RNA in LOVE
Eukaryotes

Already had studied well

Prokaryotes

So many chances in this field!!
Molecular mechanism of anti-sense RNA

Target gene

mRNA

Protein

asRNA

Anti-sense construct
Binding of anti-sense RNA and mRNA

A LOVE STORY!!!
There was a girl, asRNA.
She fell in love with mRNA.

However,
She had a strong rival, Ribosome.
One day, Cupid found such poor as RNA. Cupid casted a spell over her.

Owing to wonderful magic, she got a little confidence and courage.
mRNA fell in love with changed asRNA at a first sight.

asRNA and mRNA were together happily forever.
How can I help her?
We introduce our new method!
Cupid’s magic

1. H-stem
2. Anti-sense RBS
3. Length
Cupid’s magic

1. H-stem

2. Anti-sense RBS

3. Length
Single-stranded RNA is fragile

Anti-sense construct

asRNA
Stemmed RNA is stabilized

N. Nakashima et al. (2006)
Stemmed RNA

Lower \( \Delta G \)

GC-rich 36 nt
Construct of H-stem vector

Prefix

P_{lac}^{(R0010)}

stem

Ncol

Xhol

stem

Suffix

dT^{(B0015)}

Prefix

pSB1A3

Suffix

H-stem Vector $^{(BBa\_K1524100)}$
How to use

Prefix

H-stem

Target

Sense

Anti-sense RNA

XhoI

NcoI

IPTG

P_{lac} (R0010)

dT (B0015)

Suffix

Anti-sense

stem

stem
Cupid’s magic

1. H-stem
2. Anti-sense RBS
3. Length
Specific asRNA for protein A

Common asRNA

Specific asRNA for protein B

Common asRNA
Common asRNA suppresses wide range of protein expressions
More than 2/3 iGEMer use B0034 as RBS

Ratio of RBS used in BioBrick Parts

- B0034: 67%
- B0030: 15%
- B0032: 11%
- B0031: 4%
- B0033: 3%

[Reference] igem Registry of Standard Biological Parts “Community RBS catalog” we choose available RBS from the catalog.
asB0034 contains scar sequence

Typical BioBrick Parts

anti-sense of RBS
Method

We designed anti-sense RNA for B0034 by annealing oligonucleotides.

We inserted anti-sense B0034 into stem-loop construction.
Oligonucleotides

oligonucleotide 1

5’ - CATGGCA[TCTAGTA]TTTCTCCTCTTTTC[TCTAGTC] -3’

oligonucleotide 2

3’ - [CGT]AGATCAT[AAAGAGGAGAAAG]AGATCA[GAGCT] -5’

NcoI  S/X  B0034  S/X  XhoI
We used mRFP & GFP as target gene, and evaluated whether asB0034 works for different target genes.

Target is on pSB6A1
- Copy number is 15 ~ 20 / cell

asB0034 is on pHN1257*
- Copy number is about 30 / cell
- stem-loop structure

*N. Nakashima et al.
Method for Assay

1. Cultivated a colony of transformed bacteria in 2 mL of medium until the turbidity at OD$_{600}$ reached 0.1.

2. Retrieved the bacteria and cultivated them in 2 mL of fresh medium. Added IPTG for induction.

3. In the stationary phase, we measured the fluorescence.
GFP & mRFP was suppressed by asB0034

asB0034 suppressed both GFP and mRFP.
Evaluation of Specifically Suppression

B0034-mRFP

B0032-mRFP

asB0034

asB0034
Suppression of B0034-mRFP & B0032-mRFP by asB0034

asB0034 down regulates B0034-mRFP more efficiently than B0032-mRFP.
Conclusion

Anti-sense B0034

- can down regulate wide range of target gene controlled by B0034.

- suppress B0034 more than other RBS.

We got common anti-sense RNA!!
Cupid’s magic

1. H-stem

2. Anti-sense RBS

3. Length
Making the best asRNA is difficult.

Which pattern is the best?
**Method**

- **XhoI**
- **NcoI**
- **30nt**
- **60nt**
- **90nt**
- **120nt**

**Genetic Constructs**:

- \( P_{tet} \)
- **stem**
- **NcoI**
- **mRFP**
- **stem**
- **dT**
Method for Assay

1. Cultivated a colony of transformed bacteria in 2 mL of medium until the turbidity at $OD_{600}$ reached 0.1.

2. Retrieved the bacteria and cultivated them in 2 mL of fresh medium. Added IPTG for induction.

3. In the stationary phase, we measured the fluorescence.

\[ OD_{600} = 0.1 \]
Results

Length Variation

mRNA expression (a. u.)

<table>
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<tr>
<th>Length Varia5on</th>
<th>0</th>
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<th>2000000</th>
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<th>3000000</th>
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On going work

PCR system + E. coli polymerase
97°C → 0°C → 37°C
Our method

Selection
- You would get the anti-sense you want

Making diversity
- E. coli polymerase
- R-random primer

Amplifying products
- Common PCR
- Random fragments as primers
Conclusion

Length variation

• We found a good approach to get powerful anti-sense.

Let’s make powerful anti-sense!!
Cupid’s magic

1. H-stem
2. Anti-sense RBS
3. Length
Outreach
General public got correct knowledge for gene recombination !!!
Special Thanks

Nakashima N. and Tamura T.  
He is a professor, JAPAN, who came to provide pHN1257 vector for us. 

Citizen, who came to Hokkaido University Festival  
We held workshop. 

Sapporo Kita high-school students and teachers  
We held a session about gene recombination.
Achievements

- Team registration.
- Complete Judging form.
- Team Wiki.
- Poster and a talk for the iGEM are ready.
- We described all attributes clearly.
- We built, characterized, and documented new parts: BioBrick.
- We experimentally validated new BioBrick Parts.
- We documented the characterization of those parts.
- We submitted those new parts to the iGEM.
- We consider problems about ethics.
- We considered our problem in Safety and Discussion.
- We improved the function of an existing BioBrick Part or Device.
- We collaborated with eight teams as iGEM Japan in The 86th Annual Meeting of The Genetics Society of Japan in Nagahama, Shiga.
- We described a new approach that our team used to address some questions and evaluate our approach; Safety and Discussion.

Satisfied criteria for gold medal!!
anti-sense RNA’s love blossomed by Cupid magic.