CUHK IGEM TEAM 2014
Project: ABCDE

Presented by
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Ricky Chan Wai Lun (Wet lab team)
Joe Ho Cho Tai (Modeling team)
Elaine Chiu Yee Ting (Human Practice team)
ABCDE System

AzotoBacter vinelandii Cluster-transformable & Deoxygenated protein Expression system (ABCDE system)
Introduction
Problem: Energy and Environment

Carbon dioxide reduction to methane and coupling with acetylene to form propylene catalyzed by remodeled nitrogenase

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Hydrogenase III (H₂ase)

Byproduct of nitrogenase

Needed for CH₄ production

Nitrogenase (N₂ase)

ADP

Nitrogenase (N₂ase)

MoFe protein reduced

Fe protein oxidized

H₂ oxidized

Ferredoxin oxidized

Ferredoxin reduced

ATP

CO₂

CH₄

Supported by Guiral et al., 2005
Problem of Introducing Nitrogenase

• Oxygen sensitive (degenerated by oxygen)
• Anaerobic condition is needed
Previous iGEM Team

2013 Bielefeld-Germany iGEM team

2013 Copenhagen iGEM team
Solution: ABCDE System

*Azoto* Bacter vinelandii Cluster-transformable & Deoxygenated protein Expression system

(ABCDE system)
Why *Azotobacter vinelandii*

- Intracellular anaerobic environment
- Stable genome integration
- Safety level: group 1 bacteria
- *nifH* promoter
  (ammonium-repressible)

AzotoBacter vinelandii Cluster-transformable & Deoxygenated protein Expression system (ABCDE system)
Biobricks and Characterization

Ammonium-Repressible T7 Protein Expression System
### ABCDE: Basic Parts

<table>
<thead>
<tr>
<th>Parts</th>
<th>Description</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBa_K1314000</td>
<td>Ammonia repressible nifH promoter</td>
<td>Submitted and accepted</td>
</tr>
<tr>
<td>BBa_K1314002</td>
<td>150bp random sequence (sequence for homology)</td>
<td>Submitted and accepted</td>
</tr>
<tr>
<td>BBa_K1314003</td>
<td>part of mRFP(sequence for homology)</td>
<td>Not submitted</td>
</tr>
<tr>
<td>BBa_K1314012</td>
<td>nif K homologous sequence</td>
<td>Not submitted</td>
</tr>
</tbody>
</table>
Characterization of BBa_K1314000: nifH Promoter

NIF REGULON

transcriptional regulation mechanism of nifHDK operon in A. vinelandii

Characterization of BBa_K1314000: nifH Promoter

Gel photo of EcoRI PstI double digest check of BBa_K1314000

nifH regulated RFP expression(left) versus constitutive RFP expression(right) in DH5α
### ABCDE: Composite Parts

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</thead>
<tbody>
<tr>
<td>BBa_K1314001</td>
<td>nifH promoter with mRFP reporter</td>
<td>Submitted and accepted</td>
</tr>
<tr>
<td>BBa_K1314004</td>
<td>Ptet mRFP with random sequence</td>
<td>Submitted and accepted</td>
</tr>
<tr>
<td>BBa_K1314005</td>
<td>Ammonia- repressible T7 RNA polymerase generator(strong)</td>
<td>Submitted and accepted</td>
</tr>
<tr>
<td>BBa_K1314006</td>
<td>Ammonia- repressible T7 RNA polymerase generator(weak)</td>
<td>Submitted and accepted</td>
</tr>
<tr>
<td>BBa_K1314007</td>
<td>Ammonia- repressible T7 RNA polymerase generator(strong) with ptet mRFP reporter</td>
<td>Submitted and accepted</td>
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<tr>
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<td>Ammonia- repressible T7 RNA polymerase generator(weak) with ptet mRFP reporter</td>
<td>Submitted and accepted</td>
</tr>
<tr>
<td>BBa_K1314009</td>
<td>Amilcp reporter with part of rfp</td>
<td>Submitted and accepted</td>
</tr>
<tr>
<td>BBa_K1314010</td>
<td>T7 promoter with random sequence</td>
<td>Submitted and accepted</td>
</tr>
</tbody>
</table>
## ABCDE: Composite Parts

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<th>Status</th>
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<tbody>
<tr>
<td>BBa_K1314010</td>
<td>T7 promoter with random sequence</td>
<td>Submitted and accepted</td>
</tr>
<tr>
<td>BBa_K1314011</td>
<td>T7 promoter amilcp reporter with homologous sequence</td>
<td>Submitted and accepted</td>
</tr>
<tr>
<td>BBa_K1314013</td>
<td>Ammonia- repressible T7 RNA polymerase generator(strong) with ptet mRFP reporter( site-specific integration version)</td>
<td>Submitted and accepted</td>
</tr>
<tr>
<td>BBa_K1314014</td>
<td>Ammonia- repressible T7 RNA polymerase generator(weak) with ptet mRFP reporter( site-specific integration version)</td>
<td>Submitted and accepted</td>
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</table>
BBa_K1314013/14: T7 RNA Polymerase into \textit{A. vinelandii}
Circular DNA of BBa_K1314013 with Ampicillin resistance gene

