Team Biwako Nagahama
Poster area: C 45
5th October 2013
Do you know how to make “paper”? 
BY TREES
Paper production = Cutting trees
Effects of cutting trees

- Global Warming
- Floods
- Landslides
- And other Ecological dis-balances
Our thinking

Synthetic Biological Approach

Agrobacteria + E. Coli

AgRe Paper + EColink
AgRePaper
AgRePaper Project contents

1. What’s AgRePaper?
2. What’s Agrobacteria?
3. Synthesis and degradation system
4. What’s Shuttle vector?
1. What's AgRePaper?

**Agrobacteria** Recycle **Paper**
2. **What’s Agrobacteria?**

**A**
Agrobacteria attached to carrot cells

**B**
Tumor cells resulted by infection of agrobacteria
Result: Induction of Glucans from Agrobacteria

A: Curdlan Sheet
B: White film within glucan
C: Glucan pellet with Congo red
3. Synthesis and degradation system

Cellulose

Curdlan

Cellulose

Curdlan

OM

CelC

D-glucose

IM

BglX

CelB

Celllobiose

CelA

UDP-glucose

Lipid-P-(Glu)x

CrdS

CelC

UDP-glucose

CelC

Cellulose

Cellulose

Curdlan

Curdlan
Result:

Synthesis and degradation system

B: Lane 1 and 2: sample maker
Lane 3: *CelC* gene attached to prefix-suffix PCR production (1,122 bp) is produced from *Agrobacterium tumefaciens* C58.

C: Lane 1 and 2: sample maker
Lane 3: *CrdS* gene (2,257 bp) is produced from *Agrobacterium tumefaciens* C58.
4. What’s Shuttle vector

pBl107
9 kbp

- **E**
- **X**
- **S**
- **P**

<table>
<thead>
<tr>
<th>Notation</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>BioBrick cloning site: prefix</td>
</tr>
<tr>
<td>X</td>
<td>BioBrick cloning site: suffix</td>
</tr>
<tr>
<td>S</td>
<td>replication origin in <em>E.coli</em></td>
</tr>
<tr>
<td>P</td>
<td>replication origin in agrobacteria</td>
</tr>
<tr>
<td>ColE1</td>
<td></td>
</tr>
<tr>
<td>OriV</td>
<td></td>
</tr>
<tr>
<td>NPT III</td>
<td>kanamycin resistance gene</td>
</tr>
<tr>
<td>NPT III</td>
<td></td>
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</tbody>
</table>
Result: What’s Shuttle vector?

A  *E. coli* JM109/pBI107-RFP (Red fluorescence Protein)

B  *A. tumefaciens*/pBI107-RFP on kan plate
Planning to Create Ecolink!
What’s myoglobin?

Change in color of meat

DNA fragment synthesis

Ribosome binding site

Encoding Myoglobin

Incorporation of an efficient ribosome binding site

Modification of codon usage suitable to *E. coli* (mMb).

Ref: High-level expression of sperm whale myoglobin in *Escherichia coli*  BARRY A. SPRINGER AND STEPHEN G. SLIGAR Department of Biochemistry, University of Illinois, Urbana, IL 61801 Communicated by Gregorio Weber, September 4, 1987
Result of pUC18-mMb

※mMb : Mb gene was isolated from sperm whale with modification of codon usage suitable to *E. coli* (mMb).
Result of Point mutation and Sequencing
Construction of T7 cassette

This cassette is constructed by all Biobrick part.
Future work

<table>
<thead>
<tr>
<th>Bonds</th>
<th>Compound</th>
<th>Color</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe^{++} Ferrous (covalent)</td>
<td>:H₂O</td>
<td>Purple</td>
<td>Reduced myoglobin</td>
</tr>
<tr>
<td>:O₂</td>
<td></td>
<td>Red</td>
<td>Oxymyoglobin</td>
</tr>
<tr>
<td>:NO</td>
<td></td>
<td>Cured pink</td>
<td>Nitric oxide myoglobin</td>
</tr>
<tr>
<td>:CO</td>
<td></td>
<td>Red</td>
<td>Carboxymyoglobin</td>
</tr>
<tr>
<td>Fe^{+++} Ferric (ionic)</td>
<td>-CN</td>
<td>Red</td>
<td>Cyanmetmyoglobin</td>
</tr>
<tr>
<td>-OH</td>
<td></td>
<td>Brown</td>
<td>Metmyoglobin</td>
</tr>
<tr>
<td>-SH</td>
<td></td>
<td>Green</td>
<td>Sulfmyoglobin</td>
</tr>
</tbody>
</table>

Human Practice

- Co-operation with other iGEM teams within Japan.
- Explanation about Synthetic Biology and iGEM competition to High School teachers.
- Fun with elementary school students.
Thank you for listening our presentation

Will be waiting at Poster area C45