Construction repetitive sequences

To make the repetitive sequences, we used Microgene Polymerization Reaction (MPR).

Various length of LacI binding site were constructed at once.
2. Construction of **Timer vector** which contains GFP generator and Construction of **Lacl expression vector**

**Timer vector**

**Lacl expression vector**

*BYP5005* High copy number vector with LEU2
Functional analysis of our parts

Ex) BBa_K210007….Parts for adaption to a shuttle vector of E.coli and budding yeast.

Yeast makes colonies
The functional analysis of GFP

Circular Timer vector (-)  Circular Timer vector (+)

intrinsic fluorescence

Timer vector’s GFP fluorescence
Timer vector works successfully

The transformed yeast by linear Timer vector
Timer vector works successfully

Timer vector works as we expected.

- Timer vector is distributed to daughter cells.
- Timer vector expresses Ura3.
- LacI protects linear Timer vector.
5. Conclusion
Conclusion

1. Construction of repetitive sequences

2. Construction of Timer vector and LacI expression vector

3. Analysis of the constructed Timer vector in yeast

4. Future work
Cells

By iGEM Kyoto
Introduction

Nobody succeeded in synthesizing a cell mitochondrion. Can mitochondrion be a new cell model, “LIPOCHONDRIA”? Nobody has succeeded in synthesizing a cell.
Why mitochondria?

Mitochondria

• Are by far simpler than their host cells (they own minimal sized genome)

• Take in proteins from the cytoplasm

Could be the first step to establish a brand new method
Strategy

Step 1.
The liposome penetrates through the cell membrane.

Step 2.
It starts to import proteins in the cytoplasm through translocases.
Step1. The liposome penetrates through the cell membrane.
Design of HIV TAT(LALAAAA)₃ peptide

Functional peptide

anchor

Parts #
K210009

HIV-TAT  His Tag (LALAAAA) x 3

Cell membrane
TAT-Liposome penetrates through cell membrane

Merge

Fluorescent
Step 2. It starts to import proteins in the cytoplasm through translocases.
The function of mitochondrial translocase

Signal sequence

GFP

Signal peptide for yeast TIM complex

T7 promoter

RBS

GFP

terminator
Localization of sig-GFP in isolated mitochondria

+gene(sig-GFP) -gene

Mitotracker orange

GFP
**In vivo**

Cell membrane

Sig-GFP plasmid

Sig-GFP
Localization of sig-GFP in HeLa cells

Mito tracker orange  GFP  Merge

Sig-GFP

Normal GFP
Reconstruction of mitochondrial translocase on a liposome
Summary

- TAT(+) - liposome localizes inside HeLa cells when incubated together.

- sig-GFP localizes in isolated yeast mitochondria. (*in vitro* function)

- sig-GFP expressed in HeLa cells localizes in mitochondria. (*in vivo* function)
Future Work

Expressed!!

Artificial Genome
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A pair of MPR primers are synthesized. They are reacted under conditions similar to PCR (but without any template DNA).

**primer1**
5´-GAAATGTGAGCGCTCACAACCTGGCGGGGAG-3´

**primer2**
5´-CACACCTAGCTGACTGCCACCCGGGAC-3´

**Microgene**
GAAATGTGAGCGCTCACAACCTGGCGGGGAGCCACAGTCGACCCTAGGTGTGACGCACTAGCTGACTGCCACCCGGGAC-...

**Repetitive sequence**

Chapter 4 Results
Experimental conditions in MPR

The temperature during MPR influences the length of the acquired sequence. We programmed 72°C.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Duration</th>
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</thead>
<tbody>
<tr>
<td>94°C</td>
<td>10min</td>
</tr>
<tr>
<td>63°C</td>
<td>10min</td>
</tr>
<tr>
<td>94°C</td>
<td>10sec</td>
</tr>
<tr>
<td>x°C</td>
<td>1min</td>
</tr>
<tr>
<td>63°C</td>
<td>7min</td>
</tr>
<tr>
<td>4°C</td>
<td>forever</td>
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45 cycle

5000bp

1000bp

<table>
<thead>
<tr>
<th>Sample</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
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<tbody>
<tr>
<td>temperature (X)</td>
<td>55</td>
<td>57.8</td>
<td>62.2</td>
<td>65.1</td>
<td>69.4</td>
<td>72</td>
</tr>
</tbody>
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