Site-Directed Mutagenesis of Malate Dehydrogenase: A Class Project

Dr. Bruce Heyen, Emily Veach, Jon Zatorski, Chesley Rowlett, Ryan Burch, and Andrew Gemmaka

Challenge:

Make your own mutant protein: 1. Design the mutation 2. You have 8 weeks to create the protein Order supplies, Express the protein in a host, Purify, Characterize.

Continuing the Malate dehydrogenase project

Goals:

- To engineer an dehydrogenase enzyme with new substrate specificity towards lactate
- 2. To propose a catalytic mechanism for *E. coli* malate dehydrogenase



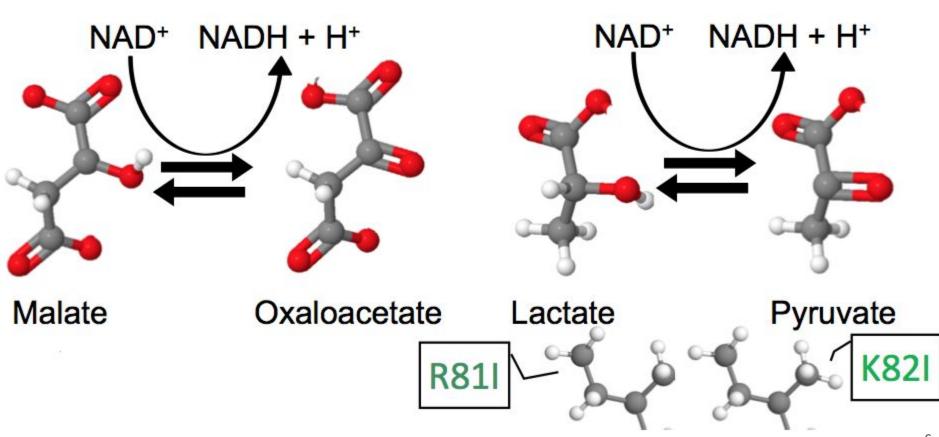
MDH DSSP-Site Record. 00000 GAAGGIGQALAL PSGSELS POBMKV LYDI AVKIKGF SGEDATPALEGADVVL I SAGVA PDB 70 DSSI Site Record O PDERKPGMDRSDL FNVNAGIVKNLVQQVAKTCPKACIGIITNPVNTTVAIAAEVLKKAGVYDKNKLFGVTTLDIICSNTFVAE PD8 81 110 120 130 150 160 140 DSSP Site Record PDELKGKQPGEVE PVIGGHSGVTILPLLSQVPGVSFTEQEVADLTKRIQNAGTEVVEAKAGGGSATLSMGQAAARFGLSLVR PD8 161 210 220 170 180 150 260 230 240 DSSP Site Record PD# AL Q G E Q G V V E C A Y V E G D G Q Y A R F F S Q P L L L G K N G V E E R K S I G T L S A F E Q N A L E G M L D T L K K D I A L G Q E F V N K PD8 241 310 312 250 260 270 280 290 300

PDB: 1IB6

Hypothesis:

A R81I/K82I dimutant will **create a hydrophobic pocket** in the active site that will lead to a change in substrate specificity from malate to lactate.



