DESIGNING A SHUTTLE VECTOR FOR PROTEIN PRODUCTION IN *PICHIA PASTORIS*

*i*PICHIA

pastoris

Georgia State University

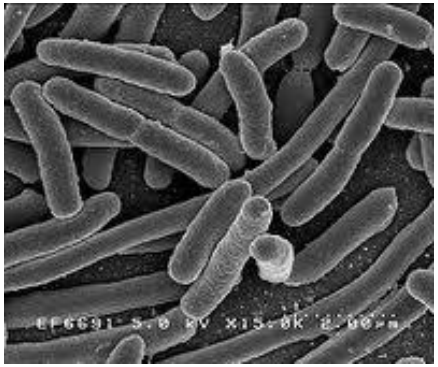
School Background

- Georgia State University was founded as an extension of the Georgia School of Technology's "Evening School of Commerce" back in 1913
- It has now become the second largest research institution in the University System of Georgia
- GSU 's has a 34 acre campus in the heart of Downtown Atlanta
- This year GSU got a record high enrollment of above 30,000 students
- This is GSU's second year participating in iGEM.

Petite. H Parker Science Building

Introduction

- Historically two organisms have been most commonly used as hosts for recombinant protein production
  - *Escherichia coli*



Saccharomyces cerevisiae

- Limitations of *E. coli*
  - No post – translational modifications
- Limitations of *S. cerevisiae*
  - Hyperglycosylation
  - Low yields

Advantages of Using *Pichia pastoris*

- Inexpensive to culture
- High production of foreign proteins
- Post-translational modification capacity
- Strongly inducible promoters
  - AOXI and AOXII

Promoter

- Alcohol Oxidase I promoter
  - Strongly inducible promoter
    - Activated by methanol
    - Inactivated in the presence of glucose
  - Controls expression of Alcohol Oxidase

