Genetically Engineered $\text{H}_2$ Detector

Mississippi State University
Present Areas of Interest

- Departmental Goal – Produce oil for biodiesel
- Dr. To wanted to find out if we could engineer bacteria to produce biodiesel efficiently
- We decided to start small
Team Goals

- Learn procedure for genetically engineered machines
- Combine knowledge from several departments
  - ABE, BCH, ChE, ECE
- Develop network with MIT and other iGEM partners to allow for future collaboration
Our Team
Our Team - Professors

- Agricultural and Biological Engineering
  - Dr. Filip To
- Biochemistry
  - Dr. Din-Pow Ma
- Electrical and Computer Engineering
  - Dr. Bob Reese
- Chemical Engineering
  - Dr. Todd French
Our Team - Students

- Agricultural and Biological Engineering
  - Graduate
    - Brendan Flynn, Robert Morris
  - Undergraduate
    - Teri Vaughn, Lauren Beatty, Scott Tran, Joe Chen, Sam Pote, Paul Kimbrough

- Biochemistry
  - Graduate
    - Victor Ho
Desired Machine Function

- We wanted to design a machine to detect the presence of H$_2$
- The machine would function when “turned on” by an inducer
- The machine would produce quantifiable fluorescence dependent on H$_2$ concentration
Uses

- Quantifiable detection of H$_2$
- This function could possibly be incorporated into bacteria used in the production of H$_2$ in the future
Design - Parts

Our Composite Part - J43001

H₂ Promoter
LacI QPI
YFP Reporter

J45503 Q04121 E0430
## Design – Part Descriptions

<table>
<thead>
<tr>
<th>Part Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>J45503</td>
<td>Cold shock promoter and H$_2$ promoter</td>
</tr>
<tr>
<td>Q04121</td>
<td>LacI QPI (Quad Part Inverter), composite</td>
</tr>
<tr>
<td>E0430</td>
<td>YFP output device, composite</td>
</tr>
</tbody>
</table>
## Design – Subparts – Q04121

<table>
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<tr>
<th>Subpart</th>
<th>Description</th>
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<tbody>
<tr>
<td>B0034</td>
<td>Strong RBS</td>
</tr>
<tr>
<td>C0012</td>
<td>LacI coding region</td>
</tr>
<tr>
<td>B0015</td>
<td>Double terminator</td>
</tr>
<tr>
<td>R0011</td>
<td>Strong promoter, Repressed by LacI, Induced by IPTG</td>
</tr>
</tbody>
</table>

Q04121 = B0034, C0012, B0015, R0011
### Design – Subparts – E0430

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<td>B0034</td>
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<td>B0015</td>
<td>Double terminator</td>
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E0430 = B0034 \(\rightarrow\) E0030 \(\rightarrow\) B0015
Design - Components

hybB
H₂ Promoter
LacI repress or gene
LacI promoter
Ribosome Binding Site
Terminator
YFP reporter
Lac promoter naturally shows an active but low transcription level
J43000 In Action

IPTG is a synthetic inducer
J43000 In Action

IPTG induces a high level of transcription at the Lac Promoter
A large amount of YFP is transcribed and translated.
J43000 In Action

Hydrogen is introduced to the machine
J43000 In Action

Hydrogen turns on the hybB promoter
J43000 In Action

The lacI gene is transcribed
J43000 In Action

LacI repressor inhibits the lac promoter

Competitive Inhibition
J43000 In Action

Lac promoter transcription is reduced, leading to lower YFP transcription

Competitive Inhibition

H₂

lacI

IPTG

YFP

YFP

YFP

YFP

YFP

YFP
J43000 In Action

Hydrogen concentration is increased

Competitive Inhibition

\[ \text{IPTG} \quad \text{lacI} \]

H_{2} H_{2} H_{2}
Lac promoter is further repressed, leading to lower YFP production.

Competitive Inhibition
J43000 In Action

Machine is exposed to a high \( \text{H}_2 \) concentration

Competitive Inhibition

- \( \text{H}_2 \)
- \( \text{H}_2 \)
- \( \text{H}_2 \)
- \( \text{lacI} \)
- \( \text{IPTG} \)
- \( \text{YFP} \)
- \( \text{YFP} \)
- \( \text{YFP} \)
J43000 In Action

Lac promoter is strongly repressed, YFP production is reduced to minimum

Competitive Inhibition

H₂  H₂  H₂  H₂  H₂

lacI  IPTG  YFP
Testing Our Machine

Requirements

- Introduction of inducer (IPTG)
  - Provides a broader range in fluorescence levels
- Varied H\textsubscript{2} concentrations
- Fluorescence quantification
Testing Our Machine

○ Gasing
  ● Cells were grown in septum topped vacuum ready tubes
  ● A vacuum pump is used to extract air from the tube
  ● Each tube is filled with a specific $H_2$ concentration
Testing Our Machine

- Photos
  - The samples were exposed to light wavelengths close to YFP absorption wavelength
  - Pictures were taken using a digital camera with fixed settings
  - Pictures were taken before, immediately after, and 5 hours after gassing
Our Machine

Visible fluorescence compared to unmodified E. coli
Testing Our Machine

- **Analysis**
  - MatLAB pixel analysis
    - Averaged pixel value for a selected area
  - 5 data sets were taken for each sample
  - Fluorescence change was calculated for pre-gassing samples and samples 5 hours after gassing
  - Changes were zeroed to wild type E.Coli fluorescence changes
Results

Fluorescence for Different Hydrogen Concentrations after 5 Hours Zeroed to Wild Type (Standard Error Shown)

Change in Pixel Value (Inverted)
Results – Functional Machine

- Minimal YFP production without addition of IPTG
- Strong YFP production with addition of IPTG
- Proportional reduction in fluorescence with addition of $H_2$
Further Work Required

- Fluorescence in 100% H₂ was actually higher than fluorescence in 75% H₂
  - Why?
- Positive results were obtained after incubating for 5 hours
  - Detection is NOT immediate
- More precise testing using wavelengths specific to YFP absorption and emission
Acknowledgements

○ Advisors
  ● Dr. Filip To, Dr. Din-Pow Ma, Dr. Todd French
○ MSU Bagley College of Engineering
○ MSU College of Agriculture and Life Sciences
○ iGEM Ambassador James Brown
○ iGEM Staff
Future Ideas

- Controlled Lipid Synthesis
  - Produce efficient energy source from inefficient organic energy sources
- Water Splitting
  - Efficient, portable H₂ production
- Tar Digestion
  - Yield cleaner energy sources
- Insulin and/or Blood Sugar regulator
  - Diabetes Control