BBa_K1314013/14: Making the Vector

Linearized DNA of BBa_K1314013 with Ampicillin resistance gene by cutting XhoI

Single Digestion using XhoI
BBa_K1314013/14: Modifying the A. vinelandii genome

genomic sequence of nifHDK operon in wild type A. vinelandii

Linearized DNA of BBa_K1314013 with Ampicillin resistance gene by cutting XhoI

K1314013 XhoI cut
6465 bp

Characterization of BBa_K1314013/14

Gel electrophoresis of EcoRI PstI double digestion check of BBa_K1314013/14
Characterization of BBa_K1314013/14

**Ammonium replete condition**
- BBa_K1314014 (no growth)
- BBa_K1314001 (RFP expression but poor growth)
- Well grown but no red RFP expression

**Ammonium depleted condition**
- BBa_K1314014 (no growth)
- BBa_K1314001 (RFP expression but poor growth)
- Wildtype A.vinelandii
  - Normal nitrogenase expression
Introduce gene under T7 promoter into *A. vinelandii*

Part of genome sequence of *A. vinelandii* with BBa_K1314013 integrated

Strain Colour change from red to blue

Sequence of DNA Vector BBa_K1314011
Characterization of BBa_K1314011

BL21 BFP expression of K1314011 (right, 10mM IPTG added) and negative control (left, no IPTG added)
### BBa_K1314015: Vector for insertion of desired protein for T7 expression

**Plasmid Backbone**

<table>
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<th>Description</th>
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</thead>
<tbody>
<tr>
<td>Part:BBa_K1314015</td>
<td>Homology integration toolkit</td>
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</table>

![EcoRI PstI double digest check gel photo](image)

- 3000bp
- 2000bp
- ~2500bp insert
Genome Integration Has Never Been Easier

Quick method for making desired vector

Restriction + Ligation = Vector for integration
Modeling

ABCDE (Protein Expression) - solve the contradiction

ABCDE system (Methane Energy) - rate & efficiency
Problem in protein expression

α-RNA polymerase \(\rightarrow\) nifH promoter \(\rightarrow\) T7polymerase \(\rightarrow\) T7 promoter

needs ammonia

repressed by ammonia
Solution: Separate into two stages

Stage one
Producing T7 polymerase

Stage two
Producing target proteins

ABCDE (Protein Expression) - solve the contradiction
We have the following equation for the rate of change of [N]:

\[
\frac{d[N]}{dt} = -A \frac{d[T7]}{dt} - B \frac{d[TP]}{dt} - \frac{C[N]}{D + [N]}
\]

- A: nitrogen needed to expression one T7 polymerase;
- B: nitrogen needed to expression one target protein;
- C: maximum speed of nitrogen consumption of unmodified bacteria;
- D: concentration needed for the rate of nitrogen consumption equals to half of maximum speed.

Stage one: producing T7 polymerase

\[ \frac{dT7}{dt} = E[N]e^{-F[N]} \]
Stage two: producing target protein

\[ \frac{d[TP]}{dt} = V_{max}([T7]) \]

Concentration vs. time graph with the equation shown on the graph.
Analysis: rate equations for CH₄ production

\[
\frac{d[CH_4]}{dt} = K_1[H^+][CO_2] = K_1K_2[H^+] = K[H^+] \\
\frac{d[H^+]}{dt} = K_3[H_2]/[oxidized ferredoxin] - k'' \frac{d[CH_4]}{dt} \\
= K_3K_4[CH_4]/[oxidized ferredoxin] - k'' \frac{d[CH_4]}{dt} \\
= K_3K_4K_5[CH_4] - k'' \frac{d[CH_4]}{dt} \\
= K'[CH_4] - k'' \frac{d[CH_4]}{dt}
\]

\[K_1 = \text{rate constant}; \ K_2 = [CO_2]^n; \quad K = K_1K_2; \]
\[K_3 = \text{rate constant}; \ [H^+] = K_4[CH_4]; \ [oxidized \ ferredoxin] = K_5; \quad K' = K_3K_4K_5. \quad K'' = K_4 + 8\]
$[CH_4] = c_1 \exp(c_2 t) + c_3 \exp(c_4 t)$
Result: Energy Efficiency

Energy Output ($E_{\text{output}}$):

$E_c$: combustion energy of CH$_4$

Energy input ($E_{\text{input}}$):

$E_1$: ATP energy for enzyme activity – producing CH$_4$
$E_2$: ATP energy for producing H$_2$
$E_m$: Metabolic energy
$E_E$: Environmental consumption

Energy Efficiency = \[
\frac{E_{\text{output}}}{E_{\text{input}}} = \frac{E_c}{E_1 + E_2 + E_m + E_E} = 5.36\% \]

~ 2 times of photosynthesis in crop plants (2%)

Reference: Govindjee, What is photosynthesis?
Human Practice and Safety
Collaboration

Cooperate with CityU_HK, Hong_Kong_HKUST, Berlin
-Cloning
-Modelling
-Ideas exchanging

Material Exchanging Platform
Collaboration

Invitrogen 1kb plus ladder

BBa_K1472614 (3384bp)

4000bp 3000bp

Colony PCR with biobrick sequencing primer

~3600bp
What do people think about our project
Do you think this project is beneficial to the human being?

