Biofuels in Bacteria and Complex Genetic Circuits

- Biofuels in Bacteria
- Bacterial Etch-a-Sketch
Biofuels in Bacteria
The Energy Industry - Problems Today
Biofuels: Butanol Approach
Advantages of Bio-butanol

• Higher energy density than other biofuels
• Higher air-fuel ratio than other biofuels
• Can be transported using existing fuel infrastructure
  – Resists water absorption, is non-corrosive
Previous Efforts in Bio-butanol Production

Dr. Chaim Weizmann, First President of Israel
  – Discovered reversible n-butanol-producing *Clostridium acetobutylicum*

Dr. James C. Liao
  – Introduced a bio-butanol-producing pathway into *E. coli*
  – Created a mutant NADH accumulating strain of *E. coli*

Dr. Michelle Chang
  – Optimized chimeric bio-butanol pathway
Dr. James C. Liao

• Contribution of a mutant strain of *E. coli*, JCL299
  – Mutant strain that has genetic modifications enabling NADH accumulation in the bacteria.
  – The bacteria was able to use the excess NADH as a driving factor of the reversible n-butanol pathway
Dr. Michelle Chang

- Optimized production of n-butanol by introducing enzymes that operate predominately in the forward direction
Rutgers iGEM

- Introduced the forward-driven pathway into the Liao NADH-accumulating mutant strain.

- The excess NADH available in the mutant strain drives the irreversible pathway forward to obtain optimal butanol production.
Bacterial Transformation of Plasmids

Transformation of three-plasmid system into JCL299 mutant strain

Transformation of three-plasmid system into DH5 alpha wild type strain
Methods

• n-Butanol was extracted with hexane from filtered supernatant of the culture

• n-butanol was quantified by Clarus 680 Gas Chromatography Flame Ionization Detection
Hexane Extraction of n-Butanol

Blue – Indicates butanol hexane extraction
Red – Indicates butanol hexane extraction in SOB media

![Graph showing the relationship between integral area and \( \mu g \) Butanol \( ml^{-1} \).]

- Blue line with equation \( y = 202.99x - 881.37 \) and \( R^2 = 0.986 \)
- Red line with equation \( y = 25.518x + 186.34 \) and \( R^2 = 0.9956 \)
Optimized Bio-butanol Production in NADH-accumulating Strain

Hybrid Pathway in DH5 alpha

Hybrid Pathway in NADH strain
Bio-butanol Project Conclusion

• Butanol production pathway is more active in a strain of *E. coli* engineered to accumulate NADH
Part Submission

- **Ter Gene**
  - Crotonyl-CoA → Butyryl-CoA
  - Forces the pathway forward, non-reversible step

- **FDH Gene**
  - Formate dehydrogenase
  - Catalyzes the oxidation of formate with reduction of NAD+ to NADH
Future Plan

• Introduce FDH gene into non-reversible pathway
• Test against NADH-accumulating strain
BACTERIAL
ETCH-A-SKETCH
Project Goal

470 nm light → Logic Circuit → Output

Memory Element
Challenges

- Light
- Color
- Sensitivity
- Selectivity
- Speed
- Noise
Circuit Design Challenges

- **Light** – need bacteria to respond to light
- Color
- Sensitivity
- Selectivity
- Speed
- Noise
Circuit Design Challenges

• Light – need bacteria to respond to light
• **Color** – need it to make color
• Sensitivity
• Selectivity
• Speed
• Noise
Circuit Design Challenges

- Light – need bacteria to respond to light
- Color – need it to make color
- **Sensitivity** – need it to respond to a *short* pulse
- Selectivity
- Speed
- Noise
Circuit Design Challenges

- Light – need bacteria to respond to light
- Color – need it to make color
- Sensitivity – need it to respond to a short pulse
- **Selectivity** – don’t want it to respond to ambient light
- Speed
- Noise
Circuit Design Challenges

• Light – need bacteria to respond to light
• Color – need it to make color
• Sensitivity – need it to respond to a short pulse
• Selectivity – don’t want it to respond to ambient light
• Speed – want to see the color quickly
• Noise
Circuit Design Challenges

• Light – need bacteria to respond to light
• Color – need it to make color
• Sensitivity – need it to respond to a short pulse
• Selectivity – don’t want it to respond to ambient light
• Speed – want to see the color quickly
• **Noise** – don’t want to see random splotches of color
Light input $\rightarrow$ Signal amplification $\rightarrow$ Color output

Light $\rightarrow$ LovTAP $\rightarrow$ Locking Switch $\rightarrow$ mRFP1
LovTAP – Light-inducible repressor

Image from Strickland et al. PNAS 2008
Locking switch – Signal amplifier

- Based on Peking 2009 bistable switch
Locking switch – Signal amplifier

- By default, cl434 levels are high
Locking switch – Signal amplifier

• Dropping cl434 levels flips the switch
Locking switch – Signal amplifier

- Dropping cl434 levels flips the switch
Locking switch – Signal amplifier

- Dropping cl434 levels flips the switch
Locking switch – Signal amplifier

• Once switch is flipped, it is stuck
Locking switch – Signal amplifier

- Once switch is flipped, it is stuck
mRFP1 – Strong color output

- mRFP1
- Fluoresces red but visible in ambient lighting
- Expressed from T7 promoter
In the light

Const. → LovTAP

ptrpL → cl434

pRM → cl

trpR → T7 Polymerase

mRFP1 → pT7

Red arrows represent repression, green arrows represent activation.
Results

• Two-plasmid circuit
• Fourteen-step ligation plan
Results

Plasmid 1

Key

Green # = Completed
Red # = Not completed
Results
Plasmid 2

Key
Green # = Completed
Red # = Not completed
Application – Biosensor

Input

Light
Chemicals

Output

Color
Enzymatic activity
Cell growth/Development

Locking switch
Future Plan

• Finish constructing the second plasmid
• Assay the circuit
• Propose different modifications to the circuit to function as specific biosensor
Questions?