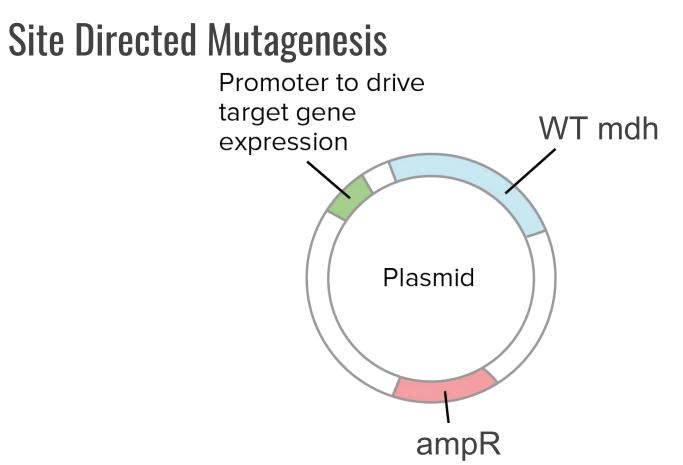


Methods



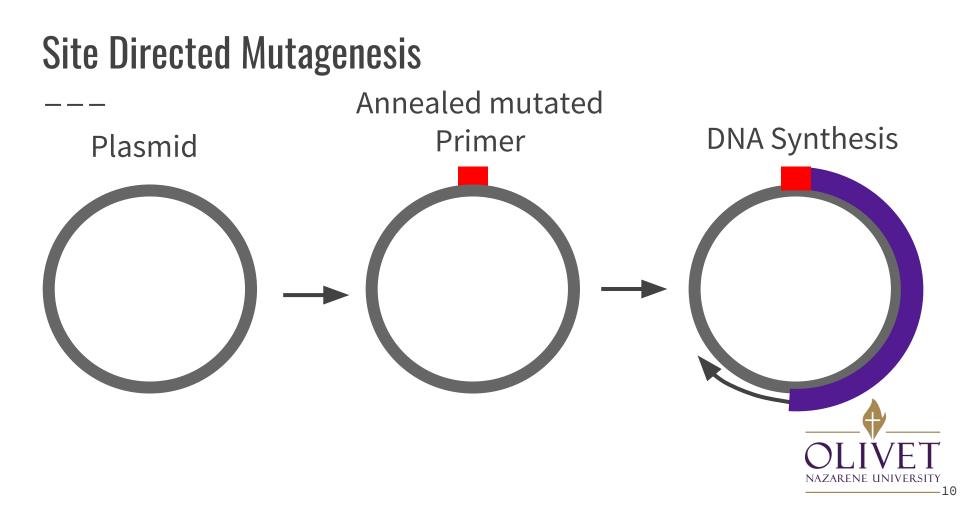
Site Directed Mutagenesis

• Mutating the plasmids





Bacterial transformation & selection https://www.khanacademy.org/science/biology/biotech-dna-technology/dna-cloning-tutorial/a/bacterial-transformation-selection (accessed Apr 12, 2018).







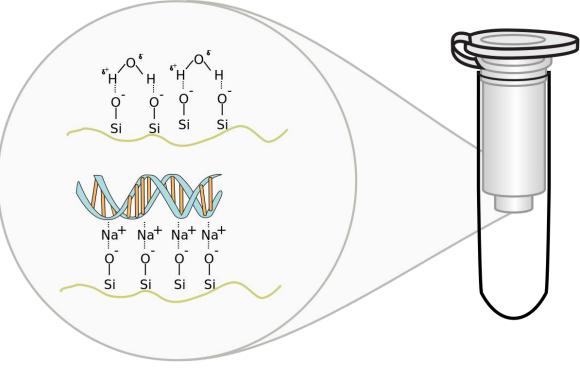
Polymerase chain reaction (PCR)

https://www.khanacademy.org/science/biology/biotech-dna-technology/dna-sequencing-pcr-electrophoresis/a/polymerase-chain-reaction-pcr (accessed Apr 12, 2018).

Purifying the Mutagenic Plasmids



Spin Column





Purifying DNA via a Spin Column

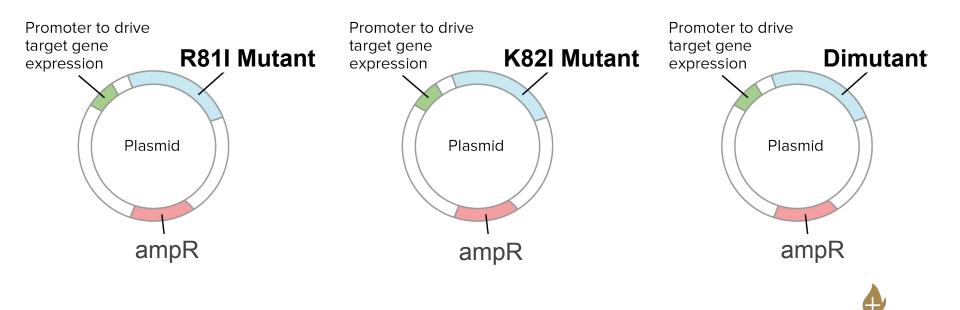


PCR product



GibThai. HiYieldTM Gel/PCR DNA Extraction Kit http://www.gibthai.com/product/product_detail/457?company_id=44 (accessed Apr 12, 2018).

