E. coil
A super E. coli for producing oil
NJU-QIBEBT
World needs energy!
World needs energy!
• World needs energy!

• Oil will be exhausted!
• World needs energy!
• Oil will be exhausted!
• Let’s solve the problem!
Background

- Renewable sources
  - solar
  - wind
  - hydropower
  - biomass
bio-energy

plant-based

microorganism-based

bacteria

fatty acid biosynthesis
We chose *E. coli* as the host organism.
**Background**

**WHY *E. coli***

1. well-developed technique

2. thorough understanding

3. mature protocols

4. diversity of carbon utilization and rapid growth rate
Why fatty acid?

- Precursor of biodiesel
- Raw material for chemical industry
- Necessary for human beings
Background

• To produce more fatty acids

HOW

• To transport fatty acids out of *E. coli*

• To detect if *E. coli* works
Production
Pumping out
Quality control
Conclusion

Modules
Module 1: Production
• Our goal

normal *E. coli*  \[\rightarrow\]  *E. coli* factory  

*Level Up!*
• Our goal
• Our design
• Our test
• Our goal

- higher production of fatty acids than usual
- different kinds of fatty acids according to our demand

*E. coli* factory
• Our goal

• Our design: Several genes in fatty acids biosynthetic pathway

• Our test
Our design: Several genes in fatty acids biosynthetic pathway.
Our design: Several genes in fatty acids biosynthetic pathway

- Rate-limiting step
- Inhibition
- Key enzyme
- Products

higher production of fatty acids than usual
**Our design**: Several genes in fatty acids biosynthetic pathway different kinds of fatty acids according to our demand

**Thioesterase gene**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sources</th>
<th>Substrate Specificity (carbon chain length)</th>
</tr>
</thead>
<tbody>
<tr>
<td><code>tes A</code></td>
<td><em>E. coli</em></td>
<td>C14/C16/C18</td>
</tr>
<tr>
<td>Ac <code>tesA</code></td>
<td><em>Acinetobacter baylyi</em></td>
<td>C8/12/14/16</td>
</tr>
<tr>
<td><code>bte</code></td>
<td><em>Umbellularia californica</em></td>
<td>C12/14</td>
</tr>
<tr>
<td>Cp <code>fatB1</code></td>
<td><em>Cuphea palustris</em></td>
<td>C8/C10</td>
</tr>
<tr>
<td>Uc <code>fatB1</code></td>
<td><em>Umbellularia californica</em></td>
<td>C12</td>
</tr>
<tr>
<td>At <code>fatA</code></td>
<td><em>Arabidopsis thaliana</em></td>
<td>C16/C18</td>
</tr>
</tbody>
</table>
Our design: Several genes in fatty acids biosynthetic pathway lead to different kinds of fatty acids according to our demand.
• **Our goals**: higher production of fatty acids than usual
different kinds of fatty acids according to our demand

• **Our design**: Several genes in fatty acids biosynthetic pathway
  
  \( \textit{bte, AtfatA, fabA, fabB} \)

  Make it controllable

• **Our test**
• Our design: Make it controllable

Method: operon structure

- $P_{lac}$
  - RBS
  - AtFatA
  - Term

- $P_{ara}$
  - RBS
  - BTE
  - Term

- $P_{trp}$
  - RBS
  - FabA
  - FabB
  - Term
Our test: Plasmid: *lac*+ RBS+ *AtfatA* CDS+ Term (C16 & C18)

- mRNA level
- Proportion of fatty acids
- Comparison with wild type *E.coli*
• **Our goals**: higher production of fatty acids than usual different kinds of fatty acids according to our demand

• **Our design**: Several genes in fatty acids biosynthetic pathway
  
  *(bte, AtfatA, fabA, fabB)*

  Make it controllable

• **Our test**: **QPCR for mRNA & GC-MS for product fatty acids**

  Plasmid: *ara*+ RBS+ *bte* CDS+ Term

  Plasmid: *lac*+ RBS+ *AtfatA* CDS+ Term

  Plasmid: *trp*+ RBS+ *fabA* CDS+ *fabB* CDS + Term
Our test: Plasmid: *lac*+ RBS+ *AtfatA* CDS+ Term (C16 & C18)

- mRNA level
- Proportion of fatty acids
- Comparison with wild type *E.coli*
Our test: Plasmid: \textit{lac+ RBS+ AtfatA CDS+ Term} (C16 & C18)

