The Team
The Team
The Team
Hydrogen

- Highly efficient energy carrier
- Production requires
  - hypoxogenic conditions
  - And consumes redox potentials, and protons
Nitrogen Fixation

- Vital Process
- It's also COSTLY
- Requires hypoxoxygenic conditions too
PROJECT
Phaseolus vulgaris nodule

Nutrient Supply

- *Phaseolus vulgaris* (bean plant) provides *Rhizobium etli* (natural nitrogen fixator), carbon sources in exchange for nitrous compounds.
Constrains

1. Couple with Rhizobium physiology.
   – Codon usage.
   – High hydrogenase production
   – Cross-talk with redox potentials
Constrains

2. Express system when microaerobic environment is present (nodulating stage).

3. Lack of standardized parts for Rhizobials, including vectors and promoter
Avoiding translational cross-talk

• Codon Adaptation Index (CAI).

– Nodulating Vs Constitutively Expressed genes
• Optimization of the 5' UTR
  – Lower $\Delta G$ for 5’UTR avoiding complex structures in RBS
MR. GENE

GENEARTH
THE GENE OF YOUR CHOICE
Construction

Operon 1

Prefix  pNifH  RBS  HydA  FeOx  TAG  TAATAA  RBS  PFOR  TAG  TAATAA  Double terminator

Operon 2

pLacZ  RBS  HydEF  TAATAA  RBS  HydG  TAATAA  Double terminator  Suffix
Construction

Operon 1

Operon 2
Construction

Operon 1

Operon 2
Low oxygen inducible promoter in Rhizobia
Activity

• Submitted and works as expected in low oxygen concentration
Activity

- Submitted and works as expected in low oxygen concentration
How to introduce in R. etli?

• Standardization pBBRMCS-5, a useful plasmid in free living and nodulating Rhizobium.

• Standard region for plasmid made from pSB1T3
• ~4:1 ratio with pSB1T3.
• DH5α
• ~4:1 ratio with pSB1T3.
• DH5α
Bioparts in progress

• High stable plasmid with region repC-ctRNA.

• Anderson’s collection in Rhizobia.
Multi-Scale Model

- Niche
  - Game Theory
  - Cellular Automaton

- Cell
  - Markov Model
  - C vs N: Cellular Automaton

- Metabolism
  - Flux Balance Analysis
METABOLIC SCALE

Flux Balance Analysis & Extreme Pathways

MODEL
Metabolism: FBA
Electron Transport

Hydrogen

Carbon

Electron Transport
FBA

• Less effective C metabolism (130%)
• Nitrogen Fixation unaffected, or enhanced
• Increased flow in Redox potentials (antioxidants!!)
Markov Model & Cellular Automaton

CELL SCALE
Cell: Plasmid Stability

• Given:

The initial inoculation at t=0 is of first daughter cells, i.e. second generation
Doubling time is of 40 minutes, conservative estimate for M9 glucose medium
There are no Gain-Of-Function mutations that generate phenotype without plasmid
The Total count is given by the number of colonies in AB-free medium, i.e. the positive control

<table>
<thead>
<tr>
<th>Hour</th>
<th>Colonies in Antibiotic-Free Medium</th>
<th>Colonies in Antibiotic-Present Medium</th>
<th>Ratio</th>
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<tr>
<td>0</td>
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</tr>
<tr>
<td>72</td>
<td>100</td>
<td>99</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Markov Chain Framework
Plasmid Stability
\[ P(x)=0.999774369 \]
Cell: N fixation comparison

WildType: 50 plants

TransGenic: 70 plants
NICHE SCALE

Game Theory & Cellular Automaton
Niche: Game Theory

• Payoff Matrix:
  – Snowdrift Game
  – WT: only N fixation, no H production
  – TG: only produces H, no N fixation
Niche: Game Theory

3 Nash Equilibria

1. TransGenic Fixation
   Unfeasible: no one fixes $N$

2. WildType Fixation
   Unfeasible: TG is more efficient

3. Mixed State
   Feasible!

<table>
<thead>
<tr>
<th>WildType</th>
<th>Stasis</th>
<th>Reproduce</th>
</tr>
</thead>
<tbody>
<tr>
<td>TransGenic Stasis</td>
<td>0/0</td>
<td>0/+</td>
</tr>
<tr>
<td>Reproduce</td>
<td>0/+</td>
<td>-/-</td>
</tr>
</tbody>
</table>
Niche: Game Theory

Mixed State:
If Players unable to change «States», Mixed Equilibrium becomes an Evolutionary Steady State.

*Id est:* resistant to «Mutations»

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<th>Reproduce</th>
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<tr>
<td>Reproduce</td>
<td></td>
<td>+/-</td>
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</tbody>
</table>
Niche: Cellular Automaton
MODELING END
Planning

Involve people
Planning

Current Scenario?

Involve people
Planning

Current Scenario?

Involve people
Some Background

Mexico Can’t rely on oil or fossil fuels for much longer

Relies too much on Fertilizer for agricultural activities

Mexico is supposed to have commitments with the environmental care
Hydrobium etli in Cuatzoquitengo
Overview

Pursued the ideal of applying our potential developments on the ground.
Hydrobium etli goes Cuatzo

- Small community in the Sierra Mixteca
- Population 1,618
Cuatzo has very few Spanish speakers.

Their main activity is agriculture.
Traditional cultivation of the bean plant (experience)
Taking the energy services to communities like Cuatzo is a challenge.
Outreach Practice

Community Teachers talked to the assembly about the project

We addressed the communal authorities with the details
“It could be a dream come true, we wouldn’t have to worry about electricity shortage anymore”

“It could be truly beneficial for us if you could prove that the little plant can give us electricity”

“We wouldn’t have to depend on the Federal Commission of Electricity anymore”
“What is the distance that must be kept between the house and the plant?”

“What about our original bean crops? What would happen if they mixed?

“Are there special cares? Do we have to fumigate?”

“The plant has a lifetime, we wouldn’t know how to maintain it living for longer, if that was the case”
What did we learn?

• Investigating and developing solutions for people’s necessities is not enough.

• We must involve them in the whole process ➔ They are eager to participate!
Bio-Sintetizarte
Bio-SintetizARTe
Planning Bio-SintetizArte
Finding the artists

- Encouraged everyone’s participation
- Small Synthetic Biology Definition
- Invited to a talk about SynBio at our campus
Bio-Sintetizarte

At The Museum
September 30th: Inauguration
Enlaza exposición la biología y el arte

Llega el género musical al cineclub del museo

Reto para la biología
The Art
"I am inspired through the observation of the cells and nature itself"

Yuria Tamayo

"On the one hand the scientific progress is extraordinary...

The Uterus Insects

...on the other hand, it’s almost a betrayal to the feminine nature of life"

www.francescadallabenetta.com
Summing up

• Bio-Sintetizarte let us to know how are we, and our work seen.

• Reached people in an elegant, sensitive, beautiful manner.

• Called attention to Synthetic Biology
Making everyone a part of Synthetic Biology
Project Wrap Up

- Hydrogen Build: In progress, 20%
- Rhizobial Kit: In progress, 50%
- Impact Exploration: Done
Sponsor Thanks

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• Dr. Guillermo Dávila & his Lab

Our Instructors
Our Advisors
Our Team