PLANTS vs HCHO

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University of Electronic Science and Technology of China
According to our survey

People want plants to deal with HCHO!

- Price: 24.8%
- Safety: 57.7%
- Efficiency: 42.7%
- How to grow: 22.4%
HCHO Terminator

Gene: HPS, PHI, FDH, FALDH

Super Power: Absorb Formaldehyde
Gas Swallower

Gene: AHA2

Super Power: Larger Stoma Absorb more gas
Gene Guard

Gene: ADCP

Super Power: Safe Gene
Design & Result
Design

Normal Plant

Folate-independent pathway

FDH + FALDH

Breath HCHO

Plants vs HCHO

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Folate-Independent Pathway in Plants

- SMM cycle
  - Ser \( \rightarrow \) 5,10-CH\(_2\)-THF
  - 5,10=CH-THF
  - 10-HCO-THF
  - Glyoxylate

- Calvin cycle
  - CO\(_2\)+H\(_2\)O

- Folate-mediated pathway
  - HCOOH
  - Formyl-GSH
  - HM-GSH

- Folate-independent pathway
  - HCHO
  - CH\(_3\)OH
  - FALDH

- FDH
RuMP Pathway in Bacteria

Ru5P → HCHO → HPS → Hu6P → PHI → F6P → Cell Constituents

Ru5P → RuBP → CO₂ → Calvin Cycle → 3-PGA → FBP → F6P

Xu5P → Ru5P → HCHO → HPS → Hu6P → PHI → F6P → Cell Constituents
**Design**

1. CO₂ enters through stomas.
2. **AtAHAS** increases to form bigger stomas.
3. **Normal Plant**
   - **RuMP pathway** (+HPS-PHI)
   - **Folate-independent pathway** (+FDH+FALDH)
4. Male Sterile
   - **AdCP**
5. **Breath HCHO**
   - **Super Plant**

*If it is out of lab*
Give the Plant a Big “Mouth”

WT  

GC1::AHA2

Scale bar: 10 μm

Wang et al., 2014 PNAS
Design

CO₂ → Stomata → Bigger Stomata

Normal Plant

RuMP pathway

Breath HCHO

Folate-independent pathway

HPS-PHI

Male Sterile

*if it is out of lab

Super Plant

UDP-Chi → AdCP

Plants vs HCHO

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Normal Plant

*Adcp

Male Sterile

*if it is out of lab
Biosafety Guard

Shukla, P., et al 2014 Funct Integr Genomics
How to assemble these parts together?
Gene Stacking

piGEM001
P35S+P2A+T2A+F2A
+nptII+T-HSP+T-35S+T-nos

+AtAHA2+PGC1
+PTA29+AdCP

piGEM002

+TCP02-HPS-PHI

+TCP01-BbFALDH

HPS-PHI+AtFDH+BbFALDH+

AtFDH+

piGEM003

piGEM004

piGEM005

piGEM006

piGEM007

piGEM008

piGEM009

piGEM010

piGEM011

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Design

**Backbone**

- **35S promoter**
- **LB**
- **HSP terminator**
- **Nos terminator**
- **35s terminator**
- **npt II**
- **GSG-X2A X: T, P, F**
- **TA29 promoter**
- **AHA2**
- **AdCP**
- **RB**

**Translation Enhancer**

35S pro-MASS: BBa_K1537015

Pre-existing Part: BBa_K414002

2A peptide: BBa_K1537016/BBa_K1537017

Pre-existing Part: BBa_K1199016/BBa_K1199046

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Mono-gene Expression Vectors

<table>
<thead>
<tr>
<th>Location</th>
<th>Gene</th>
<th>HPS-PHI</th>
<th>FDH</th>
<th>FALDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroplast</td>
<td>piGEM003</td>
<td>piGEM004</td>
<td>piGEM005</td>
<td></td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>piGEM006</td>
<td>piGEM007</td>
<td>piGEM008</td>
<td></td>
</tr>
</tbody>
</table>

Translation Enhancer: GSG-X2A
Transit peptide: piGEM008

35S promoter
GC1 promoter
TA29 promoter

LB
RB
Multi-gene Expression Vectors

piGEM009

35S promoter

HPS-PH1

FDH

FALDH

npt II

T

T

AHA2

AdCP

RB

GC1 promoter

TA29 promoter

piGEM010

35S promoter

HPS-PH1

FDH

FALDH

npt II

T

T

AHA2

AdCP

RB

GC1 promoter

TA29 promoter

piGEM011

35S promoter

HPS-PH1

FDH

FALDH

npt II

T

T

AHA2

AdCP

RB

GC1 promoter

TA29 promoter

Translation Enhancer

Transit peptide

GSG-X2A X: T, P, F

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Vectors Verification by Digestion
A. tumefaciens Mediated Tobacco Transformation

