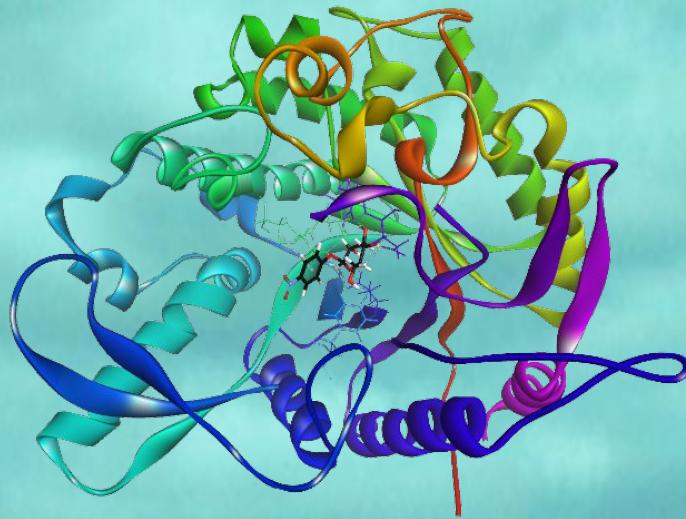


Design-2-Data CURE

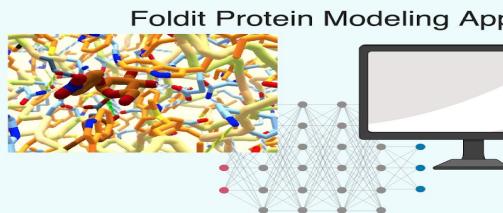


By Cameron McCoy and Aria Khoshjabinzadeh

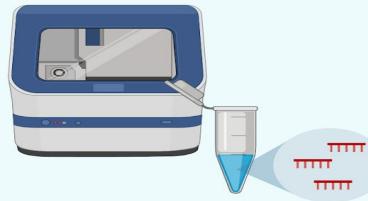
Goal: test the accuracy of protein modeling softwares predictability in measuring an enzyme's catalytic efficiency (how much product is formed) when a single amino acid is substituted with another amino acid.

- Beta-Glucosidase (BglB) variants will be added to a Data Set originating from UC Davis which is used to train new design algorithms for functional and stability predictions
- As of 2019 there are over 129 mutants
- This allows for a broader exploration of the structure-function relationships in BglB

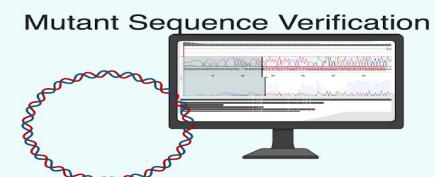
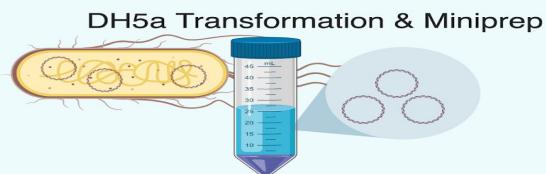
Design



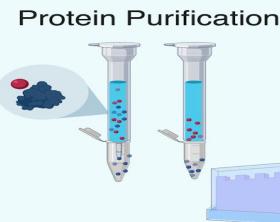
Oligo Synthesis



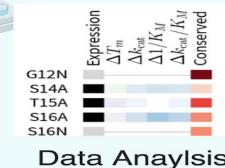
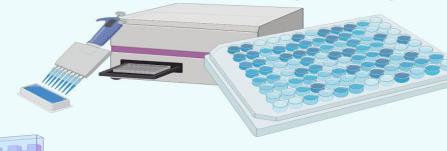
Build



Test



Kinetic and Thermal Stability Assays



Design



- The protein modeling software that was used is called Foldit Standalone.
- A design of Beta-glucosidase was uploaded to the program
- Any amino acid near the active site of the enzyme was clicked on and swapped with another amino acid

Original Amino Acid Residue	New Amino Acid Residue	Mutant Name
Tryptophan	Tyrosine	W325Y
Tryptophan	Lysine	W325K
Tryptophan	Histidine	W409H

Beta-Glucosidase (BglB)

Family 1 Glycoside Hydrolase

Comes from *Paenibacillus polyxma*

- Plant growth promoting Rhizobacteria (PGPR)
- Reduces the occurrence or severity of plant disease

