Asia Jamboree

Gold Medal and Best New Biobrick or Device, Engineered
Codon Switch
Controlling Protein Biosynthesis
Background
mRNA

aaRS
Codon Usage Frequencies in *E.coli*

**Rare Codons**

*fig* by Chunchun
Rare tRNA

mRNA

Rare Codon

fig by Chunchun
Problem

mRNA

Rare Codons
mRNA

New Application

Rare Codons
Rare-Codon Switch
mRNA

aaRS

Rare Codons
Rare Codons

Charged tRNA

aaRS

Rare Codon

Rare Codons
tRNA

aaRS

Rare Codons

Rare Codon
tRNA

aaRS

Rare Codons
Native ArgRS

Arg

tRNAArg-AGG

RFP

mRNA

6AGG codons

RFP-6AGG
Overexpressing tRNA_{Arg}
Bright RFP Merged

RFP

RFP-6AGG

Over-expressing tRNA^{Arg} RFP-6AGG
AspRS

Asp

tRNA^{Asp}-GAC

CUG

mRNA

anticodon recognition domain
Modified AspRS

tRNA\textsuperscript{Asp-AGG}

mRNA

UCC

Asp

AGG
RFP-6AGG

Luciferase-4AGG
Modified AspRS

Asp

tRNA_{Asp-AGG}

AGG codons

RFP/ luciferase

mRNA

RFP-6AGG/ luciferase-4AGG
Bright RFP Merged

RFP

RFP-6AGG tRNA\textsuperscript{Asp}-AGG

RFP-6AGG tRNA\textsuperscript{Asp}-AGG Modified AspRS
Modified AspRS | tRNA$^{\text{Asp}}$-AGG | Luciferase-4AGG
--- | --- | ---
- | + | +
Rare Codons

tRNA

aaRS

Rare Codon Switch

Rare Codons
2xAGG-luciferase
4xAGG-luciferase
6xAGG-luciferase
8xAGG-luciferase
Influence of Inserted Rare Codon Number

**Model**

- **Product Protein**
  - 2 AGG
  - 4 AGG
  - 6 AGG
  - 8 AGG

**Experiment**

- **Relative Light Units**
  - 2 AGG
  - 4 AGG
  - 6 AGG
  - 8 AGG

**Predicted Product**

- **Relative Protein Amount**
  - Number of Rare Codons: 2, 4, 6, 8

**Actual Product**

- **Relative Light Units**
  - Number of Rare Codons: 2, 4, 6, 8
Influence of Inserted Rare Codon Number
Predictions Made by Characterization Curve

![Graph showing predictions for induced and non-induced states based on the number of rare codons. The graph plots relative light units against the number of rare codons per unit. The x-axis represents the number of rare codons, while the y-axis represents the relative light units. The graph shows a linear relationship for induced states with a higher light output compared to the non-induced states, which remain constant.]
Rare Codon Locations

- 8AGG
- 4AGG
- 30bp
- 111bp
- 4AGG
- 4AGG
Tandem Copy Number
Higher Yield

Lower Background

Graphs showing Relative Light Units over time (hour) for different conditions.

- Left graph: Comparison of 2*4AGG and 4AGG with data points and curves.
- Right graph: Comparison of 8AGG and 2*4AGG with data points and curves.
Rare-Codon Switch

Strict Switch

Stop-Codon Switch

Initial-Codon Switch
Stop-Codon Switch
luciferase -TAG
tRNA\textsuperscript{Asp}-GAC

AspRS

anticodon recognition domain

mRNA

CUG

GAC
tRNA_{Asp-GAC}

mRNA

GAC

CUG

tRNA_{Asp-TAG}

mRNA

UAG

Modified AspRS

Asp

tRNA^{Asp-TAG}

AUC

mRNA
Modified AspRS

Asp

tRNA$^\text{Asp-\text{TAG}}$

Luciferase-TAG
Stop-Codon Switch

**Modified AspRS**
- tRNA$^{\text{Asp-TAG}}$
  - Luciferase-TAG
  +

Relative Light Units

- $0.00E+00$
- $1.00E+05$
- $2.00E+05$
- $3.00E+05$
- $4.00E+05$
- $5.00E+05$
- $6.00E+05$
Initial-Codon Switch
KanR-CGA
tRNA^{fMet}-ATG
MetRS
Met
UAC
AUG
mRNA

anticodon recognition domain
Modified MetRS

Met

tRNA^{fMet}-CGA

GCU

mRNA

CUG

tRNA^{fMet}-CGA

CGA
Initial-Codon Switch

Survived

Not survived

Initial-Codon Switch ✓
## Achievements

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Applications
Applications

Expansion of Regulating Tools for Synthetic Biology

- Multi-Level Regulation
- Direct and Precise Regulation

New Methods for Protein Function Research

- One Codon Different Amino Acids
- One mRNA Different Proteins
Multi-Level Regulation

- AGA
- AGG
- CGA
- CUA
- Rare codon type
- Rare codon number
- Rare tRNA amount
- Different Strength of induction

Protein Expression Level

1, 2, 3, 4, 5, 6, 7, 8, 9
Direct and Precise Manipulation
Positive Feedback
Positive Feedback

(n)AGG

Luciferase
tRNA<sup>Arg</sup> gene
Positive Feedback

![Graph showing positive feedback with two lines: one for PT7-luc-6AGG-tRNA<sup>Arg</sup> and one for PT7-luc-6AGG. The y-axis represents Relative Light Units, and the x-axis represents Time (hour).]
Positive Feedback
Protein Function Research
One Codon
Different Amino Acids
One mRNA
Different Proteins
mRNA

RBS

UAG

Applications

No Codon Switch

Stop-Codon Switch

Initial-Codon Switch

Peptide part 1

Complete peptide

Peptide part 2
## Conclusions

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Human Practice
Professors
Classmates
Other iGEM teams

New ideas
Broader views
Deeper thoughts
New friends
High School Activities

Presentation

Questionnaire

Interaction
It is also your stage!
Do you have interest in synthetic biology?

- Before the presentation:
- After the presentation:

**percentage**
Q: How to avoid the miscalculation of tRNA, if we introduce tRNA and aaRS pair?
A: The miscalculation can be avoided by the aaRS’s ability to recognize the other parts of the tRNA than anticodon. (We put it in a plain way so that they can understand it with ease)
Q: What is the application of the project?
A: For example, sometimes changing amino-acid on active center of an enzyme would have a great effect on enzyme activity. Our project provides an easy way to introduce point mutation at the active center.
Q: Is the project done by the experiment or just a theoretical research?
A: What we have shown is our experiment result. We have spent a lot of time in the lab on our project. iGEM is not only a competition of ideas, but of fulfilling them as well.
Q: What is the significance of this competition to the senior school students?
A: Taking part in iGEM is a good chance for the students to learn about academic thinking ways and helps them make decision about their future career. By showing the usefulness of synthetic biology, iGEM raises students’ confidence to further study biology.
Q: It makes the living creature as the toys of the human-beings, doesn’t it?
A: No, living creature could never be the toy of the human-being, no matter how much we can manipulate living creatures by the method of genetic engineering and synthetic biology. We pay as much attention to bio-safety and ethics as to the experiment and technology.
Problems for high school students to participate in iGEM:

- Academic pressure
- Lack of experimental skills
- Lack of funding
- Limited professional knowledge
Our Team
Acknowledgement
Thank You!