Designing a Shuttle Vector for Protein Production in *Pichia pastoris*

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Abstract

Pichia pastoris is a methylotrophic yeast that is used as an alternate host to *Saccharomyces cerevisiae* and *Escherichia coli* for protein production. This organism possesses the ability to produce large amounts of foreign proteins while keeping endogenous protein production to a minimum. This makes it an attractive host for protein production. Designing a vector to insert genes into this yeast would allow iGEM users to harness the protein producing ability of this organism. Keeping this in mind, the goal of the Georgia State University iGEM team was to develop a shuttle vector for *P. pastoris*.

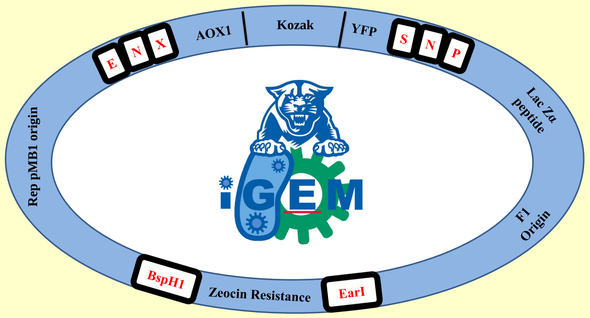
Modifications were made to a previously submitted protein fusion vector to make it an appropriate vector for *P. pastoris*. The theoretical construct of the vector has been successfully completed and presented here. Even though progress was made in the wet lab production of this vector, we were not able to submit the vector for this year's competition. However, enough insight has been obtained from the conducted experiments to submit the vector to iGEM in the near future.

Introduction

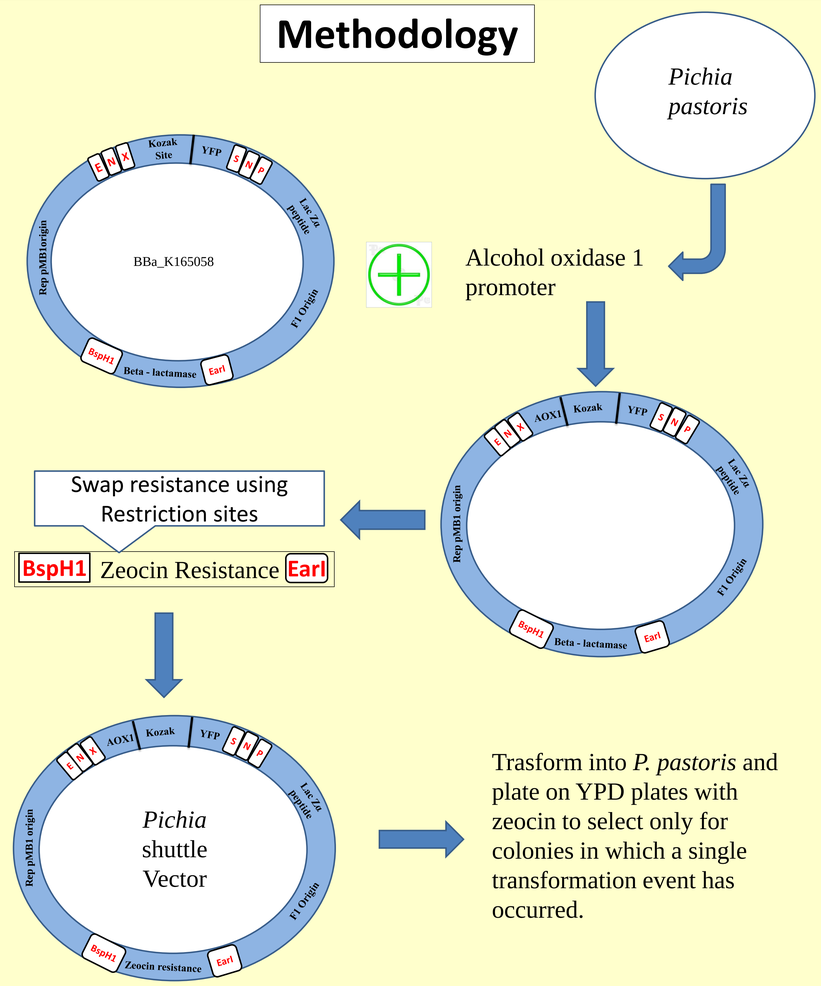
The advent of recombinant DNA technology has been a major breakthrough in science. Human proteins such as insulin can be produced in large amounts by introducing the gene for human insulin into other organisms. Both prokaryotic and eukaryotic organisms have been used as hosts for this purpose. However, one of the problems that researchers often encounter while using prokaryotic organisms as host systems for eukaryotic protein production is the lack of post translational modifications in these organisms. Historically *Saccharomyces cerevisiae* has been used as a eukaryotic host organism. There are however several limitation to using *S. cerevisiae* as a host organism. Firstly, the yield of protein produced using this organism has been reported to be only 1 to 5%. Also, purification of protein produced by this organism is extremely difficult and leads to an even smaller yield.