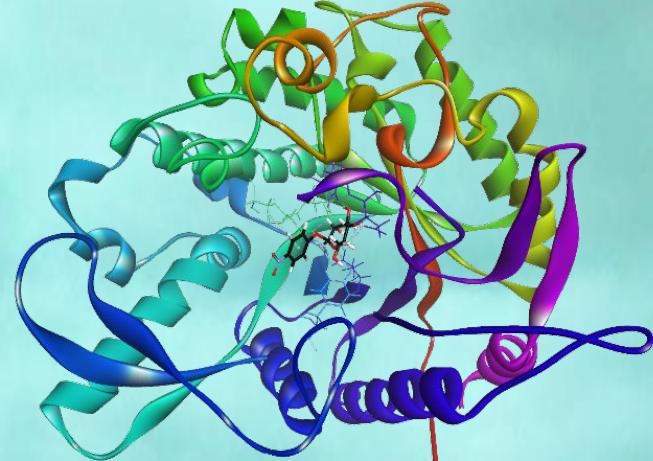


Fig. 1. Beta-Glucosidase B (target enzyme)

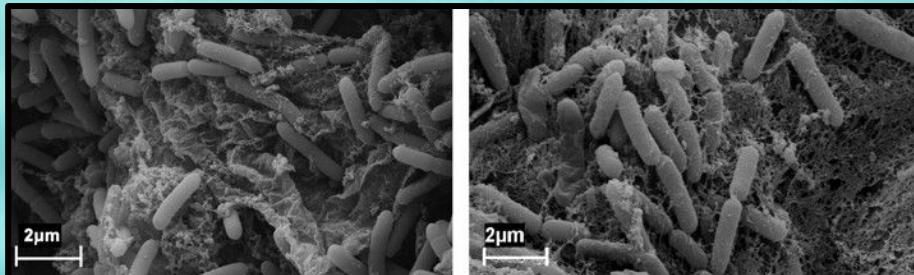


Fig. 2. Plant roots colonized by *P. polyxma*

Ordering Oligos

DNA oligos were ordered from Eurofins that encode for the mutations in the *bglB* gene (33-mer)

TMCC001 W325K: TTCCGGATGAATTCTTTACCCATATCGGTAAC

Wildtype: TTCCGGATGAATTCCCAACCCATATCGGTAAC

TMCC003 W325Y: TTCCGGATGAATTCTATAACCCATATCGGTAAC

TMCC002 W409H: ACGTTGCTATAACCATGTGCCATTCAAAGTT

Wildtype: ACGTTGCTATAACCCATGCCATTCAAAGTT



The background features a wide expanse of light-colored sand dunes stretching towards a horizon under a sky filled with heavy, textured clouds.

BUILD

Plasmid Construction

- ① 5' phosphorylation of oligo with Kinase reaction
- ② Annealed oligo to viral vector pET-29b(+) with 5 flanking amino acid codons
- ③ Polymerized a complementary strand of DNA with free nucleotides using polymerase and ligase

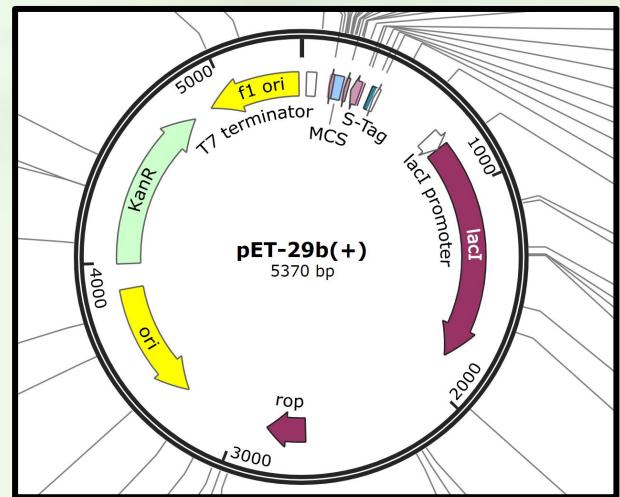
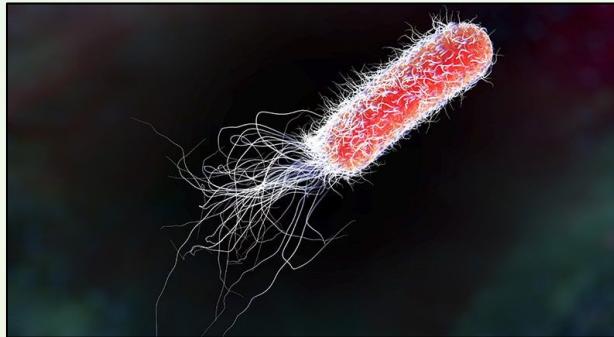


Fig 1. pET-29b(+)

