Pichia pastoris: A model chassis for future iGEM users
Why our iGEM team loves vaccines...

- Vaccination is one of the most cost-effective health interventions.
- An estimated 2.5 million deaths are prevented through vaccinations every year.
- They can be delivered with very high coverage to prevent disease in many marginalized populations.
Yet... vaccine development

- Can take more than 10 years, depending on the disease.
- **Steps:**
  - discovery
  - process engineering
  - toxicology and animal studies to human trials.
  - **production**
  - distribution

Many challenges of vaccine development can be solved with the yeast:

*Pichia pastoris*
A little bit about *Pichia pastoris*...

- **Pichia pastoris**: unicellular methlyotrophic yeast
- Gained popularity in the 1980s after industry harnessed powerful promoters inducing necessary genes for methanol utilization.
- Has since been successfully used for academic and industrial purposes to produce hundreds of heterologous proteins
Pichia pastoris is a solution because...

- **High yield** of potential vaccine antigens which can lower prices, solving the issue of large-scale production.

- Great deal of **post translational modification** of complex proteins that prokaryotic *E. coli* can not accomplish.

- Strong preference of *P. pastoris* for aerobic growth, a key physiological trait that greatly **facilitates its culturing** at high cell densities relative to fermentative yeasts (i.e. *Saccharomyces cerevisiae*).
Our two specific goals are...

1. To make iGEM standardized parts compatible with the use of *Pichia pastoris*

2. To use this standardized platform technology for vaccine production
Our approach for efficient part design

1. X ———— S
2. X ———— SNP
3. ENX ———— S
4. ENX ———— SNP
Alcohol Oxidase 1 promoter (pAOX1)

- **Natural Function**: This promoter regulates expression of Alcohol Oxidase 1. Induces high levels of expression when induced by methanol.

- **Application**: Heterologous protein expression with Pichia pastoris has been primarily accomplished under the control of the pAOX1. This promoter provides not only high yields but tight regulation and has been a very significant contributor to the success of P. pastoris as a system for heterologous protein expression.

**Part**: BBa_K431007

**Category**: Promoter

**Status**: In progress
Natural Function: This promoter regulates expression of glyceraldehyde 3-phosphate dehydrogenase. This constitutive promoter produces high levels of GAPDH as it is needed for glycolysis.

Application: The promoter for glyceraldehyde 3-phosphate dehydrogenase has been successfully used as an alternative to pAOX1. Comparable levels of protein expression are obtained. It also offers the benefit of being a constitutive promoter that does not require the use of methanol.
Formaldehyde Dehydrogenase

- **Natural Function**: This enzyme is used by the cell to convert formaldehyde to formate.

- **Application**: This part is useful with Pichia pastoris for 2 reasons.
  - 1. Selectivity of transformants with compatible strain
  - 2. Screen for transformants by increasing formaldehyde concentrations in the media.
    - No need for multiple transformations and antibiotic resistance
    - Obtain multiple copies of your cassette

**Part**: BBa_K431010
**Category**: Coding Region
**Status**: In progress
**Natural Function**: Biosynthesis of Histidine. Catalyzes the following reaction

\[ \text{L-histidinol} + \text{H}_2\text{O} + 2 \text{NAD} \rightarrow \text{L-histidine} + 2 \text{NADH}. \]

**Application**: Selectivity of transformed cells. Part is compatible with auxotrophic mutant strain GS115 (Δhis4).

**Problem**: This part occurs in nature with two unwanted restriction sites (XbaI sites @ 1941 and 2474).
Histidinol Dehydrogenase (His4)

**Part:** BBa_K431006  
**Category:** Promoter  
**Status:** In progress

**Approach**
- Isolate with primers, add **EcoRI** and **SpeI** sites

**PCR Reaction**

- **EcoRI** Forward Primer #1
  - 5’ TAAGGAATCCATGACATTTCCTTGCTACCTGCA
  - 3’
- **SpeI** Reverse Primer #1
  - 5’ GGCAGCTAGTTATTTATTCTCCATACGAACCT TAAC
  - 3’

- **Histidinol Dehydrogenase (2.6 kb)**
- **Unwanted Xba I Sites**
**Histidinol Dehydrogenase (His4)**

**Part:** BBa_K431006  
**Category:** Promoter  
**Status:** In progress

**Approach**

- Insert into plasmid, mutate out unwanted Xba I Sites

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**Diagram**

- **EcoRI**
- **Spe I**
- Histidinol Dehydrogenase (2.6 kB)
- Unwanted Xba I Sites
- Site Directed Mutagenesis

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Approach

3. Final PCR with Biobrick and alternative ends
Influenza A
hemagglutinin head

- **Natural Function**: This part is a conserved antigenic region among influenza virus sequences.

- **Applications**: This part will be converted into a biobrick part and utilized to exemplify our systems potential to produce vaccines with pichia pastoris.

- **Design**: This part will be purchased as a synthesized DNA strand. This will allow us to eliminate two unwanted restriction enzyme sites as well as avoid working with a potentially hazardous virus.
Antigen Design

We compared previously isolated and sequenced Influenza A H1N1 strains.

A conserved region comprised of the Hemagglutinin globular head was identified.

This region of the virus has been shown to produce an immune response.

Previously isolated sequence of hemagglutinin globular head
Antigen Design

- The next step was to design this part to fit the registry standard.
- Two unwanted restriction sites were identified.
- To eliminate these sites, we did a nucleotide swap while maintaining the same amino acid sequence.
Antigen Design

Schematic illustrating protein folding

Biobrick part

E/N/X C

HA Globular head Antigen

C S/N/P

• Hemagglutinin Antigen
The Pichia system: A promising approach to produce vaccines

- Application
Conclusions

- We are working to standardize a platform technology beneficial to diverse applications.

**Applications**
- Vaccines
  - Antigen testing
  - Vaccine production
- Heterolgous protein
  - Hundreds of proteins have been successfully produced in *P. pastoris*!
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References


