Designing, Cloning and Isolation of Mutant B-Glucosidase Enzymes

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Overview

Contributions to Design 2 Data (D2D) took place over the Spring 2021 semester at Truckee Meadows Community College. This project introduced students to biotechnological laboratory methods, while assisting a national project in gathering data on protein folding and assisting the predictability of protein folding software algorithms. The overarching goal of this national project is to test the accuracy of protein modeling software predictability in measuring an enzyme's catalytic efficiency (how well the enzyme works) when a single amino acid is substituted with another amino acid.

In Spring 2021, we performed the design and building stages of the D2D project. Novel mutants of Beta-Glucosidase B (BglB) were modeled within a protein folding software called FoldIt and then wet lab procedures were performed. DNA Oligos were ordered after designing mutants in FoldIt. The mutants were built through kunkel mutagenesis and *E. coli* were chemically transformed with the plasmids containing BglB Mutants.

Post-sequencing will consist of producing protein from our confirmed plasmids. Future functional and stability testing (testing stage) will be used to help verify and improve design algorithms for protein fold predictions in FoldIt. Protein folding software such as FoldIt are invaluable as they can be used to advance medical or agricultural research such as drug development. Foldit Education and Game modes had an impact on students during beta-testing for Biol 190L and 251L wherein the gamification of protein folding concepts helped solidify this information.

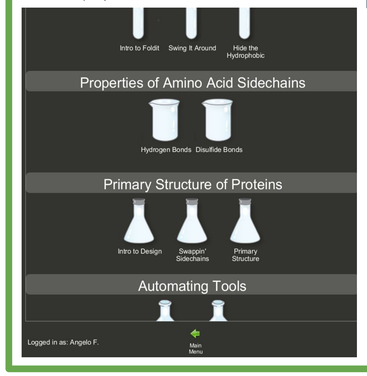
Introduction

Protein folding programs such as FoldIt can be used to advance medical and agricultural research, for example in drug development. We are adding and testing new variants of an enzyme (Beta-Glucosidase B; BglB) to a database originating from UC Davis in a national collaboration (1, 2). This national project is named Design to Data (D2D) as illustrated below. In Spring 2021, we performed the design and building stages of the D2D project. The future functional and stability results (testing stage) will be used to help verify and improve design algorithms for protein fold predictions.

Methods

Foldit Education

- Tutorial Mode
- 29 tutorials total
- Has a greater impact on a users ability to manipulate a protein without theory
- Will learn basic protein folding factors (emphasizing & visualizing lecture concepts)



Foldit Game Program

- Campaign gameplay
- 39 Levels total
- Each level has a specific problem to solve that relates to protein chemistry (puzzles)
- More challenging due to application of amino acids and spatial awareness

Methods

BglB mutants were designed in a protein modeling software known as FoldIt. DNA oligos that encode for the BglB mutations were ordered from Eurofins. The BglB mutants were first built using these DNA oligos and added to a plasmid (via Kunkel mutagenesis - oligo phosphorylation, annealing, and polymerization). Then DH5a *E. coli* were chemically transformed with these plasmids containing the BglB mutants. After transformations, the plasmids containing the BglB mutants are going to be extracted from bacteria using Qiagen Mini Prep kits.

Foldit Education and Game modules were developed to expand the design component of this course undergraduate research experience (CURE) to complement protein folding concepts from lecture. As seen below, the Foldit program provides a game-like twist to reinforcing protein folding concepts.

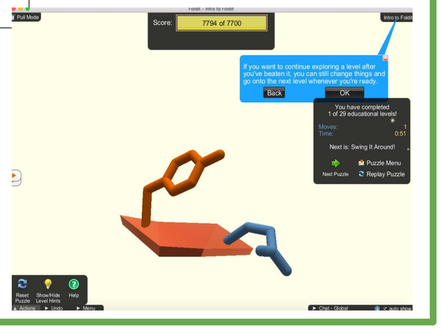
15mL and 2mL tubes containing 2uL size sample

Results

The BglB mutants were first built using DNA oligos using FoldIt. The plasmid with the BglB mutants were created via Kunkel mutagenesis. DH5a *E. coli* were chemically transformed with these plasmids containing the BglB mutants. Results of a successful transformation is pictured below.

Mutant	Original a.a.	Mutated a.a
E406D	Glutamic Acid (acidic; hydrophilic)	Aspartic Acid (acidic; hydrophilic)
W325K	Tryptophan (non-polar; hydrophobic)	Lysine (basic; hydrophilic)
E406W	Glutamic Acid (acidic; hydrophilic)	Tryptophan (non-polar; hydrophobic)
H178D (pic on the right)	Histidine (basic; hydrophilic)	Aspartic Acid (acidic; hydrophilic)
W325Y	Tryptophan (non-polar; hydrophobic)	Tyrosine (polar; hydrophilic)
W409H	Tryptophan (non-polar; hydrophobic)	Histidine (basic; hydrophilic)

Both Foldit Education and Game modes are effective in utilizing visualization and interactivity to cement certain concepts introduced to students in biology courses; students are asked to provide screenshots of their progress within the game (Foldit Education screenshot below). The Foldit Education module has been beta-tested by three sections of biology courses (190L/251L) at TMCC. The Foldit Game mode involving a COVID-19 science puzzle will be beta-tested at the end of the spring 2021 semester to emphasize practical uses of this program. These Foldit modules have been well-received and collected positive feedback from students that have already completed the modules.

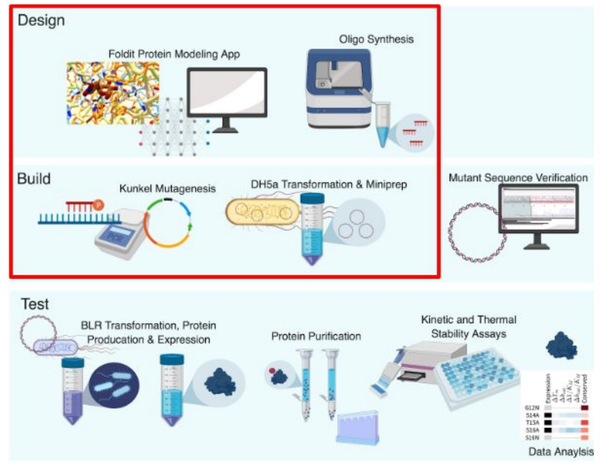


Conclusions

In Spring 2021, we performed the design and building stages of the D2D project (illustrated in next column). Plasmids containing the BglB mutants were built and transformed into *E. coli*. One mutant plasmid is currently being isolated using Mini Prep (H178D).

Both Foldit Education and Game modes are assisting students in reinforcing concepts related to protein folding and properties. Foldit Education was helpful for students in substantiating introductory concepts of protein folding, such as hydrophilic and hydrophobic behavior, and protein structure. These Foldit modules have been well-received and collected positive feedback from students that have already completed them.

Conclusions



The future functional and stability results (testing stage) will be used to help verify the BglB mutants and improve design algorithms for protein fold predictions. Our work of testing new variants of an enzyme (Beta-Glucosidase B; BglB) will be added to a database in a national D2D project. Protein folding programs such as FoldIt can be used to advance medical and agricultural research, for example in drug development, which highlights the importance of this project.

References

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