Total: \textbf{0.141mg/L}  
Total: \textbf{20.3mg/L}
Our test: Plasmid: *ara+ RBS+ bte CDS+ Term* (*C12 & C14*)

- mRNA level
- Proportion of fatty acids
- Comparison with wild type *E.coli*

![Graph showing mRNA Level of bte Gene](chart)
• **Our test:** Plasmid: *ara+ RBS+ bte CDS+ Term* (C12 & C14)
  - mRNA level
  - Proportion of fatty acids
  - Comparison with wild type *E.coli*
**Our test:** Plasmid: *ara+ RBS+ bte CDS+ Term (C12 & C14)*

- mRNA level
- Proportion of fatty acids
- **Comparison with wild type** *E. coli*
Our test: Plasmid: trp+ RBS+ fabA CDS+ fabB CDS + Term (unsaturated fatty acids)
Module 2: Pumping out
- Our goal: **pump out fatty acid outside the membrane**
- Our design
- Our modification
• Our goal: pump out fatty acid outside the membrane
• Our design: use MsbA and fadD promoter
• Our modification
Our design: use MsbA and *fadD* promoter

- MsbA: ATP-binding cassette transporter
- *fadD* promoter
  - No acyl-CoAs
  - Plus acyl-CoAs

Ref: Youjun Feng et al. *PLOS ONE*
Our design: use MsbA and \textit{fadD} promoter

- MsbA: ATP-binding cassette transporter

Ref: Andrew Ward \textit{et al.} \textit{PNAS}
Our design: use MsbA and \textit{fadD} promoter

\begin{itemize}
\item MsbA: ATP-binding cassette transporter
\item \textit{fadD} promoter
\item Our circuit
\end{itemize}

\textbf{Produce high amounts of fatty acids}

\textit{X-FFA}
Our Modification: point mutation of cutting site EcoRI

\[ msbA \text{ .seq } ..ACTGGCGACGTG\text{GAATTCCGCAATG}.. \]

PM at this site

\[ PM \text{ msbA .seq } ..ACTGGCGACGTG\text{GAGTTCCGCAATG}.. \]
Module 3: Quality control
• Our goal: monitor the amounts of fatty acids
• Our design
• Our test
• Model
• **Our goal**: monitor the amounts of fatty acids

• **Our design**: *E. coli* traffic light

• **Our test**

• **Model**
• Our design: *E. coli* traffic light

- **Principle**

  ![Diagram showing the regulation of FadD and FabB genes by FadR in the presence and absence of free fatty acids.](image-url)
Our design: *E. coli* traffic light

- Principle
- Our Blueprint: GFP & RFP
• **Our design**: *E. coli* traffic light
  - Principle
  - Our Blueprint: GFP & RFP
  - Our Circuit
• Our test: fluorescence detection
- Our test: fluorescence detection
- **Our test:** fluorescence detection

![Graph showing fluorescence intensity over growth time](image)

- PfabB & PfadD
- Synthesis & degradation → fluorescence intensity
## Modeling

<table>
<thead>
<tr>
<th>Time</th>
<th>RFP Fluorescence</th>
<th>GFP Fluorescence</th>
<th>Fatty Acid Concentration</th>
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<tbody>
<tr>
<td>2</td>
<td>1.055</td>
<td>4.123</td>
<td>0.119</td>
</tr>
<tr>
<td>3</td>
<td>0.671</td>
<td>3.578</td>
<td>0.126</td>
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<tr>
<td>4</td>
<td>0.912</td>
<td>3.735</td>
<td>0.183</td>
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<tr>
<td>6</td>
<td>1.107</td>
<td>4.039</td>
<td>0.302</td>
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<tr>
<td>8</td>
<td>1.349</td>
<td>4.325</td>
<td>0.263</td>
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<tr>
<td>12</td>
<td>1.954</td>
<td>4.976</td>
<td>0.265</td>
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<tr>
<td>18</td>
<td>1.828</td>
<td>4.940</td>
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<tr>
<td>24</td>
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<td>5.570</td>
<td>0.271</td>
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<tr>
<td>36</td>
<td>1.854</td>
<td>5.286</td>
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<td>48</td>
<td>1.770</td>
<td>4.025</td>
<td>0.245</td>
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<tr>
<td>60</td>
<td>1.845</td>
<td>4.077</td>
<td>0.233</td>
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<tr>
<td>72</td>
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<td>3.705</td>
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<tr>
<td>84</td>
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<td>3.702</td>
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<tr>
<td>96</td>
<td>2.104</td>
<td>3.536</td>
<td>0.222</td>
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<tr>
<td>108</td>
<td>2.086</td>
<td>3.528</td>
<td>0.205</td>
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<tr>
<td>120</td>
<td>2.223</td>
<td>3.429</td>
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<tr>
<td>132</td>
<td>2.304</td>
<td>3.442</td>
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<tr>
<td>144</td>
<td>2.518</td>
<td>3.209</td>
<td>0.199</td>
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### Modeling

