Optimizing DNA Concentrations using minipreps for sequencing and enzyme activity assays of BglB mutants

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Abstract

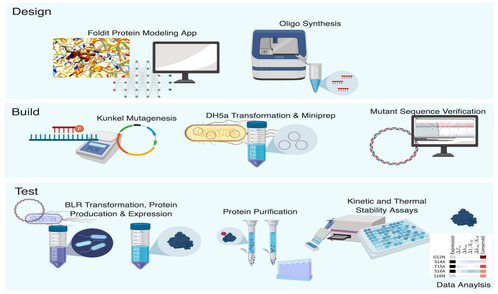
Contributions to Design 2 Data (D2D) took place over the Fall 2021 and Spring 2022 semesters at Truckee Meadows Community College. This project introduced students to biotechnological laboratory methods, while assisting a national project in gathering data on protein folding and assisting the predictability 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 and to provide an authentic research experience in the classroom.

In Fall 2021 and Spring 2022, we performed the build and test 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. The optimization of miniprep helped to increase DNA concentration around twofold and likelihood of confirmed mutations.

BglB proteins were expressed and purified after DNA concentration. Enzyme activity results for our mutant, E406W, suggested that the mutation stopped expression; the other mutants, W325Y and W409N, had lower enzymatic activity (lower catalytic efficiency of 20 vs. 100 mM^{-1} min^{-1} [k_{cat}/K_M] for mutants vs. WT). Protein folding software such as FoldIt are invaluable as they can be used to advance medical or agricultural research such as drug development. This research streamlined the D2D workflow in the classroom, which provides an authentic research experience.

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; workflow illustrated in Conclusions). In Spring 2022, we optimized the miniprep stage of the D2D project (second half of the build it stage). Additionally, we performed the kinetic and thermostability assays to determine the catalytic efficiency and heat stability of our enzymes compared to wildtype BglB.



Methods

- **Heat-Shock Transformation:** DH5a *E. coli* were transformed with plasmids containing the BglB mutants using a heat-shock protocol.
- **QIAGEN Miniprep:** Plasmids containing the BglB mutants are extracted from DH5a *E. coli* using QIAGEN miniprep kits.
  - Several changes were made to the miniprep protocol, all of which were helpful in yielding higher DNA concentrations - 1) Adjusted TB broth formulation and altered the amount of volume used for the overnight cultures, 2) pouring the supernatant onto the spin column, and 3) increased the elution time to 5 minutes.
- **Protein Expression & Purification:** BglB mutants were expressed in BLR *E. coli* and BglB proteins were purified using a Nickel column.
- **Enzyme Activity Assays:** The catalytic efficiency (k_{cat}/K_M) and thermal stability (T_{50}) were calculated by observing BglB enzyme conversion of substrate (PNG) into a yellow product. Enzyme activity was measured over a 15 minute period via the absorbance of the yellow product at 420 nm.

Results

The miniprep procedure was optimized by changing:

- the volume of the cells grown, the incubation time, the method of transference of the supernatant liquid, the elution time, and the TB formulation.

Each of these changes led to an increase in the DNA concentration as shown in tables 1 & 2.

	27-Oct-2021 (Class Research)	05-Nov-2021 (Our Research)	10-Nov-2021 (Class Research)	21-Jan-2022 (Our Research)
Overnight Growth Volume	2 mL	5 mL	5 mL	5 mL
Overnight Growth Duration	22-24 h	22-24 h	22-24 h	16-18 h
Miniprep Supernatant Transfer Method	Using Pipette	Pour	Pour	Pour
Miniprep Elution Buffer Time	1 min	5 mins	5 mins	5 mins
TB Formulation	Old w/ Glycerin	Old w/ Glycerin	Old w/ Glycerin	New w/o Glycerin

Table 1 - Miniprep Optimizations and Controls

	27-Oct-2021 (Class Research)	05-Nov-2021 (Our Research)	10-Nov-2021 (Class Research)	21-Jan-2022 (Our Research)
Range (ng/uL)	5.70-34.30	14.80-33.10	13.40-47.80	36.90-75.60
Average (ng/uL)	18.71	25.73	29.77	49.47
Samples > 25 ng/uL (for sequencing)	5/28 = 17.9 %	3/6 = ~50.0%	23/29 = 79.3%	6/6 = ~100.0%

Table 2 - Resulting Percentage of Utilizable Plasmid DNA Concentrations After Each Optimization

The enzyme activity assays were performed next:

- E406W had no protein expression, and both W409N and W325Y had lowered catalytic activity compared to wildtype.

These results are illustrated in Table 3 & Figure 1.

	Enzyme Activity	
DNA Sample	Catalytic Efficiency (k_{cat}/K_M in mM^{-1} min^{-1})	Thermal Stability (T_{50} in oC)
WT	102.60	41.0 ± 0.2
E406W	N/A (No Protein Expression)	N/A (No Protein Expression)
W409N	22.44	40.2 ± 0.2
W325Y	21.42	40.8 ± 0.2

Table 3 - Results of Enzyme Activity in WT and Mutants

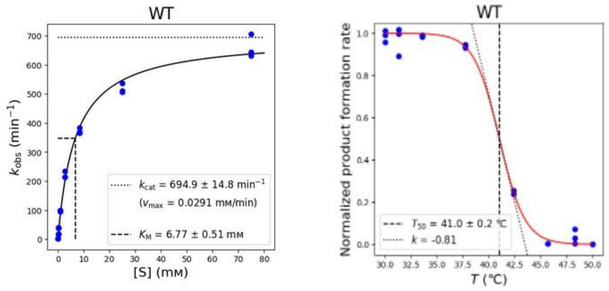


Figure 1 - Catalytic Effeciency and Thermal stability of wildtype BglB

Conclusions

Between Fall 2021 and March 2022, we performed the remainder of the workflow of D2D, but first focused on the optimization of the Miniprep step in the DNA transformation process. This was done by:

- increasing the volume of cells grown, decreasing the amount of incubation time, pouring out the supernatant liquid instead to pipetting it out, extending the elution time from 1 minute to 5 minutes, and using a different TB that did not contain Glycerin.

In doing so, students were much more able to achieve large enough plasmid DNA concentrations and higher likelihood of confirmed mutants in order to move on to the "Test" module of this project.

Enzyme activity assays were then performed on mutants E406W, W409N, and W325Y.

- E406W had no protein expression, while both W409N and W325Y had lowered catalytic activity when compared to wildtype BglB.

The verification of our BglB mutants will help 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. This research streamlined the D2D workflow in the classroom, which provides an authentic research experience.

References

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