Co-cultivation

Selection

Rooting
Positive Tobacco Plantlets of Each Transgenic Line

piGEM003  piGEM004  piGEM005  piGEM006  piGEM007

piGEM008  piGEM009  piGEM010  piGEM011  Wildtype
## Result

### Transformation Efficiency of Each Vector

<table>
<thead>
<tr>
<th>Lines</th>
<th>No. of transformed tobacco Leaf disc</th>
<th>Rate of Kan-resistant plantlet (%)</th>
<th>Rate of PCR positive plantlet (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>piGEM003</td>
<td>150</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>piGEM004</td>
<td>150</td>
<td>38.7</td>
<td>20.0</td>
</tr>
<tr>
<td>piGEM005</td>
<td>150</td>
<td>61.3</td>
<td>100.0</td>
</tr>
<tr>
<td>piGEM006</td>
<td>150</td>
<td>41.3</td>
<td>100.0</td>
</tr>
<tr>
<td>piGEM007</td>
<td>150</td>
<td>22.7</td>
<td>16.7</td>
</tr>
<tr>
<td>piGEM008</td>
<td>50</td>
<td>30.0</td>
<td>22.2</td>
</tr>
<tr>
<td>piGEM009</td>
<td>150</td>
<td>56.7</td>
<td>83.3</td>
</tr>
<tr>
<td>piGEM010</td>
<td>150</td>
<td>40.0</td>
<td>100.0</td>
</tr>
<tr>
<td>piGEM011</td>
<td>200</td>
<td>21.5</td>
<td>100.0</td>
</tr>
</tbody>
</table>

* Rate of Kan-resistant plantlet = No. of Kan-resistant plantlet / No. of transformed tobacco Leaf disc

**Rate of positive seedlings = No. of PCR positive plantlet / No. of Kan-resistant plantlet
Expression of Key Enzyme Genes in Tobacco

The RT-PCR result of piGEM010-1 transgenic line was shown as an example. All other transgenic plants showed similar results.
Enhanced Formaldehyde Tolerance

Before

After

piGEM010

35S promoter

LB

GC1 promoter

TA29 promoter

HPS

FDH

FALDH

npt II

T

T

AHA2

AdCP

FALDH

37% 10μl HCHO solution

Transgenic

Wildtype

Before

After

A

HCHO

A

HCHO

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Enhanced Formaldehyde Absorbance

piGEM010

35S promoter

GC1 promoter  TA29 promoter

Before

After
Ongoing Work

- HCHO tolerance comparison of transgenic plants carrying different vectors
- The effect of different cellular location of the key enzymes on HCHO metabolism efficiency
- AdCP gene function as pollen abortion
Future Work

- Give the plants a big “mouth”
- Chloroplast transformation
- Application on other plants
Conclusion

- Submitted 12 high quality and well-characterized Standard Biobricks, including 2 improved biobricks;

- Constructed 11 vectors;

- Got positive transgenic plants;

- Integrated two pathways using synthetic biology method;

- HCHO ‘terminator’ in transgenic plants
- RuMP Pathway
- Folate-Independent Pathway
- The Stomatal Pathway
Theoretically, the bacterial RuMP pathway can be integrated with the Calvin cycle for formaldehyde consumption.
Folate-Independent Pathway

Dynamic equation

\[
\frac{dX_i}{dt} = \sum \gamma_i (l) J_l + p_i \left( X_i^0 - X_i \right)
\]

\[
\frac{dX}{dt} = k_0 X_0 - (k_0 + k_1) X
\]

\[
\frac{dY}{dt} = k_1 X - \left[ a k_2 + \left( 1 - a \right) k_3 \right] Y
\]

FALDH/ FDH are the key enzymes in formaldehyde metabolism via Floate-independent pathway.
The Stomatal Pathway

The formaldehyde molecular diffusion velocity distribution meets the maxwell speed distribution function in normal conditions.

\[ d\Omega dS dt = f(v_x, v_y, v_z) v_x dv_x v_y dv_y v_z dv_z \]

\[ d\Omega = f(v_x, v_y, v_z) v_x dv_x v_y dv_y v_z \]

\[ f(v_x, v_y, v_z) = \frac{n}{\left(\frac{m}{2\pi kT}\right)^{\frac{3}{2}}} e^{-\frac{m(v_x^2 + v_y^2 + v_z^2)}{2kT}} \]

The reason is the mass transport caused by the uneven distribution of formaldehyde density.

The gene AtAHA2 is a key component to intensify the net absorption rate \( P \).
Human Practice

1. Questionnaire
2. Lecture
3. Knowledge quiz
4. Communication
5. Video

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Human Practice

1

Questionnaire
A Questionnaire to find out Which way would people like to deal with HCHO? Do they trust synthetic biology?
Almost All of them showed support about our project.
Human Practice

Lecture at The No.7 Middle School of Chengdu about Synthetic Biology

2 Lecture

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Knowledge quiz

Synthetic biology knowledge for more people
Human Practice

4

Communication

Communate with iGEM team from Sichuan University
Make a cartoon video about our project for more people to know it.
Save the planet with plants!
Thank you!