Site Directed Mutagenesis

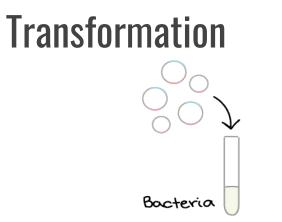


Bacterial transformation & selection https://www.khanacademy.org/science/biology/biotech-dna-technology/dna-cloning-tutorial/a/bacterial-transformation-selection (accessed Apr 12, 2018).

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Transformation

• Inserting the plasmids into the cells

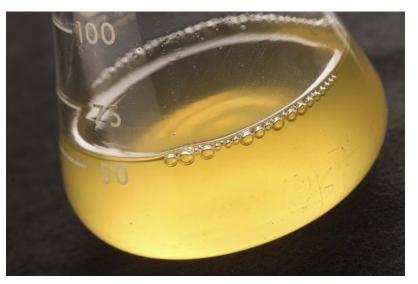




Bacterial transformation & selection https://www.khanacademy.org/science/biology/biotech-dna-technology/dna-cloning-tutorial/a/bacterial-transformation-selection (accessed Apr 12, 2018).

Cell Growth

- Ampicillin Broth
- Selects for transformed cells





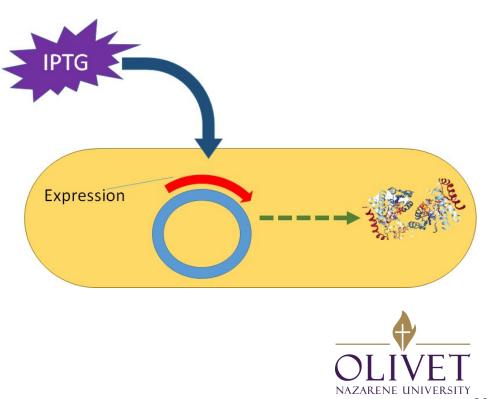
NASA - NASA Ames Research Center Public Affairs Office https://www.nasa.gov/centers/ames/news/releases/2002/02images/bionano/bionano3.html (accessed Apr 12, 2018).

Expression

• Expressing MDH

Protein Expression

- When lactose is present, the gene is expressed
- IPTG is a lactose analog that binds with higher affinity
- This allows for high gene expression

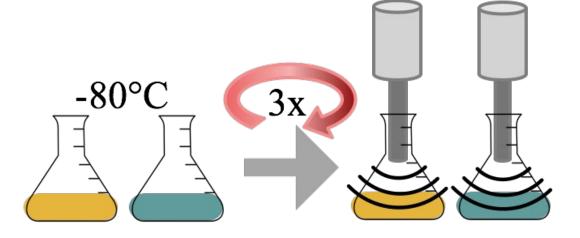


Purification

• Isolating protein

Cell Lysis

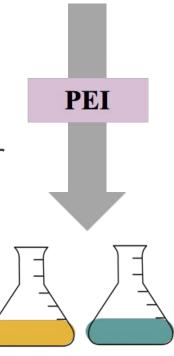
- Freeze
- Thaw
- Sonicate





Precipitation

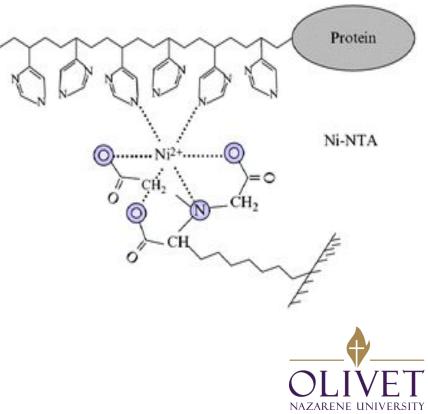
- Added protease inhibitor
- PEI precipitates DNA





Purification

- The protein was engineered to have a histidine tag
- Histidine has an imidazole R-group, which is attracted to our Ni-NTA purification column.



