Make it or Break it:
Diesel Production and Gluten Destruction the Synthetic Biology Way
Washington iGEM 2011 Objectives

- Diesel Production
- Gluten Destruction
- iGEM Toolkits
- Community Outreach
Making it: work on diesel production

Diesel Production

- Gluten Destruction
- iGEM Toolkits
- Community Outreach
Society is dependent upon limited petroleum reserves.

- Marine
- Flight
- Combustion
- Long Distance Trucking
- Petroleum Reserves
- Alkanes

The diagram illustrates the combustion of petroleum reserves, leading to the emission of CO₂, and its use in various sectors such as marine, flight, and long-distance trucking.
Integrating CO$_2$ into fuel production
Biofuels are renewable, yet also inefficient, incompatible, and corrosive.
An ideal fuel is both renewable and efficient.

- **Renewability**
- **Energy Density**
- **Compatibility**

- **Petroleum**
- **Microbially-Produced Alkanes**
- **Biofuel**
Microbial production of alkanes from fatty acids

Cellular Fatty Acid Biosynthesis

- Acyl-ACP Reductase
- AAR
- Aldehyde DeCarbonylase
- ADC

The basic alkane production pathway

- Strong constitutive promoter
- RBS
- ADC
- RBS
- AAR

Bba_K590026 → Bba_K590031 → Bba_K590032

Bba_K590025
Analyzing alkane production with GCMS
Analyzing alkane production with GCMS
Analyzing alkane production with GCMS

Gas Chromatograph

Mass Spectrometer

Experimental Spectrum

Reference Spectrum

Average of 9.287 to 9.316 min.: 71.0 ms

Pentadecane

Head to Tail MF = 764
RMF = 909
ADC expression alone is not sufficient for hydrocarbon production
AAR expression results in production of a C14 alcohol
Alkane production from the PetroBrick

We converted sugar into diesel!
Initial alkane yield

Alkane Titer (mg/L)

- C13
- C15
- C17-ene
Optimized alkane yield

- **Original Conditions**
- **Optimized Conditions**

<table>
<thead>
<tr>
<th>Alkane Yield (mg/L)</th>
<th>C13 Alkane</th>
<th>C15 Alkane</th>
<th>C17 Alkane</th>
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<tr>
<td></td>
<td>0.4</td>
<td>6.7</td>
<td>0</td>
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<tr>
<td></td>
<td>1.7</td>
<td>160.3</td>
<td>4.3</td>
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</tbody>
</table>

Achieved 171 mg/L diesel yield
System modifications

Alternative Chassis

Enzyme Localization

System Optimization

Decarbonylase

Redesign

Alternative Aldehyde

Branched Alkanes

Decarbonylase Redesign
From making it to breaking it: gluten destruction

- Diesel Production
- Gluten Destruction
- iGEM Toolkits
- Community Outreach
Gluten intolerance is a common and difficult problem
Indigestible gluten peptides trigger an immune response.

Gluten
Indigestible gluten peptides trigger an immune response.
A protein therapeutic is in clinical trials... but is has low activity at pH 4

- SC PEP from *Sphingomonas capsulata*
- Low activity in acidic conditions
- Good activity on PQLP
Kumamolisin is optimal at pH 4...
but it has unknown activity on PQLP

- Endopeptidase from *Alicyclobacillus sendaiensis*
- High activity in acidic conditions
- Unknown activity on PQLP
An ideal treatment is optimal at low pH and has PQLP activity.
We used computational tools to redesign Kumamolisin for PQLP

Native active site

G319S variant

To download your copy of Foldit, go to http://Fold.It
To test our designs, we developed a whole cell lysate assay

Over 100 novel mutants tested!
Activity of mutants from whole cell lysate screen

- N291D
- D169G
- G319S, D358G, D368H

Fold Change from Native Kumamolisin

Native Kumamolisin
The top mutants were purified and characterized: over 10-fold improvement.

![Bar graph showing fold improvements over WT Kumamolisin](image-url)

- **N291D**: Approximately 12-fold improvement
- **S354N, D358G, D368H**: Approximately 6-fold improvement
- **G319S, D358G, D368H**: Approximately 4-fold improvement
- **Kumamolisin**: Approximately 1-fold improvement
- **SC PEP**: Approximately 1-fold improvement
Greater than 100-fold improvement with a combinatorial mutant

118x activity

0.15x activity

1x activity

784x better than what’s in clinical trials
UW iGEM Objectives

- Diesel Production
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Gibson Assembly Toolkit

Gibson Assembly

Insert

Standard Biobrick Vectors

RFC 10

Gibson Assembly Vectors

RFC 21 (BglBrick)

...GCGGCCGC...

...CGCCGGCG...

BglBrick

...CTGCTGCGGAGATCT...

...AACAGGGTTCTCGAG...

plasmids

1A3

1C3

3K3

4A5

4C5

SB

SB

SB

SB

SB

SB

GA

GA

GA

GA

GA

Ga

pGB plasmids

pGB1A3

pGB1C3

pGB3K3

pGB4A5

pGB4C5

“plasmids for Gibson Assembly”
Improved cloning efficiency with new pGA vectors

Gibson Assembly Efficiencies

- pSB Vector Cloning
- pGA Vector Cloning

- pSB1A3
- pGA1A3

GFP

<table>
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<tr>
<th>Efficiency</th>
<th>pSB</th>
<th>pGA</th>
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<tbody>
<tr>
<td>12%</td>
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<tr>
<td>99%</td>
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</table>
Goal: Build magnetic *E. coli* – “MagnetoColi”

Extracted 18 genes from *mamAB* operon that encode scaffold polymerization, vesicle formation, and biomineralization*

*Thanks to the Komeili lab (Berkeley) for the genomic DNA! (*Magnetospirillum magneticum*, AMB-1)
Magnetosome Toolkit

gene characterization

sfGFP-MamK scaffold polymerization

Membrane localized sfGFP-MamI, binds MamK

Full Characterization & Assembly

MagnetoColi!
UW iGEM 2011 Objectives

Diesel Production

Gluten Destruction

iGEM Toolkits

Community Outreach
Showing synthetic biology to the community!

Engineering Discovery Days

Bennett Elementary School
Teaching the community about cloning!

- Balloon Cell
- Ribbon Vector
- Cut
- DNA Insert
- Linearized Plasmid
- Ligate
- Transform
Teaching the community about cloning!

Completed Cell with New Vector!
What We Accomplished

- Diesel Production
- Gluten Destruction
- iGEM Toolkit
- Community Outreach
What We Accomplished

- Made diesel from sugar
- Engineered enzyme to improve activity 100 fold
- Gibson Assembly Toolkit
- Magnetosome Toolkit
- Showed community synthetic biology is awesome
Biobricks Submitted

Total Biobricks
65!!!
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Questions??