Using Frying Oil to Product High Value Products in an engineered strain of *Escherichia coli*
2014 Team

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Our Story
Overview of Project

Breaking down used frying oil to produce a value-added terpenoid

Spent frying oil can be used to power cars, but there are still 3 billion gallons of spent frying oil produced each year in the U.S.

2. Photograph courtesy of Dan Dickinson. Used with permission under creative commons with attribution.
Project Components

1. Biosensor
2. Breakdown of frying oil
3. High-value product
4. Kill switch
Biosensor - Goal

- Detect the presence of fatty acids
- Function as the promoter for breakdown
- No unnecessary stress without oils present
Biosensor - Background


- **Fatty Acids Absent**: No GFP production
- **Fatty Acids Present**: GFP Produced
Biosensor - Approach and Results

- Assemble 4 promoters from 4 sequenced oligonucleotides
  - Chose PAR and PFL1
- Link to GFP for testing

Future Work:
- Use fully synthesized gene
- Integrate into breakdown
Breakdown of Frying Oil - Goal

- Breakdown used frying oil products
- Create metabolic intermediate

Fatty Acid $\xrightarrow{\text{Beta-Oxidation}}$ Acetyl-CoA $\xrightarrow{\text{Mevalonate Pathway}}$
Breakdown of Frying Oil

- Frying oil is made of triacylglycerol
  - heating makes
    - diacylglycerol
    - fatty acids
- The fatty acids are broken down by the beta oxidation cycle
- FadD is the rate limiting step

Upregulate Beta-Oxidation

- Test by linking to lac promoter
- Eventually link to biosensor promoter
- Creates acetyl-CoA for use in high-value product manufacturing

High-Value Product: Goal

- Acetyl-CoA
  → IPP
  → Terpenoid

High-Value Product: Background

- 2 pathways
- Mevalonate and MEP

High-Value Product: Approach

- Created plasmids to put mevalonate pathway into *E. coli*
- Based on constructing two operons from yeast genes (*S. cerevisiae*)

Kill Switch: Goal

- Toggleable low-leak kill switch
  - Specific conditions
  - Low stress
  - Highly-efficient
Kill Switch: Background

- Tryptophan Repressor (BBa_K588001)
  - Control of KillerRed expression
- KillerRed gene
  - Reactive Oxygen Species

Kill Switch: Approach

- Trp + GFP Plasmid
- Trp+ KR Plasmid
- Kill curve of KR plasmid

Low Stress Design

- Allocates resources to necessary functions
  - Promotes growth and survival
  - Prevents cell from producing products when unnecessary
  - Reserves energy for cell
Workplace safety programs can decrease chances of injury by up to 50%.

2. Photograph courtesy of Michigan School of Natural Resources and Environment. Used with permission under creative commons with attribution.
Future Directions

- Combination of components into single continuous pathway
- Optimization of pathway
- Optimization of environmental conditions
- Economic feasibility analysis
Human Practices

● University of Colorado - Boulder collaboration
● Tested one of their biobricks before submission
Human Practices

- Outreach at elementary school
- Conducted experiments with Science Club
Thank you to our advisers, iGEM, sponsors, and other teams for making this competition and our project possible.
Acetyl-CoA Production

Produce one acetyl-CoA per 2 carbons in the fatty acid backbone

Image courtesy of PharmaChange.info
Terpenoids

Created from combination of isoprene units via condensation reaction

1. Images (left and top right) courtesy of Cyberlipid Center
2. Image (bottom right) courtesy of Simon Cotton

Examples of terpenoids
Plant vs Bacterial Extraction

- Current yields: ~500 mg/L
- Order of magnitude increase in yield may be possible

2. Image (left) courtesy of German Plant Breeders’ Association
3. Image (right) courtesy of Scientific American Magazine
Determining Kill Switch

- KillerRed Info:
  - Ordered via Evrogen
  - 239 aa length, 27kDa
  - Maximum Wavelength: 610 nm

- Alternative Choice: Heme Pathway
  - Removal of HemH gene causes protoporphyrin overproduction
  - Excess protoporphyrin causes ROS buildup resulting in cell lysis