The Optimization of a Novel Approach for Bio-Brick Expression in a Eukaryotic System

Shivani Shah, Julia Dave, Annie Khowaja, Countiss Miller, Tre Landry and Dr. Matthew W. Brewer*

The goal of our project is to use the pGAP vector system to express recombinant proteins in Pichia pastoris.

**Components of the pGAP Vector System**
- Zeocin resistance via Sh ble gene using both eukaryotic and prokaryotic promoters allows selection in both prokaryotic and eukaryotic cells.
- GAP promoter allows for constitutive expression in Pichia pastoris.
- α-secretion signal fused to the target protein allows secretion of the protein to the growth medium, simplifying purification.
- 6X His-tag and c-myc epitope help identify the protein on a Western blot with the help of common, commercially available antibodies.

**Advantages of Pichia vs. E. coli and other yeast**
- Eukaryotic gene expression more similar to mammals.
- Extracellular protein secretion for ease of purification.
- Faster protein production and easier manipulation than other yeast systems.

**Accomplishments**
- Submitted design of the pGAP plasmid that can be used with Standard 10 Bio-Brick parts.
- Inserted several kit plate parts into the pGAP vector system.
- Collaborated with Georgia Tech to test expression of mambalgin in E. coli.

**Future Directions**
- Continue working with the promoter made by Georgia Institute of Technology in our collaboration project. By working with that promoter we can use the pSB vector to express and secrete Mambalgin-1 from E. coli.
- Ligate Mambalgin-1 cDNA into the pGAPz vector and transform it into P. pastoris.
- Purify the protein and test its effectiveness.

**References**

**Acknowledgements**
Matthew Brewer, PhD
Maruf Hoque
Christopher Cornelison, PhD
Jessica Siemer
Georgia State University
STEM at GSU

**Hey I’m Pichia, and I can do it better.**