

Title: Mamba Juice: Expression of Exogenous Mambalgin Peptide Using the pGAPZ α Vector System

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Introduction: Pharmaceutical companies have invested considerable financial resources in developing analgesics. Often, the compounds used in these medicines are naturally occurring, such as aspirin and opioids. Mambalgin-1, an analgesic peptide component of the African Black Mamba venom, has been shown to have comparable analgesic effects to morphine but does not induce the debilitating withdrawal affects. Extracting this peptide is expensive and dangerous work; our objective was the development of a recombinant system producing this peptide without milking snakes.

Purpose: Using recombinant DNA technology, we sought to produce high quantities of pure, functional Mambalgin-1 in a safe and cost effective manner through a cassette system in the yeast *Pichia pastoris*. Since this project is part of the International Genetically Engineered Machine (iGEM) competition and iGEM is dedicated to the sharing of DNA through a process of standardization, we also sought to standardize the pGAPZ α expression vectors for future iGEM use.

Method: The original multiple cloning sites (MCS) of the pGAPZ α vectors were removed through restriction digestion and standardized MCS were ligated into the vectors. Mambalgin-1 cDNA was then inserted into one standardized vector. Copies of the resulting plasmid were then linearized and transformed into *Pichia pastoris*.

Results: Preliminary results show successful growth of *Pichia* in presence of Zeocin, an indicator of successful transformation with pGAPZ α plasmid sequences. Standardization of the pGAPZ α C plasmid has been confirmed by analytical digestion and PCR analysis. Further, addition of the Mambalgin-1 cDNA has been confirmed by analytical digest.

Conclusion: We have modified *Pichia pastoris* expression vectors for future iGEM use. Proteins produced using these plasmids can be tagged for secretion and expressed as a *his* and *myc*-tagged fusion, allowing for easier purification and detection. Work is ongoing to verify the presence of the Mambalgin-1 cDNA in *P. pastoris*. Purified Mambalgin-1 can then be tested for functional binding to acid sensing ion channels (ASIC) and may ultimately prove useful as a potent, non-opiate analgesic.