# Mutated Leucyl-tRNA Synthetase and its Use in the Incorporation of Unnatural Amino Acids

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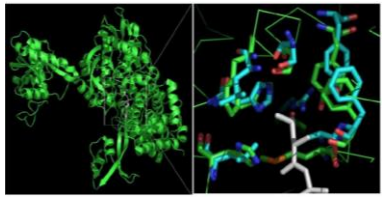
## Introduction

- The genetic code has traditionally used the standard 20 amino acids in the process of translation.
- The incorporation of unnatural amino acids could be beneficial in studying protein structure, function, and even lead to advantageous enhanced properties.
- The aminoacyl-tRNA synthetase(aaRS), which is responsible for the charging of amino acids to corresponding tRNAs, can be mutated and the tRNA can suppress the amber stop codon instead of ending translation.
- The pair of *Methanobacterium thermoautotrophicum* leucyl-tRNA synthetase (MLRS) and *Halobacterium sp. NRC-1* leucyl-tRNA shows little cross-reactivity in *E. coli*.
- The leucine binding domain and CP1 editing domain of MLRS were identified from the structural homology between *P. horikoskii and T. thermophilus* leucyl-tRNA synthetase.



Structural homology of the leucyl-tRNA synthetases derived from *T. thermophilus* and *P. horikoskii*

## Objective

- To generate a library of the MLRS whose five amino acids are randomized in the leucine binding site.
- Positive and negative selection steps will be taken to determine the best mutant that can both suppress the amber stop codon and charge only unnatural amino acids.

## Method

Generation of pSupK-MLRS-HL(TAG) ΔCP1

- A six codon (NNK) linker sequence replaces the CPI editing domain and a library of possible mutants $10^9$ was created using PCR.
- The library was inserted into the pSupK-MLRS-HL(TAG) plasmid and transformed into GH371 E.coli containing the pBREP plasmid.
- Selection using chloramphenicol reveals mutants that suppress the amber codon. Fluorescent colonies are collected and sequenced.

Construction of the N-Terminal Library

- The A32 and Y33 positions in the leucine-binding site were randomized.
- The N-Terminal library was inserted between the NdeI and MluI restriction sites of pSupK-MLRS-HL(TAG) ΔCP1.
- The library was transformed into DH10B E. coli. The N-terminal library was isolated from the colonies.

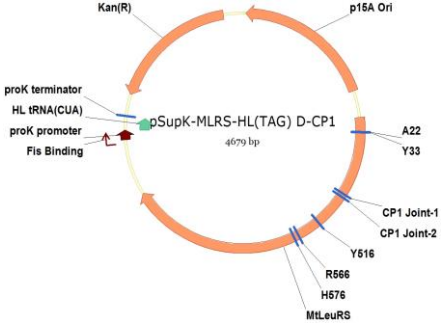
Construction of the C-Terminal Library

- A two step PCR procedure was used to generate mutations at the Y516X, R556X, and H576X positions.
- To increase the size of the insert a second PCR step was taken using DraIII F and AflII R primers.
- The library was inserted into the N-Terminal library and transformed in GH371 E. coli containing the pRCG plasmid.

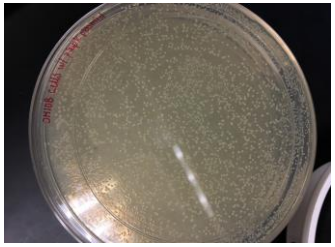
Dual Genetic Selection

- The GH371 E. coli cells containing the library is grown in the presence of chloramphenicol. The mutants that suppress the amber codon survive and fluoresce green.
- If the MLRS DCP1charge leucine to the tRNA and not the unnatural amino acid, the cells die in the presence of 5-fluorouracil.

## Results

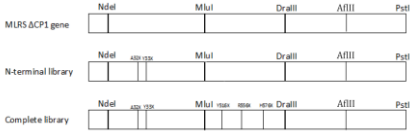



- The best pSupK-MLRS-HL(TAG) ΔCP1 mutant was sequenced and the amino acid sequence was Leu-Tyr-His-Ala-Val-Tyr.
- The N-Terminal library was transformed into DH10B *E.coli* and the plasmids were isolated.



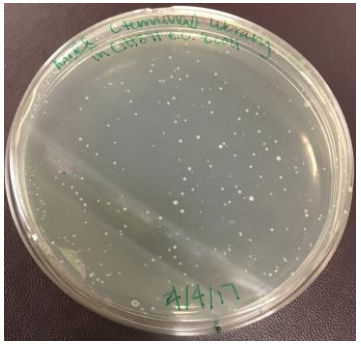
N-Terminal Library transformed in GH371 E.coli containing the pRCG plasmid

- The C-Terminal library was inserted into the AflII and MluI restriction sites of the N-Terminal library and transformed into GH371 *E. coli* cells




Relative mutation and restriction sites of the library

## Conclusion



C-Terminal library in GH371 E.coli containing the pRCG plasmid

- A library of mutant leucyl-tRNA synthetase was generated with a mutated CPI editing domain and randomized active site.
- The transformation efficiency of the C-terminal library is too low right now to cover all possible mutants.

## Future Plans

- The transformation efficiency of the library needs to improve before the selection process can proceed. Further transformations will be performed with GH371 E. coli until all possible mutants have been accounted for.
- The plasmids will be recovered and purified.
- The positive and negative selections will be performed and a best variant will be selected and sequenced.
- Further experiments will be performed using the variant in synthesizing a model protein called the Z-Domain.

## Acknowledgements

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