Engineering Variant Forms of A1AT

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Abstract

Alpha-1-antitrypsin (A1AT) deficiency (A1AD) is a genetic condition that can lead to early onset emphysema and COPD. It can often lead to other complications such as liver cirrhosis. A1AD affects around 100,000 people in the U.S. and many more worldwide, with less than 10 percent of residents in the U.S. being properly diagnosed. Diagnosis is difficult due to A1AD often being misdiagnosed as asthma, as well as the actual diagnostic tests involving multiple steps and being time consuming. This leads to the need for new diagnostic tests.

The A1AT protein M form is commercially available for study. However, the mutated versions are not, which leads to the need to produce these mutated forms. To do so, we acquired plasmid DNA containing both the M and Z forms. These genes were then cut from multiple plasmids and subsequently inserted in a bacterial plasmid for propagation in chemically competent *E. coli* cells..

Goals

- **Propagate the original plasmids**
- **Engineer the new plasmids**
- **Prove ligation worked**

Procedure

1. Transform *E. coli* cells
2. Purify and cut plasmids
3. Run through a Gel
4. Extract DNA
5. Ligate products

Plasmid Vectors

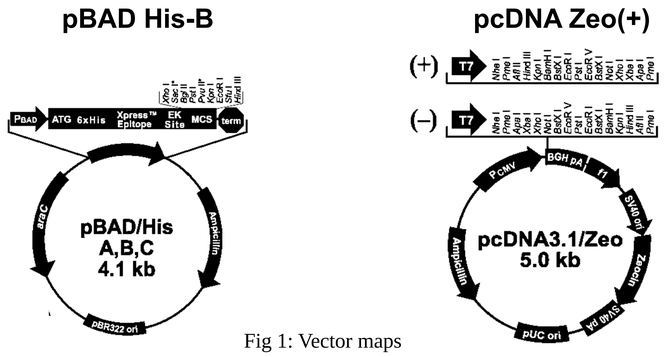


Fig 1: Vector maps

Transformed Cells

Fig 2: Isolated colony (left panel) is used to inoculate the tube containing growth media (right panel). After growth occurs, the contents are spun down into a small cell pellet (oval).

Indicator of plasmid quantity and purity ratios

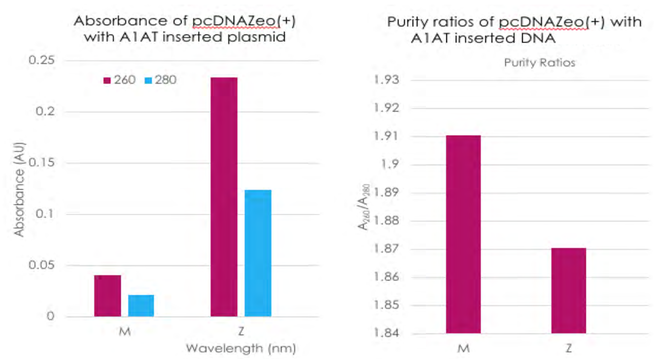
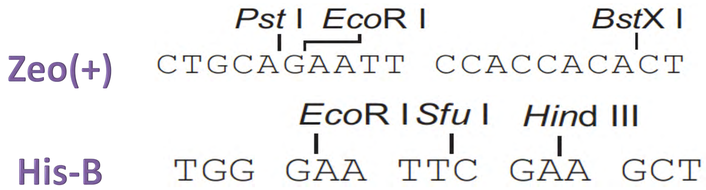


Fig 3: DNA absorbs light at 260 nm, and protein absorbs light at 280 nm (left panel). The figure on the right shows purity ratios of the protein. An ideal ratio is between 1.7 and 2.0. These results are expected because more DNA should be present than protein.

DNA Sequence



DNA gel cuts

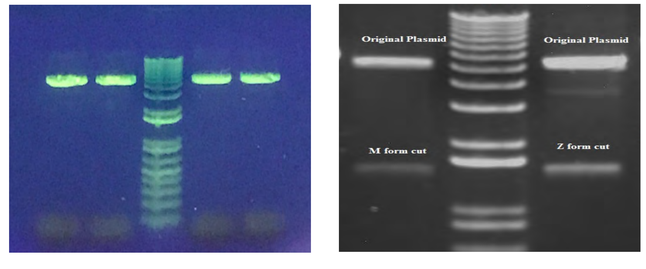
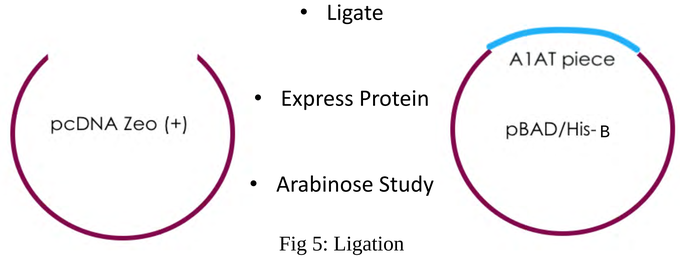


Fig 4: Agarose gel (left panel) shows pBAD/His C cut with EcoRI. Agarose gel (right panel) shows, EcoRI cut plasmids, M and Z forms from the original plasmid. The gels separate the DNA based on the sizes of each strand.

Future Work

- Ligate



- Express Protein
- Arabinose Study

Fig 5: Ligation

Conclusions

- **Plasmids expressed in bacteria**
- **Plasmids cut as expected**
- **Ligation to come**

References

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- Mr. Bryan Materi
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- pBAD/Myc-His Kit. (n.d.). Retrieved September 20, 2017, from ThermoFisher