Non-canonical amino acids (ncAAs) in nature and lab

Synthetase/tRNA pairs not well characterized

System for measuring fidelity and efficiency
### The Genetic Code: 64 Codons, 20 Amino Acids

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</table>

TAG = “Amber” codon
Translation with a Canonical Amino Acid

Amino Acid → tRNA Synthetase → tRNA → Charged tRNA → Translating Ribosome → Polypeptide
Expanding the Genetic Code

Non-canonical Amino Acid

Modified tRNA Synthetase

Charged tRNA

Recognizes “amber” codon

Translating Ribosome

Polypeptide
Non-canonical Problems and Limitations

Post-translational modification not specific enough

Mass spectrometry demands time and money

Poor characterization of existing pairs
Non-canonical Amino Acids

L-tyrosine
3-amino-L-tyrosine
4-azido-L-phenylalanine

o-(2-nitrobenzyl)-L-tyrosine
What Non-canonicals Need

A solution that is:

- Time efficient
- Cost effective
- Easy
- Accessible
Genetic Code Expansion Pack Measurement Kit

- Cheaper alternative, *in vivo*, qualitative & quantitative, and inducible.
- Uses RFP and GFP fluorescence.
- Easy to learn and use.
- Easily adapted for any tRNA synthetase/tRNA pair.
Kit Components

Control Strain:
- pStG
- pFRYC

Experimental Strain:
- pStG
- pFRY
How It Works

pFRYC – control reporter

pFRY - experimental reporter

-ncAA

+ncAA
Non-canonical Amino Acids

- 4-azido-L-phenylalanine
- o-(2-nitrobenzyl)-L-tyrosine
- 3-amino-L-tyrosine
- 4-azido-L-phenylalanine
A Good and a Bad Synthetase/tRNA Pair

GFP Relative to RFP

Normalized Fluorescence Values

-ncAA
+ncAA

AzF-FRYC AzF-FRY

APPROVED
A Good and a Bad Synthetase/tRNA Pair

GFP Relative to RFP

Normalized Fluorescence Values

AzF-FRYC  AzF-FRY

AminoY-FRYC  AminoY-FRY

APPROVED

REJECTED
Incorporation Value for ncAA tRNA Synthetase/ tRNA pairs

![Graph showing incorporation values for different ncAAs with approved and rejected labels.](image-url)
The Expanded Genetic Code Measurement Kit

Our Measurement Kit:

- Time Efficient and Cost Effective
- Can be used by undergraduates; Portable
- Characterized 7 ncAA tRNA synthetase/tRNA pairs:
  * 4 showed high fidelity, 3 showed low fidelity.
  * These 4 pairs can be confidently used.

Future Plans:

We will confirm our results to mass spectrometry.
# BioBrick Parts

## Part: BBa_K1416000

**Designed by:** Jordan Monk  
**Group:** iGEM14_Austin_Texas  
*(2014-10-06)*

The tRNA synthetase/tRNA needed for incorporating o-(2-nitrobenzyl)-L-tyrosine (ONBY) at a UAG codon

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ONBYRS promoter  
ONBYRS  
IRNA  
tRNA promoter  
terminator

**Assembly Compatibility:**  
10 12 24 23 25 1000

## Part: BBa_K1416001

**Designed by:** Jordan Monk  
**Group:** iGEM14_Austin_Texas  
*(2014-10-06)*

The tRNA synthetase/tRNA needed for incorporating 3-iodo-L-tyrosine (IodoY) at a UAG codon

<table>
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IodoYRS promoter  
IodoYRS  
IRNA  
tRNA promoter  
terminator

**Assembly Compatibility:**  
10 12 24 23 25 1000
Utilizing a Functional ncAA tRNA Synthetase/tRNA Pair

\[ \text{o-(2-nitrobenzyl)-L-tyrosine (ONBY)} \]
ONBY: The Photocaged Tyrosine ncAA
Photocaged T7 RNA Polymerase (RNAP)

Caged T7 RNAP

365 nm Light

Active T7 RNAP

Gene

Translated Protein

Caging Group
Temporal Control of GFP Expression

Fluorescence (RFU)

0 minutes
1 minute
5 minutes
15 minutes
30 minutes

Negative control  Positive control  ambT7-GFP
Photocaging T7 RNAP with ONBY works, but the system is not yet perfected
Human Practices: Caffeinated Coli

• Invitation to SXSW Create provided the team with the opportunity to reach out to the public and raise awareness for the science of synthetic biology
Data Collection

- Collect coffee samples from various coffee houses across town
- Engage coffee shop owners and employees about our project
Learning Tricks of the Trade

- Senior members engaged newer members and taught essential research techniques.
- Excellent introduction to synthetic biology
Community Response

Coffee drinkers

Home brewers

Coffee brewers

Wright Bros Brew & Brew

Relative Caffeine Content in a 12oz Cup of Coffee

- Wright Bros Brew
- Houndstooth
- Thunderbird
- Thal Fresh
- Geisha
- Cherrywood
- Epoch - Light
- Cenote - Dark
- Flipirts
- Java Live on 5th
- Hideout Coffee
- Russell's Bakery
- Lavazza Expression
- Halcyon
- Kick But\n- Average
- Once over Coffee
- Bennu
- Genuine Joe
- Palma's Coffee
- Dominican Joe's
- The Coffee Cup
- Fair Bear Coffee
- Stinson's Coffee
- Monkey Ness
- Summernoon Coffee
- Austin Java
- Jo's Coffee
- Opa Coffee
- Hot Rod Coffee
- Vintage Heart
- Le Cafe Crepe
- Corona Cafe
- Ilie Bean Coffee
- Café Rubus
- Strange Brew
Achievements

- Successful creation of the **Expanded Genetic Code Measurement Kit**
- Characterization of seven ncAA synthetase/tRNA pairs
- Successful recreation of photocaged T7 RNAP
- Successfully controlled GFP expression spatially and temporally using the photocaged T7 RNAP
- Submitted two BioBrick parts of functional ncAA synthetase/tRNA pairs
- We completed the interlab study as part of the measurement track
- We are now making the **Expanded Genetic Code Measurement Kit** (not a BioBrick) available to anyone who requests it
Acknowledgements

Special thanks to:

Razan Alnahhas
Mike Hammerling
The Barrick Lab
Sean Leonard

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College of Natural Sciences