HokkaidoU
Protein delivery into eukaryotic cells by type III secretion machines

About T3SS

- E. coli
- Needle
- Outer rings
- Neck
- Inner rings
- Inner rod
- Socket
- Cup

Dimensions:
- 100 nm
- 3 um
- 1 um
- 80 nm
Bacterial cytosol

Target cell cytosol

Target cell membrane

Outer membrane

Inner membrane

Bacterial cytosol
Gene Construct

**E. coli K-12**

[SPI-2/T3Signal-GFP/RFP]

**pBAC SPI2**

**pSB1C3**

**Secretion apparatus**

**Promoter**

**RBS**

**T3S signal**

**GFP**

**Double terminator**

**RFP reporter**

**Fused protein**

**Target cell**

**pSB1C3**
Result

phase-contrast

GFP

RFP

Merge
Objectives for iGEM 2011

I. Making injection assay easier by using onion cells sheets

II. Screening what proteins can be injected by T3SS

III. Seeking a method for verifying the injection of proteins
<table>
<thead>
<tr>
<th>Animal cells</th>
<th>Onion cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difficult to prepare</td>
<td>Easy to prepare</td>
</tr>
<tr>
<td>Weak against bacteria</td>
<td>Tolerant against bacteria</td>
</tr>
</tbody>
</table>
Objectives for iGEM 2011

I. Making injection assay easier by using onion cells sheets

II. Screening what proteins can be injected by T3SS

III. Seeking a method for verifying the injection of proteins
Wish to clone various proteins into...
Biofusion standard takes...

Too much cost and time!
What should we do?
Four requirements for cloning system

I. Inserting various kinds of BioBrick parts into another Biobrick using a SAME primer set for PCR.

II. Making it sure that the part is to be inserted in the correct direction.

III. Producing a fusion protein.

IV. Resulting construct to retain BioBrick properties.
Solution: insert Bsa I cloning site

Universal Prefix primer

insert

ENX

BioBrick

SNP

Universal Suffix primer

TetR

plasmid backbone

BioBrick

RBS

Sec. sig.

Double Terminator

ENX
Solution: insert Bsa I cloning site

Universal Prefix primer

insert

ENX

BioBrick

S

N

P

Universal Suffix primer

TetR

plasmid backbone

RBS

Sec. sig.

Bsa I cloning site

Bsa I

Bsa I

Bsa I

Bsa I
Solution: insert Bsa I cloning site
Bsa I cloning site
Bsa I cloning site

Spe I like overhang

ATCCGG

Not I like overhang

CTAGAT
Bsa I cloning site

BSAI

AGGCGGCTCCGGCG

BioBrick

ACTAGATTCTAG

BSAI

ligation scar

ligation scar
Another problem

Prefix primer

BioBrick

Suffix primer

Not I and Spe I digestion
Another problem

Prefix primer

BioBrick

Suffix primer

Not I and Spe I digestion

GGCCGC

BioBrick

ATCTAG
Another problem

Prefix primer

BioBrick

Suffix primer

Not I and Spe I digestion
Xba-byebye primer

Xba byebye primer

BioBrick

Suffix primer

Not I and Spe I digestion

in frame stop codon

insert

Xba I
Xba-byebye primer

Xba byebye primer

Not I and Spe I digestion

Work as a NEW BioBrick!
Objectives for iGEM 2011

I. Making injection assay easier by using onion cells sheets
II. Screening what proteins can be injected by T3SS
III. Seeking a method for verifying the injection of proteins
Glycogen Synthase Kinase 3β

We Used This Part

MSGRPRTTSFAES
<table>
<thead>
<tr>
<th>GSK Tag state</th>
<th>Location</th>
<th>Bacterial cytosol</th>
<th>Eukaryotic cytosol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphorylated</td>
<td>NO</td>
<td>YES</td>
<td></td>
</tr>
<tr>
<td>unmodified</td>
<td>YES</td>
<td>NO</td>
<td></td>
</tr>
</tbody>
</table>
## GSK tag and antibodies

<table>
<thead>
<tr>
<th>GSK Tag state</th>
<th>Antibodies</th>
<th>Anti phosphorylated GSK</th>
<th>Anti GSK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphorylated</td>
<td>Recognizes</td>
<td>Recognizes</td>
<td></td>
</tr>
<tr>
<td>unmodified</td>
<td>—</td>
<td></td>
<td>Recognizes</td>
</tr>
</tbody>
</table>

BIO x ART

NEW COMMUNICATION TOOL
HOW TO COMMUNICATE SCIENCE

TOUGH WORK

CLASSES
WORK SHOP
READING BOOKS
SCIENCE
WE CAN ENJOY
ART
BIO x ART
ONE MORE THING
Virtual BIO x ART Gallery
Acknowledgement

HOKKAIDO UNIVERSITY

Primary Cell

Amino Up Chemical

(有) メンデル工房
サイエンス教室

COSMO BIO CO., LTD.
Inspiration for Life Science

HOKUTO KOHKI ENGINEERING

HokkaidoU