Title: Complex Eukaryotic Protein Expression using a Prokaryotic chassis

(\textit{Escherichia coli})

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Introduction: Morphine is a common opiate administered for medicinal purpose to suppress pain. Many opiates, contribute to the common addiction problem that may arise from these preoperative drugs. Patients may become tolerant to morphine through repeated use, requiring a higher dosage to produce the same effect. Mambalgin-1 peptide, a protein component of Black Mamba snake venom poses a possible solution to morphine addiction. Since extraction is inefficient the objective is to use the \textit{Escherichia coli} (E.coli) plasmid, PSB1C3, Mambalgin-1 cDNA, along with specific ribosome binding site (RBS) sequences and primers for polymerase chain reaction (PCR) to express the protein without milking snakes.

Purpose: This project is a part of the International Genetically Engineered Machine (iGEM) competition, which consists of the standardization of DNA parts and collaboration with other universities. In our collaboration with the G.A Tech iGEM team, synthetic biology techniques were applied to induce the expression of Mambalgin in \textit{E. coli}. Using the designed prokaryotic promoter along with RBS and PCR primers to optimize the expression of Mambalgin, eukaryotic peptide, in the prokaryotic chassis to obtain a high yield of pure Mambalgin-1.

Method: Standard techniques were used including, PCR for amplification of Mambalgin cDNA, gel electrophoresis to verify gene of interest, ligation was performed to incorporate Mambalgin into standardized PSB1C3 plasmid. This vector was transformed into E. coli in order to yield efficient expression.

Results: Initial testing and sequencing (GA Tech) confirm the presence of RBS, and Mambalgin cDNA. Sequencing will confirm the presence of Mambalgin cDNA in PSB1C3 and western blot will confirm the protein by using antibodies that are specific to the Mambalgin peptide.

Conclusion: Future direction of this project is to test addictive, analgesic and immunological side effects using animal models. This testing will provide understanding of many health factors associated with Mambalgin-1 protein. This protein can be introduced to the pharmaceutical industry as a morphine replacement. Furthermore, many health care benefits can come from mass production of this protein including a decline in the problem of opiate addiction.