Investigators have recently started using *Pichia pastoris* as an alternative host organism for protein production. Use of this organism is advantageous for several reasons. One reason is that grows under similar conditions to *Saccharomyces* and therefore no new growth conditions need to be worked out for this organism. Another reason is that *P. pastoris* has the availability of strong inducible promoters that can be used for protein production. Further, post translational modifications in this organism are similar to human modifications. Finally, the purification and isolation of protein product is easily achieved in *P. pastoris*. The limitations of *E. coli* and *S. cerevisiae* have led to researchers looking for alternative hosts for protein production and some of the above mentioned properties of *Pichia pastoris* make it an ideal organism.

Pichia Shuttle Vector Design

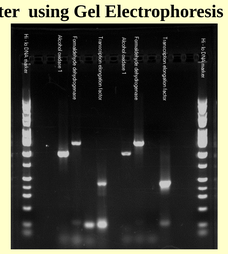


Methodology



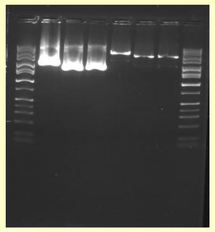
Results

Figure 1: Analysis for Isolation of Alcohol Oxidase I Promoter using Gel Electrophoresis



Four promoters were isolated from *P. pastoris* using PCR. Lanes 4, 9 were alcohol oxidase I, lanes 5, 10 were formaldehyde dehydrogenase, lanes 6, 11 were glyceraldehyde – 3 – phosphate dehydrogenase, and lanes 7, 12 were transcriptional elongation factor. A standard hi – lo DNA ladder was run in lane 1 and 14. Bands were obtained around expected length for alcohol oxidase I, Formaldehyde dehydrogenase, and Transcriptional elongation factor. However, no bands were obtained for glyceraldehydes – 3 – phosphate dehydrogenase.

Figure 2: Analysis of AOX1 insert into part BBa_K165058



The AOX1 gene was ligated into part BBa_K165058 and then transformed into *E. coli*. The plasmids were extracted from *E.coli* and analyzed using restriction digestion. Lane 2 contained uncut circular plasmid without insert. Lane 3 and 4 contained two cloned colonies with insert in circular form. Lane 5 contains the plasmid without insert cut with *Eco*RI. Lane 6 and 7 contained the plasmids with insert cut with *Eco*RI. The bands with insert appear smaller then the plain plasmid which indicated that the ligation reaction did not work

Discussion

As seen in figure 1, Alcohol oxidase I promoter was successfully isolated from *Pichia pastoris*. The primers were designed with overhangs containing restriction sites, making the isolated gene compatible with iGEM standards. The isolated AOX1 promoter was inserted upstream to a eukaryotic ribosomal binding site and yellow fluorescent protein in a protein fusion vector designed by the Silver lab. The ligated vector and promoter were cloned into chemically competent *E. coli* cells. Cloned colonies were analyzed using restriction digestion.

There were several problems encountered in the analysis of the cloned vector. Theoretically, the cloned colonies should have a higher molecular weight than the control without the added promoter. According to figure 2, this was not the case. This indicates that AOX1 gene was not inserted into the vector correctly. However, restriction digestion may not be the best method for cloning analysis. Therefore, we plan to design primers that overlap with the plasmid backbone as well as the AOX1 promoter and utilize PCR for the analysis of the clones. Additionally, properties of the cloning vector, such as presence of LacZα peptide, can be used to analyze the clones through Milller assay.

Future Work

The GSU iGEM team wants to continue working on constructing the shuttle vector that can be used for protein production in *Pichia pastoris*. We have encountered problems in analyzing the insertion of the alcohol oxidase I promoter gene into the part BBa_K165058. Our next step is to optimize analysis of the clones. Once we determine the correct insertion of the gene into the biobrick part, the next step is to swap the beta – lactamase gene with the synthesized zeocin resistance gene using restriction enzymes.

After this the next step will be to transform the vector carrying yellow fluorescent protein into *P. pastoris* using zeocin resistance as a selective marker. The yellow fluorescent protein will be used for characterization of the promoter. Furthermore, we plan on swapping the AOXI promoter with other inducible promoters such as formaldehyde dehydrogenase and transcription elongation factor. Eventually we want to be able to use this vector for production of eukaryotic proteins in *Pichia pastoris*.

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