Purification

- Combined all fractions from the elution step
- Centrifugal filtration

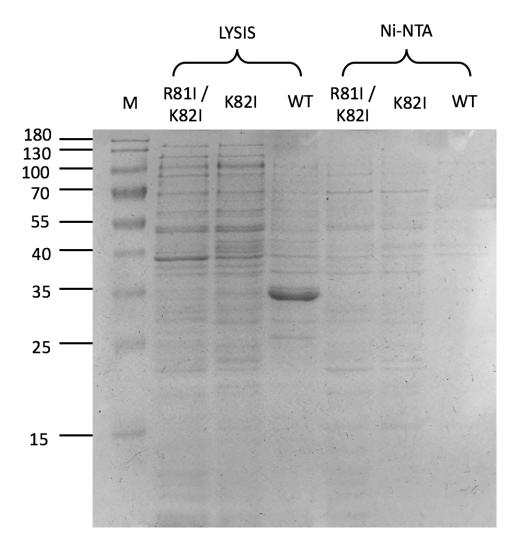


Results



SDS-PAGE

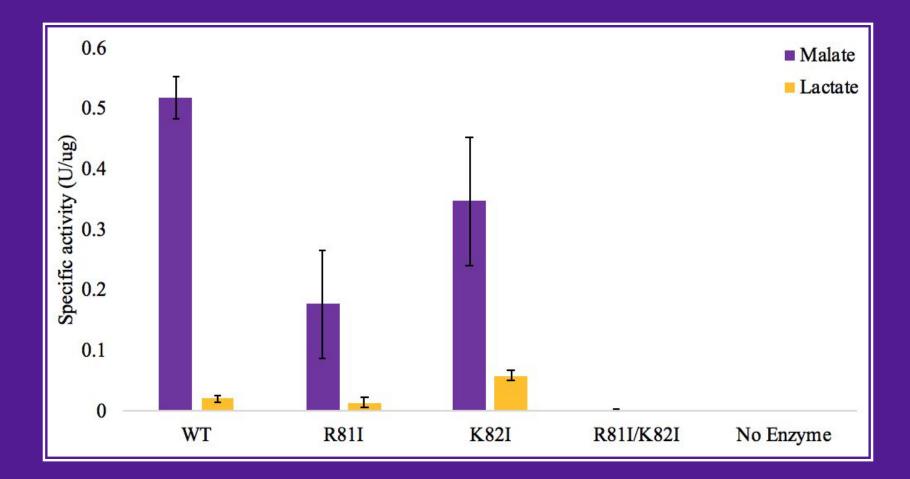
• Protein analysis





Enzyme Activity

- Bradford assay
- Comparing malate vs lactate specificity



Conclusion



Conclusion

Hypothesis not supported. Results provide a new insight into the catalytic mechanism of E. coli

- Arginine-81 may have greater influence on malate-oxaloacetate catalysis
- Lysine-82 may have greater influence on specificity
- Arg-81 was previously listed as a functional paralog shift mutation, but Lys-82 was not.



Near Future - Other methods, other proteins

- <u>Methods</u>
- pH probe enzyme assays
- Bioinformatics
- Structure techniques
 - ∘ X-ray
 - \circ Protein NMR



- Any protein
- Any organism
 CHO cells



Reflections

- Opportunity for independent research
- Importance of independent research
- Requirement for getting into grad school/jobs
- Class Bonding
- Critical thinking/ problem solving



Acknowledgements



- Olivet Nazarene University
- Department of
 - Chemistry
- Dr. Bruce J. Heyen



Questions?