Methanol $\longrightarrow$ **Formaldehyde + Hydrogen Peroxide**

Primer Design

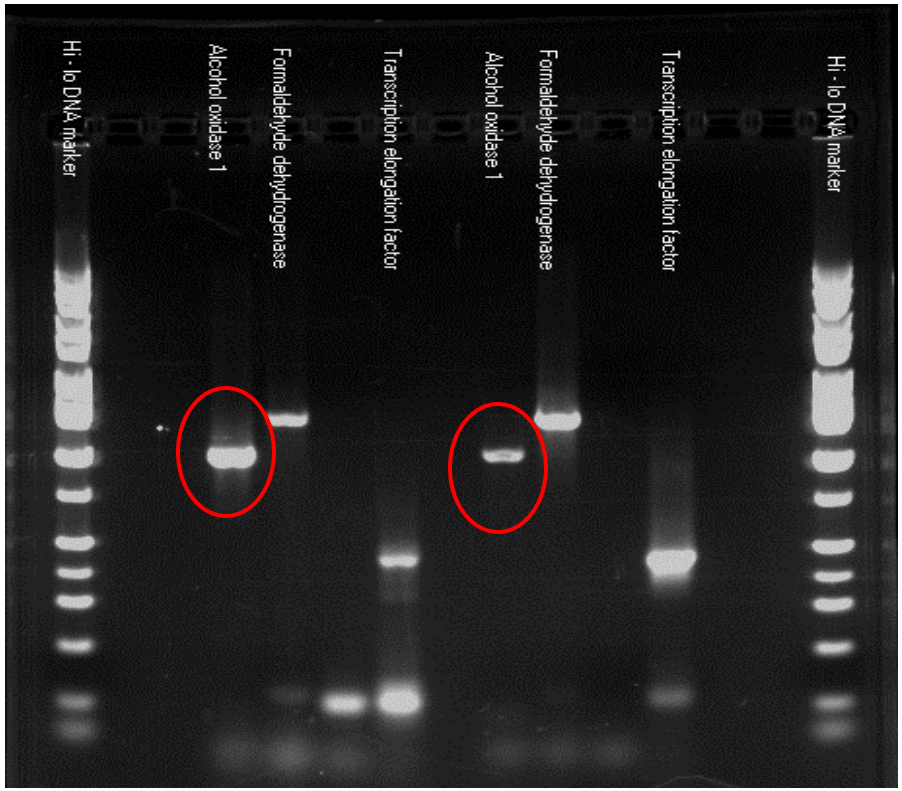
- Forward primer

- Reverse primer

- Isolated Gene

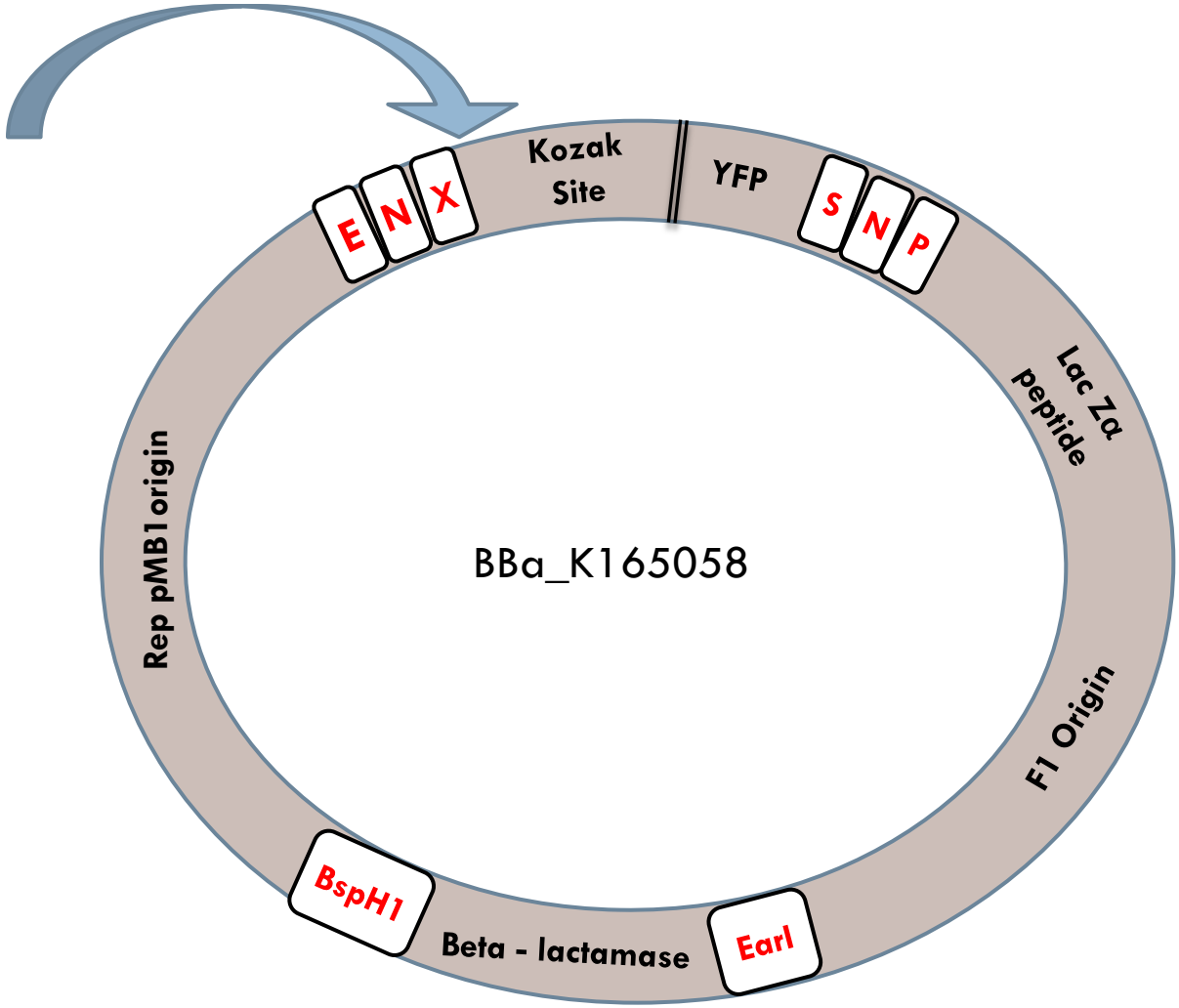
Isolation of Alcohol Oxidase I

- Alcohol Oxidase I promoter gene is 940bp
- Addition of the restriction sites using primers should add 40bp
- As seen in the figure a band was present slightly below 1000bp indicating that AOX1 was successfully isolated.