<table>
<thead>
<tr>
<th>Variant</th>
<th>Parameter Estimate</th>
<th>Standard Deviation</th>
<th>P Value</th>
<th>Statistical Quantity</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.2899</td>
<td>0.0402</td>
<td>0.0002</td>
<td>Mean Square Error</td>
<td>0.0014</td>
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<tr>
<td></td>
<td>(MSE)</td>
<td></td>
<td></td>
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<tr>
<td>x1 Coefficient</td>
<td>-0.0663</td>
<td>0.0168</td>
<td>0.0055</td>
<td>F Value</td>
<td>9.8400</td>
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<tr>
<td>x2 Coefficient</td>
<td>0.0180</td>
<td>0.0071</td>
<td>0.0386</td>
<td>P Value</td>
<td>0.0093</td>
</tr>
</tbody>
</table>
• Model

\[ \hat{y} = 0.01802x_G - 0.06631x_R + 0.28991 \] (Linear fitting)

- \( x_G \): Synthesis
- \( x_R \): Degradation
- \( \hat{y} \): Fatty Acid Concentration
Modules: How they work
Modules: Conclusion

- Quality control
- MsbA: pump out
- Power up: more fatty acids
Next Step: Cheaper Carbon Source
### Parts Submitted

<table>
<thead>
<tr>
<th>Part Code</th>
<th>Description</th>
<th>Part Code</th>
<th>Description</th>
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<tbody>
<tr>
<td>BBa_K1402000</td>
<td>MsbA-CDS</td>
<td>BBa_K1402009</td>
<td>FabB Promoter</td>
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<td>BBa_K1402001</td>
<td>RBS-MsbA-Terminator</td>
<td>BBa_K1402010</td>
<td>Lac Promoter</td>
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<td>T7-RBS-MsbA-Terminator</td>
<td>BBa_K1402011</td>
<td>FabB-RBS-GFP</td>
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<tr>
<td>BBa_K1402003</td>
<td>FadD-RBS-MsbA-Terminator</td>
<td>BBa_K1402012</td>
<td>FadD-RBS-RFP</td>
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<td>BBa_K1402004</td>
<td>AtFatA-CDS</td>
<td>BBa_K1402013</td>
<td>FadD-RFP-FabB-GFP</td>
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<td>RBS-AtFatA-Terminator</td>
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<td>BTE-CDS</td>
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<td>BBa_K1402006</td>
<td>T7-RBS-AtFatA-Terminator</td>
<td>BBa_K1402015</td>
<td>RBS-BTE-Terminator</td>
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<td>BBa_K1402007</td>
<td>Lac-RBS-AtFatA-Terminator</td>
<td>BBa_K1402016</td>
<td>Ara-RBS-BTE-Terminator</td>
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<tr>
<td>BBa_K1402008</td>
<td>FadD Promoter</td>
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Expand iGEM Family in China

Meetup at Huazhong Agricultural University

Establishment of an iGEM Club in Nanjing University

Internship in Qingdao Institute of Bioenergy and Bioprocess Technology

Small-scale Industrialization

Human Practice
Human Practice: Expand iGEM Family in China
Human Practice: Meetup at HZAU
Human Practice: Internship in QIBEBT
Human Practice: Small-scale Industrialization
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Prof. Chen Xi
Prof. Xian Mo
Prof. Zen Ke

Advisor: Liu Yanqing, Wang Xueliang, Xiong Aoli

Nanjing University
School of Life Sciences

M3 lab

Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences
THANK YOU
Q & A

Finally, give it up for E.COIL!