

Advances in biological engineering have led to the production of medically relevant proteins such as insulin and thrombin. These breakthroughs were made by bacteria, which have been the organisms of choice when producing commercially and scientifically important biological macromolecules. Although bacteria have successfully produced a number of proteins, fully functional proteins have not always been expressed. This is due to the bacteria lacking the mechanisms to perform posttranslational modifications, which yields inactive proteins. Yeasts such as *Saccharomyces cerevisiae* and *Pichia pastoris* give researchers the ability to perform this function. *P. pastoris* has a stronger inducible promoter system, which is located in the AOX1 and AOX2 genes, compared to that of *S. cerevisiae*. This system allows for transcriptional regulation that leads to a high concentration of pure exogenous protein. Our lab has modified a commercially available *Pichia* expression vector, pGAPz- $\alpha$ , to iGEM standards by the addition of a multiple cloning site (MCS) linker piece by means of restriction enzyme digest followed by ligation. Modification of the MCS to iGEM standards allowed insertion of the iGEM part encoding the red fluorescent protein (RFP) cDNA. Purification and linearization was done with EcoRI and Pst. We were then able to ligate RFP and pGAPz- $\alpha$ . A cocktail restriction digest was done in order to confirm correct ligation. The ligation of the RFP into pGAPz- $\alpha$  was done. The vector was then transformed into *Pichia* competent cells. Future possibilities would include expressing a protein that can be useful as well as advancing uses of *Pichia* over *E. coli* because of its ability to perform posttranslational modifications which is important in eukaryotes. Also, we would like to work on the pPik 9 because of that ability to be inducible as well.