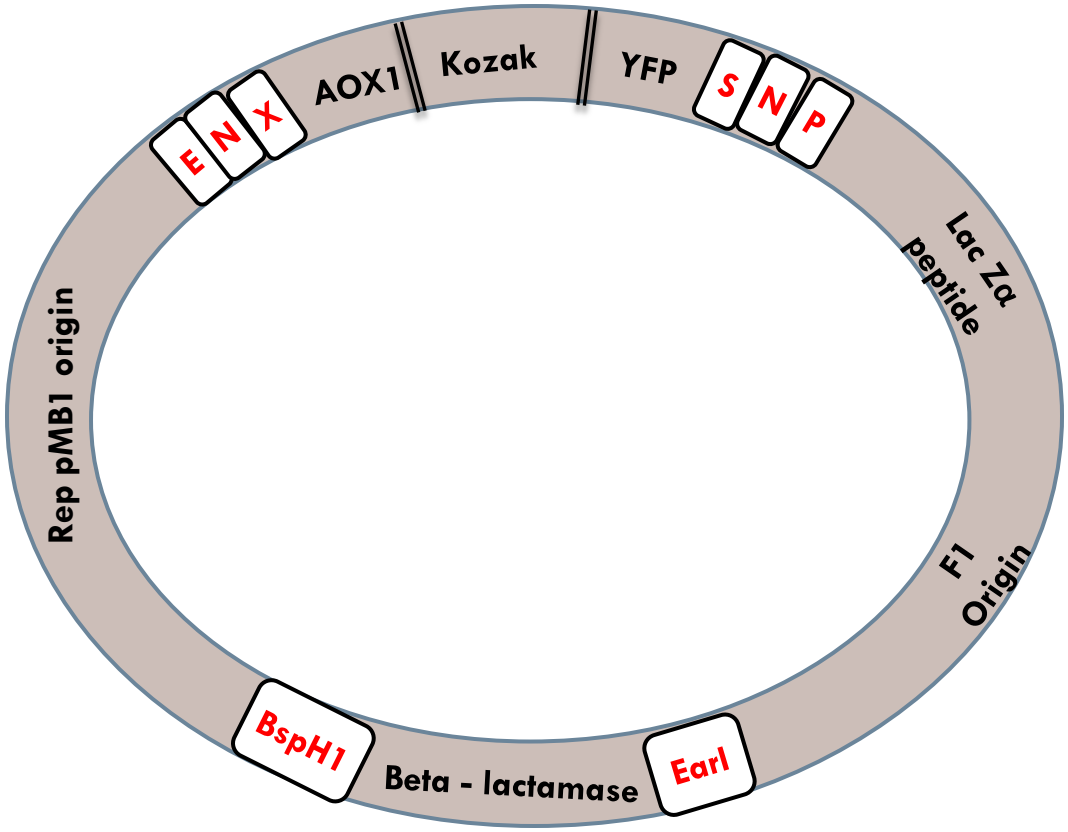


Vector Design

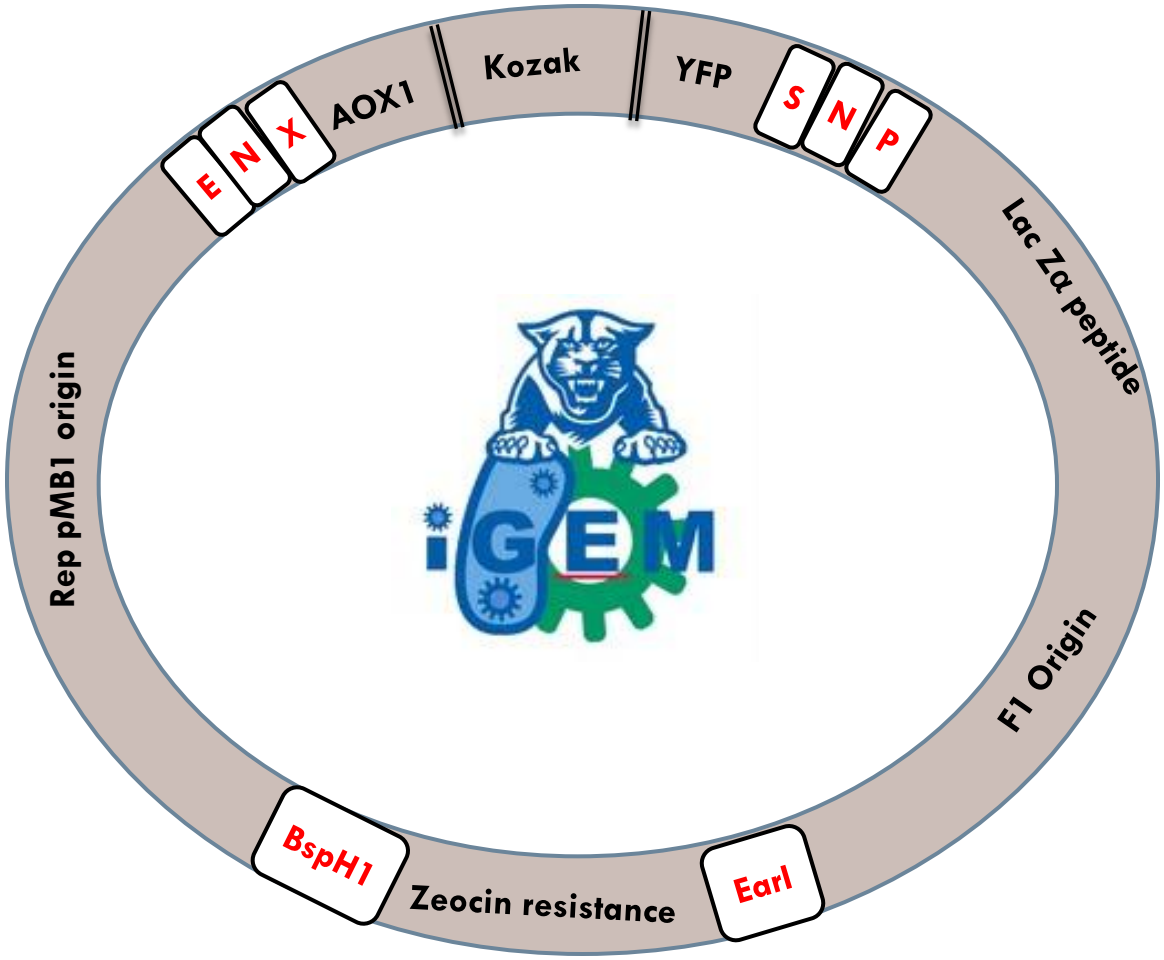
- Alcohol Oxidase I Promoter



Vector Design

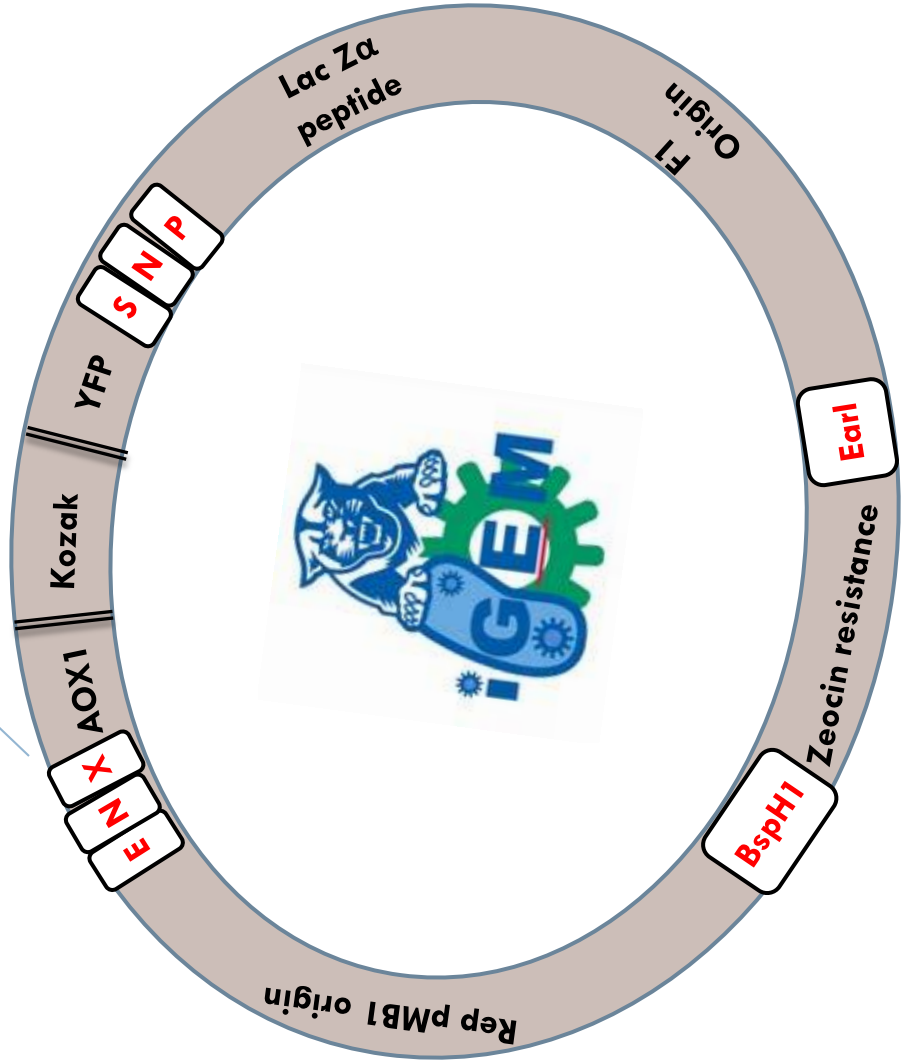


Vector Design



Homologous Recombination

Integration of the vector into the chromosome of *P. pastoris* in a single cross over event



Future Applications

- Characterize the Alcohol Oxidase promoter
  - Fluorescence of the yeast
- Swap with *Pichia* promoters
  - Example
    - Gly-3-Phosphate Dehydrogenase
    - Formaldehyde Dehydrogenase
    - Transcription Elongation Factor
- Production of eukaryotic proteins and production of vaccines

2011 Team Accomplishments

- Established GSU Synthetic Biology Club
- Created 3-semester iGEM course for future development
- Received $45,000 departmental funding for lab equipment
- Hosted multiple fundraising events
  - Kaplan Auction
  - Bake Sales
  - Keg Parties
  - Racquetball Tournament

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