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
<th>No opinion</th>
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<tbody>
<tr>
<td>HS</td>
<td>90</td>
<td>10</td>
<td>4</td>
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<tr>
<td>UG</td>
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<td>10</td>
<td>2</td>
</tr>
<tr>
<td>PG</td>
<td>10</td>
<td>5</td>
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</tr>
<tr>
<td>Other*</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

HS: High School  
UG: Under-graduate  
PG: Post-graduate  
Other: Any responders other than these three
What is the most attractive highlight to you in this project?

**Environmental friendly**

**Bacterial-based**

**Creativity**

**Practical**

**None**

**Other (Interesting)**

**HS: High School**

**UG: Under-graduate**

**PG: Post-graduate**

**Other: Any responders other than these three**

- HS (102)
- UG (408)
- PG (61)
- Other* (32)
In which aspect do you think this project is helpful and therefore worth conducting?
What will be your main concern of this project?

- Safety
- Efficiency
- Cost
- Other

HS: High School
UG: Under-graduate
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Other: Any responders other than these three
Safety

• Lab safety training course
• Proper handling of biological and chemical materials
• Proper disposal of biological and chemical waste
• Personal protective equipment
• Only organisms in risk group 1 are used
• Negative pressure laboratory
• Communication with supervisors regularly
Laboratory Demonstration
- Introduce simple lab tools
- Some simple cloning techniques
- *Focus on increasing safety awareness
Have you heard of International Genetical Engineered Machine (iGEM) or Synthetic Biology?

- Neither: 100%
- iGEM only: 80%
- Synthetic Biology Only: 60%
- Both: 40%

HS: High School
UG: Under-graduate
PG: Post-graduate
Other: Any responders other than these three
Do you think there are enough ways for public to reach Synthetic Biology?

HS: High School
UG: Under-graduate
PG: Post-graduate
Other: Any responders other than these three
In your point of view, which is the most effective way to raise public awareness towards synthetic biology?

- Leaflet
- Poster
- Workshop
- Other

- HS: High School
- UG: Under-graduate
- PG: Post-graduate
- Other: Any responders other than these three

HS (102) | UG (408) | PG (61) | Other* (32)
Outreach

University
- Energy Day
- Art Fair

High School
- Collaboration
- Workshops

Industry
- CUHK Orientation Day
- Energy Day

International
- Poster
- Collaboration
Outreach

University
  - Energy Day
    - Art Fair
  - Collaboration
  - Workshops
  - CUHK Orientation Day

High School

Industry
  - Energy Day

International
  - Poster
  - Collaboration
Energy day

Poster presentation
- Promote our project
- Receive professional comments

There are industries interested in our project
Art fair, CUHK Orientation Day, Workshops & Poster Exhibition

Leaflet Distribution
Poster Exhibition
-Promotion of iGEM and Synthetic Biology
-Promotion of Project
-Recruitment of next year iGEM team
Would you join activities related to Synthetic Biology (say, iGEM), if you were given a chance?

<table>
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More!

- First intracellular anaerobic protein expression system
- Introduce *Azotobacter vinelandii* to iGEM
- Try to make registry biobricks usable in the chassis
- Carbon fixation with the bacteria

Bronze
- Completed Judging form
- Wiki

Silver
- Characterize Bba_K1314000, Bba_K1314001, Bba_K1314011, Bba_K1314013, Bba_K1314014
- 16 biobricks submitted and accepted

Gold
- Improved and characterized E1010 and K529009
- Helped CityU to construct a functional biobrick with some characterization
- Analyzed difference between postgraduate, high school and college student
- Linking all the iGEM together by material changing platform
And Further More…

- Electricity production
- Magnetosome production
- Carbon fixation
- Nitrogen fixation
- ......More to be discovered

ABCDE is just a beginning!
Acknowledgement

Our Sponsors:
Special Acknowledgement:

• Professor CHAN King Ming (Associate Professor, School of Life Sciences)
• Professor CHAN Ting Fung (Associate Professor, School of Life Sciences)
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• Thank you Professor Dennis R. Dean and Ms. Valerie Cash for giving DNA and strain dj of *Azotobactor Vinlandii*.
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• Thank you Dr. Zhu Yuan (Dept. of Biology and Chemistry, City University of Hong Kong) for modelling work.
Thank You!