

CALIFORNIA STATE SCIENCE FAIR 2011 PROJECT SUMMARY

Name(s)
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Project Number

31214

Project Title

Evolution of a Tryptophanyl tRNA/Tryptophanyl-tRNA Synthetase Pair for Encoding Unnatural Amino Acids in E. coli

Abstract

Objectives/Goals

The purpose of this research is to create a tryptophanyl tRNA and tryptophanyl tRNA Synthetase orthogonal pair that will be able to successfully and efficiently incorporate unnatural amino acids while in E. Coli.

Methods/Materials

Various chemicals, centrifuges, an electroporator, and PCR machines were provided by the Schultz lab at The Scripps Research Institute and used in the experiment. The first stage of the project was to clone out the tRNA and Synthetase and place them into another vector. The second step was to mutate different portions of these molecules in order to increase activity and maintain non-pross reactivity. At the end of steps 1 and 2, the pair was tested on chloramphenical antibiotic plates to est their properties. The final step was to randomize the acceptor stem of the tRNA and select an optimal one by putting it through positive and negative rounds of selection.

Results

For the different stages, gels were run to see if DNA fragments were the correct length, which they all were eventually. The antibiotic tests distinguished between the more active molecules as well as the cross reactive ones. Ultimately, two optimal strains of tRNA were created.

Conclusions/Discussion

The first step of incorporating unnatural amino acids is complete. An optimal tRNA and Synthetase were created. Subsequent work would be to engineer the active site of the Synthetase to incorporate unnatural amino acids. The applications for this work are engless. Unnatural amino acids expand the scope of proteins and allow us to create new ones with enhanced functions that can, for example, cure diseases.

Summary Statement

My project created a tryptophanyl tRNA and tryptophanyl-tRNA Synthetase pair platform that would be able to successfully incorporate unnatural amino acids in E.Coli.

Help Received

I would like to thank Df. Peter G. Schultz and his staff for making it possible for me to work in their lab, Dr. Abhishek Chatterjee for being a great mentor and answered all my questions, AFCEA for providing me with a grant for this work, and my parents for driving me to the lab.