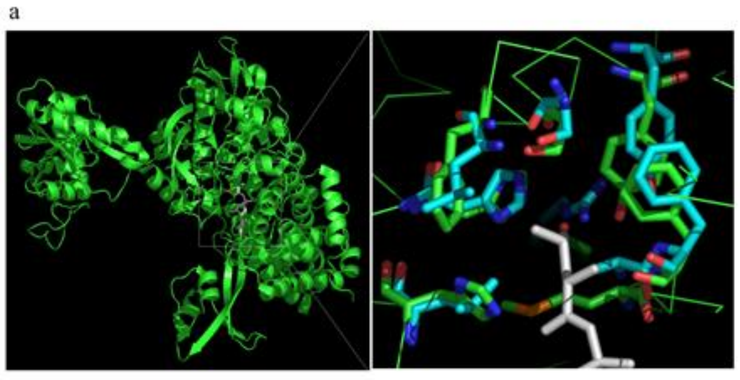
Genetic selection of leucyl-tRNA synthetase for the production of fluorescent proteins in living cells

Viet Dinh, Andrea Guedez, and Youngha Ryu
Department of Chemistry & Biochemistry

Introduction

This project is aimed to produce a molecular library of the leucyl-tRNA synthetase (LeuRS) without its editing domain. The randomization of the five amino acids in the leucine binding site generates about 34 million different LeuRS variants, from which the variants to incorporate two fluorescent amino acids will be selected by dual genetic selection (Figure 1). Fluorescent proteins are easy to detect and utilized in many different applications.



X-ray crystal structure of the *Thermus thermophilus* LeuRS

Structural homology in the leucine binding site between LeuRS's of *Thermus thermophilus* and *Pyrococcus horikoshi*

Structures of fluorescent amino acids that will be incorporated into proteins by the directed evolution of the LeuRS

Figure 1

Methods

- Overlapping extension PCR was used to obtain a library of LeuRS variants (Figures 2 and 3).
- The library was introduced to *E. coli* cells in a single step to ensure the highest possible transformation efficiency.
- Consisting of the alternating positive and negative selection, a genetic selection experiment will allow us to select the cells containing the LeuRS variants that incorporate fluorescent amino acids into proteins essential for their survival.
- After repeated selection steps, the successful variants will be isolated and tested for the effective incorporation of the unnatural amino acid with our model protein called the Z-domain.

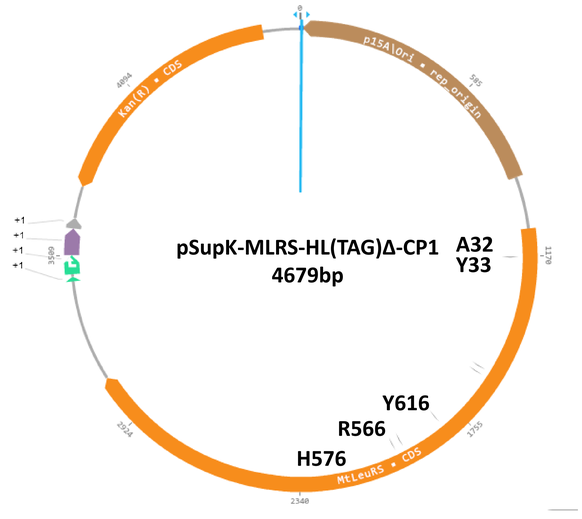


Figure 2. The map of the pSupK-MLRS-HL(TAG)Δ-CP1 plasmid.

Results

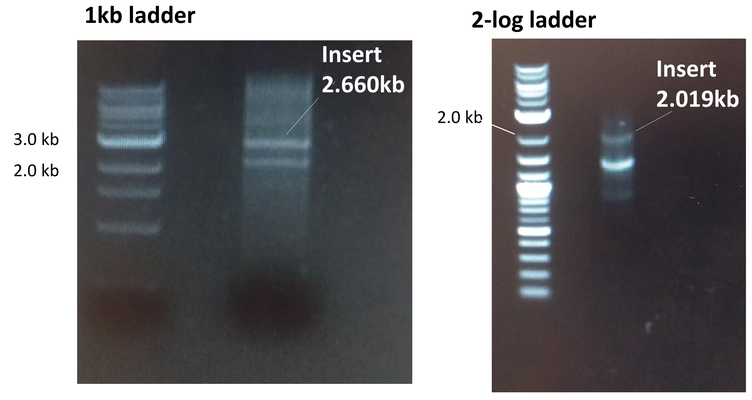


Figure 3. Construction of the pSupK-MLRS-HL(TAG)Δ-CP1 plasmid .

Summary

This project is aimed to modify LeuRS to incorporate fluorescent amino acids into proteins in order to produce fluorescent proteins in living cells. A complete library of LeuRS have been produced by overlapping extension PCR and introduced to *E. coli* in a single step. Currently, we are working on a large scale production of viable *E. coli* cells that cover the whole diversity of library. Consequently, the library of LeuRS variants will be subject to a genetic selection experiment to obtain LeuRS variants that incorporate only fluorescent amino acids into proteins.

Acknowledgements: TCU SERC