The bacteria transformed was

DH5a *E.coli*

- Provided by UC Davis
- Very good at replicating DNA



Bacterial Transformations

Using Eppendorf Electroporator to introduce plasmid into DH5a



Fig 1. Eppendorf Eporator

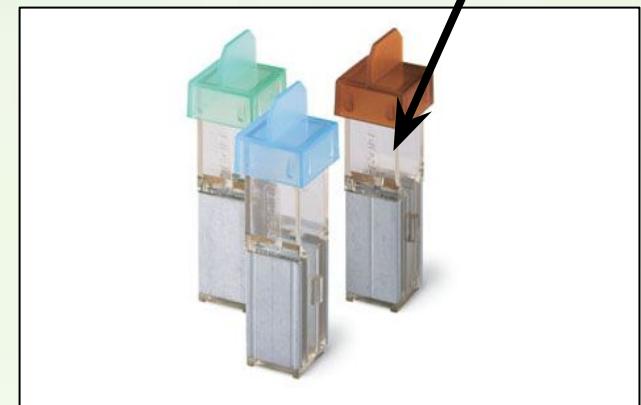


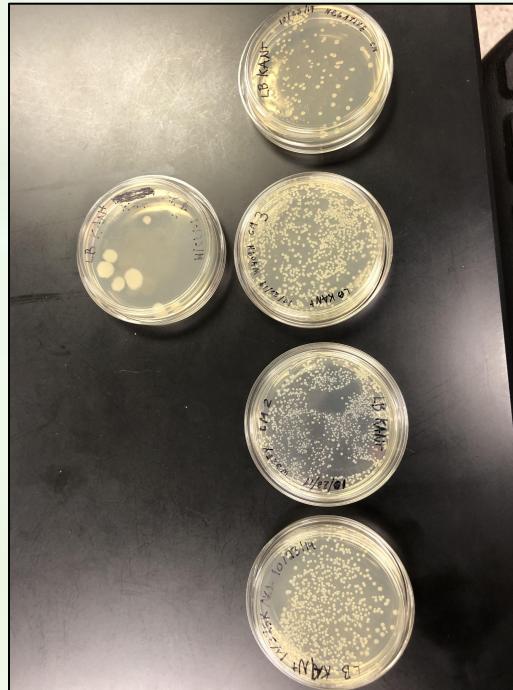
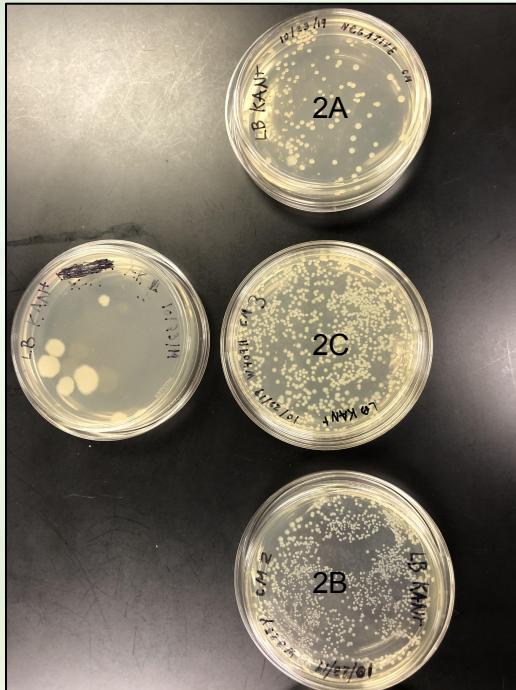
Fig 2. Electroporation Cuvettes

Mixture of DH5a
and Dialyzed DNA



**Bacterial Cells were transformed with pGlo to
test the Eppendorf used for Electroporation**

Transformed Bacteria



3 LB agar plates with Kanamycin were plated with separate colonies

MiniPrep: Plasmid Extraction



The plasmid DNA needs to be extracted from the Bacterial cells

Qiaprep 2.0 columns have a silica membrane for selective adsorption of our Plasmid DNA

- RNA, proteins, and metabolites pelleted while the supernatant contains DNA
- The leftover DNA is eluted from the membrane

Aria: 1

Cameron: 2

Sharif: 3

DNA Concentration Assay

The concentration of the DNA had to be determined to see if our MiniPrep was successful in eluting a sample of DNA pure enough to be sequenced

	1A	1B	1C	2A	2B	2C	3A	3B	3C
A260/A280	2.05	2.07	1.87	2.02	2.06	1.92	2.02	2.08	2.03
ng/uL	77.5	55.4	42.8	22.2	34.0	27.5	53.7	51.9	35.4



Fig 1. Nanodrop 2000c

Electrophoresis



5,371 base pair plasmid

WHERE DO WE
GO FROM HERE?