Constructing a Biological Ethanol and Methanol Sensor

Missouri University of Science and Technology
Missouri S&T 2008 iGEM Team

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Formerly…

University of Missouri-Rolla

Formerly…

The Missouri School of Mines
Lots of science majors…

TONS of engineering students.
iGEM is only one of many undergraduate student research teams which include:

- The Solar Car, Robotics, and Concrete Canoe Teams

Started in 2006: small, but growing organization

Cross-disciplinary

- Biological Sciences
- Chemical and Biological Engineering

University-wide involvement
Project Motivation

- St. Patrick: Patron Saint of Engineers
Project Motivation

Celebration!

Sad Pat 😞
Motivation

- Biological Breathalyzer
- Ethanol responsive gene
  - AOX1 promoter
  - Ethanol/Methanol metabolism of yeast
- Expanded project into an ethanol/methanol sensor
The Project

- Determine the concentration of ethanol and/or methanol in a closed system

- Possible applications
  - Home brewing
  - Use for monitoring gasoline additives
  - Methanol fermentation processes
Background

- Based on metabolic pathway of *P. pastoris*
  - Diauxic metabolism for MeOH and EtOH
  - EtOH preferred

**Growth and Carbon utilization of *P. pastoris***
Methanol Metabolism

- Alcohol Oxidase (AO) Enzyme
  - First key enzyme in MeOH utilization pathway
  - Two related AO enzymes produced by two separate genes: AOX1 and AOX2
  - AOX1 mRNA has higher steady-state level
  - Majority of AO enzyme produced from AOX1
Project Outline

- Join AOX1 promoter to RFP gene
- Place this system in *E. coli*
- Introduce *E. coli* cells to EtOH and MeOH
- Preferential consumption of all EtOH
  - No Fluorescence
- Secondary consumption of MeOH
  - AOX enzyme expressed
  - Fluorescence
Isolation of AOXI promoter

• Checked for standard enzyme cutting sites within AOX1 promoter

• Amplified AOXI promoter (from pPIC3K vector) using PCR

• Flanked AOXI promoter with appropriate restriction sites using primers
Isolation of AOXI promoter

• Cloned PCR product into pCR2.1 vector
Isolation of AOXI promoter

- Transformed pCR2.1 vector with promoter into competent cells

LacZ gene → β-galactosidase → cleavage of x-gal → Blue Pigment
Checking AOX1 promoter clones

- Separated AOXI promoter from pCR2.1 plasmid using EcoRI
Preparation of RFP vector

- Obtained RFP vector (BBa_J61002) from registry
- Transformed RFP vector into *E. coli* cells to amplify
Preparation of RFP vector

- Extracted amplified RFP vectors
Isolating AOXI promoter and RPF vector

Digested AOXI promoter clone and RFP vector with XbaI and SpeI
Ligation of AOXI promoter and RFP vector

- Performed overnight ligation at 16 °C
- Transformed ligation into *E. coli* cells
Current Stages of Research

- Screening cells to determine which ones contain the ligated AOX1 promoter and RFP vector
- Improving ligation method using controls and purifications.
Testing

Direct Measurement of Methanol

Length of time cells fluoresce

Indirect Measurement of Ethanol

Length of time before cells fluoresce
Overview

Creating a Correlation:

1. Introduce known concentration of ethanol and methanol to cells’ environment

2. Time until fluorescence is detected

3. Relate time to concentration of ethanol present in environment

\% Ethanol = ???
Obstacles

- Ligation of AOX1 and RFP vector
- Screening and controls
- Functionality of the part in *E. coli*
- Indirect measurement of ethanol
- Response, degradation, and lag time
Minimum concentration of ethanol
Maximum concentration of methanol
Lag time
  - Protein synthesis/folding rates
  - Rate of protein degradation
Minimum amount of enzyme before reaching detectable levels of fluorescence
Implementation in real industrial process?
What Have We Gained?

- Skills and techniques
- Collaboration and Team work
- Job opportunities
- Ties with companies
Acknowledgements

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References


3. **International Genetically Engineered Machines Competition (iGEM).**
   http://igem.org