Directed evolution of an archaea leucyl-tRNA synthetase for the incorporation of unnatural amino acids into proteins in *E. coli*

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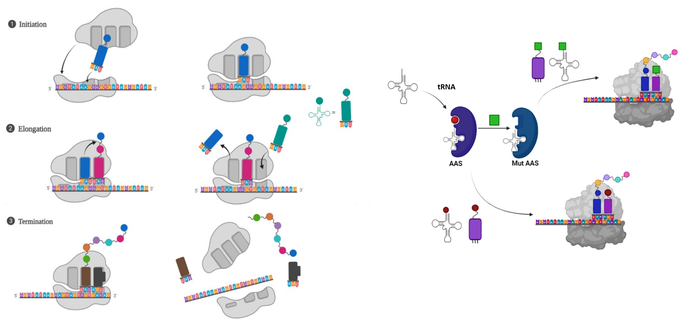
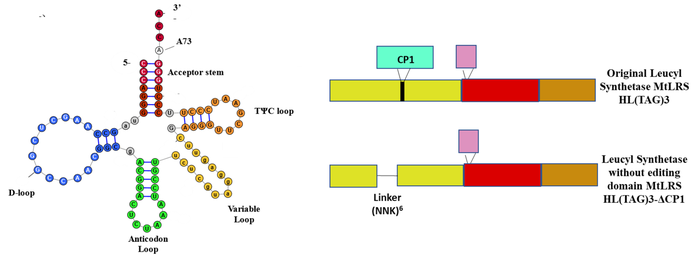
Introduction

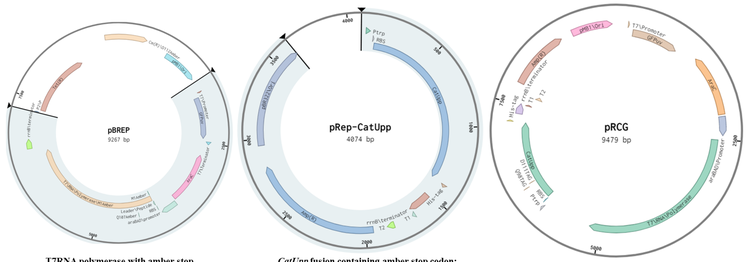
The genetic information is converted into proteins during the translation process in which the amino acids act as building blocks for the synthesis of the protein chains in a multi step process. Every amino acid is encoded by a codon or series of codons that dictate the position in which the amino acid will be incorporated. Specific enzymes called aminoacyl-tRNA synthetases (aaRS) load the corresponding amino acid onto a tRNA molecule that act as a carrier delivers it at the right position during protein synthesis.[1]



There are three codons that do not encode any amino acid, but indicate when the process is completed (stop codons). The amber stop codon, which is used only in about 7% during translation in *E. Coli* and it does not interfere with many essential genes, have been used as a blank slate to incorporate unnatural amino acids (UAAs) into proteins, allowing to gain new functions.[2] To accomplish this, an orthogonal pair of aaRS/tRNA that does not cross-react with the endogenous species but with the unnatural amino acid must be used. Libraries of possible variants are screened against the unnatural amino acid in a process in which marker genes are modified with the amber stop codon, and their ability to incorporate UAAs is tested. We explored a pair composed of a *Methanobacterium thermoautotrophicum* leucyl-tRNA synthetase and *Halobacterium sp. NRC-1* tRNA.[3]



Designing a dual genetic selection system[4]



T7RNA polymerase with amber stop codon (M1TAG, Q107TAG)

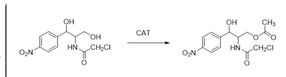
CatUpp fusion containing amber stop codon: Q98TAG D111TAG Q98TAG D111TAG

T7RNA polymerase with an amber stop codon

CatUpp fusion containing amber stop codon

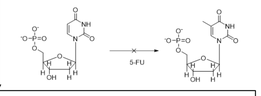
***E.coli* GH371 cells containing library**

Positive Selection — +Chloramphenicol + UAA



Survivors containing a functional synthetase express a full-length CAT protein that yields in chloramphenicol resistance

Negative Selection — +5-FU

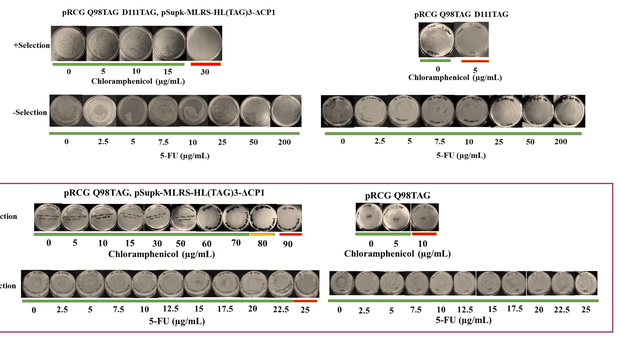


Variants that incorporate endogenous metabolites make a full length UPRT that converts 5-FU to a toxic product and cells die.

Next round of selection

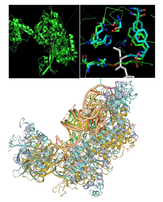
Re-test on plates with and without ligand and higher chloramphenicol concentrations

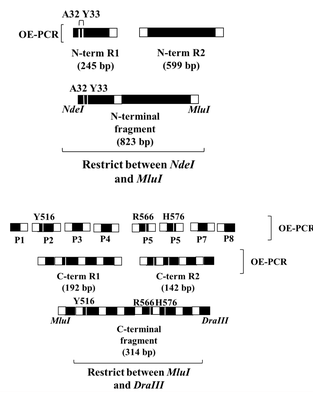
Determining the Selection Conditions



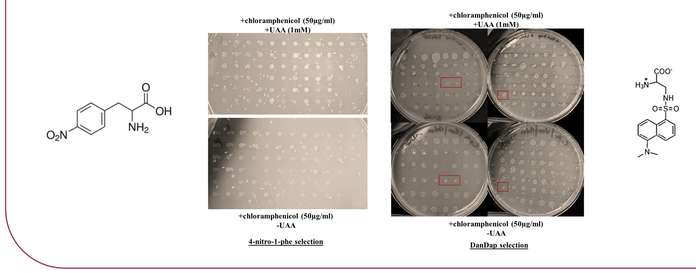
pRCG Q98TAG showed a chloramphenicol resistance up to 80 μg/mL and 5-FU sensitivity of 22.5 μg/mL

Designing a MtLRS library





Selecting the MtLRS library for Dansyl-Dap and 4-nitro-1-phe incorporation



Future Directions:

. More variants will be tested for the incorporation of 4-nitro-1-phe.
. The variants found for Dansyl-Dap selection are currently being sequenced and tested for the incorporation of Dansyl-Dap on Z-domain protein.

References:

1.Simonetti et al. *Cell. Mol. Life Sci.* **2008**, *66* (3), 423.
2.Ovaa, H.; Wals, k.. *Front. Chem.* **2014**, *2* (15), 1-12.
3.Anderson, J. C.; Schultz, P. G.. *Biochemistry* **2003**, *42* (32), 9598-9608.
4.Melançon, C. E., 3rd; Schultz, P. G. *Bioorg. Med. Chem.* **2009**, *19* (14), 3845-3847.

Acknowledgements:

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