Genetic Selection of Leucyl-tRNA Synthetase Variants to Incorporate N-ε-acetyl Lysine into Proteins

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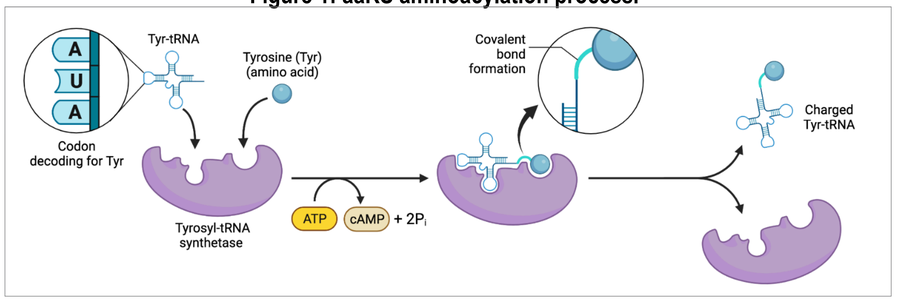
Introduction:

Proteins are synthesized by ribosomes in the process called translation. During translation, transfer RNA (tRNA) carries amino acids to the corresponding messenger RNA (mRNA) codons. To carry the correct amino acid to the right codon, the tRNA is "charged" by a specific aminoacyl tRNA synthetase (aaRS). The aaRS binds to both a specific amino acid and ATP. This leads to the formation of an aminoacyl-adenylate complex releasing pyrophosphate. A hydroxyl group of a tRNA then attacks the aminoacyl-adenylate complex, which releases AMP and subsequently tRNA that is now attached to its specific amino acid.

Figure 1: aaRS aminoacylation process.[1]



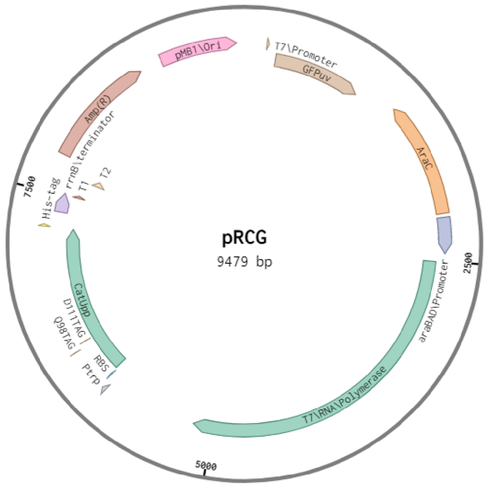
After synthesis, proteins undergo different post-translational modifications such as acetylation, lipidation, phosphorylation, etc. These modifications diversify and regulate protein's structure. This project focuses on N-ε-acetylation of lysine (AcLys), which is one of the most important post-translational modifications of proteins. One application of this study is using site-directed incorporation of AcLys to introduce novel functions to proteins.

Methanobacterium thermoautotrophicum leucyl tRNA synthetase (MLRS) variants were created in which the editing domain was removed to allow for unnatural amino acids (UAA) binding to the aaRS. In addition, *Halobacterium* sp. NRC-1 leucyl tRNA is used as the tRNA pair to the aaRS. Incorporation occurs through the use of amber stop codon (TAG), which is used in only 7% of termination in *E. Coli*. Previously, we have successfully randomized 5 positions in the MLRS active site to generate millions of different variants. These variants went through positive and negative selection rounds to test for successfuly incorporation of AcLys. Two clones made it through the selection process and are being tested for successful incorporation of AcLys at the 7th position of the Z-domain protein.

Methods:

Dual genetic screening of the MLRS libary:

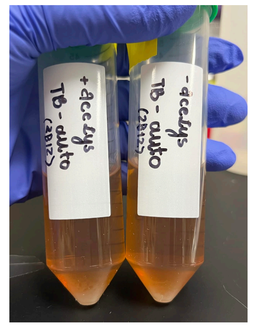
Figure 2: pRCG plasmid.[2]



- Positive selection selects for colonies with functional synthetases that charge tRNA with any natural amino acids or AcLys.
- Negative selection selects against colonies with synthetases that incorporate natural amino acids instead of AcLys. In the presence of 5-fluorouracil, cells that incoroporate natural amino acids will generate uracil phosphoribosyl transferase (UPRT), which inhibits thymidylate synthase and cause cell death.

Expression of Z-domain TAG mutant:

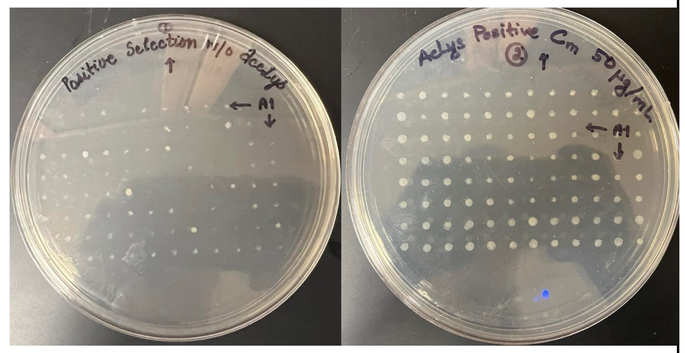
- Electrocompetent cells were transformed with DNA of the clones that made it thorugh the selection process and plated overnight.
- Cells were then placed in TB autoinduction media containing AcLys (positive) or no AcLys (negative) overnight for protein production.



Z-domain purification and concentration:

- Cells were harvested using centrifugation and lysed using sonication.
- The cell lysate was purified and visualized using SDS-PAGE.

Results:

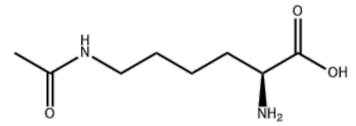


- AcLys	+ AcLys
+ Chloramphenicol	+ Chloramphenicol

Future Directions:

Further study of AcLys incorporation:

- Z-domain purification
- Mass spectrometry
- Study of function



References:

1. Reece, J. B., & Campbell, N. A. (2011). Campbell biology. Boston: Benjamin Cummings / Pearson.
2. Guedez, P. Directed Evolution of Synthetic Riboswitches and a Leucyl tRNA Synthetase. Ph. D. Dissertation, Texas Christian University, Fort Worth, TX, 2022.

Acknowledgement:

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