Plants as Biosensors

Nevada iSense 2010
World population is increasing but land for food production is decreasing.

Figure 3. Population and Arable Land in Developing Countries

Growth of population
Billions, 1961–2050

Arable land per capita
Hectares, 1961–2050

Source: UNFPA/FAO

Population Reports
How can we help?

Plant Biosensors can be part of the solution

Environmental Sensing Genes
“Thanks Evolution”

Fluorescent Protein Signals
“Thanks Molecular Biology”
Entering a “New Kingdom”
The 2010 Goals

1. **Crawl before you walk**: Create some of the first plant-compatible iGEM parts.

2. **Your new best friends**: Characterize the feasibility of Tobacco BY-2 cells as a viable plant model.

3. **Making sense of it all**: Begin implementation of Boolean networking models.
iGEM Compatible Plant Parts

• Why do we need plant parts?
  – Plant specific promoters
  – Plant specific ribosome binding sites (AACAATGGC)
  – Plants need Agrobacterium transformation vectors

None of the available iGEM parts are suitable for genetic engineering in plants
Promoters

- **RD29A**
  - Cold, drought and salinity induced transcription factor
  - BBa_K414008

- **DREB1C**
  - Cold stress induced transcription factor
  - BBa_K414007

- **CaMV 35s**
  - Constitutive Promoter
  - BBa_K414006
• **RD29A**
  – Cold, drought and salinity induced transcription factor
  – BBa_K414008
• **DREB1C**
  - Cold stress induced transcription factor
  - BBa_K414007
• **CaMV 35s**
  – Constitutive Promoter
  – BBa_K414006
Kozak Sequence

- Prokaryotic organisms use Shine/Dalgarno sequence

- Terrestrial Plant specific ribosome binding sites (AAAAAAAAAAAACAATGGC)
Kozak + Reporter Gene

- **Kozak + eYFP**
  - BBa_K414004
- **Kozak + GFP**
  - BBa_K414001
- **Kozak + mCherry**
  - BBa_K414003

These reporter genes contain a Ribosome Binding Site (Kozak Sequence) specific for plant expression.
Plant Transformation Vector

- pBIB HYG
Plant Transformation Vector

- piGEM10
Tobacco BY-2 Cells

The Perfect Model Organism for Plant Systems
What are BY-2 Cells?

- Plant cell line derived from *Nicotiana tabacum*.
- Non-green
- Rapidly growing
- Undifferentiated cells
- Proliferate mitotically
- Compared to HeLa cells for Humans
Why are BY-2 Cells an Ideal Model System?

- Easily transformed
- Single cell representation of multi-cellular organism
- Colorless
- Only proliferate *in vitro*
Agrobacterium tumefaciens

- Plant pathogen
- Horizontal gene transfer vector for plants
- Random integration into host genome
- High transformation efficiency
Agrobacterium transformation

piGEM cold

Kanamycin
Transformation Results

Transformed cells after 20 days

Expression in cold-stressed cells
Fluorometry Assay

- Measure stress treatment in real time
  - Cold
  - Salt
Modeling Goal

- Analyze fluorometry assays and identify important promoters

- Promoter1 + Reporter
- Promoter2 + Reporter
- Promoter3 + Reporter
- Promoter4 + Reporter

e.g. RD29A tied to RFP
Overview of cold-response transcription factors

- Early response
  - ICE1
- Early/mid response
  - DREB1s A, B, C
  - RD29A
- Late response?
  - RAP2.1, RAP2.6, ZAT6
### Boolean Networks

#### Collect data

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#### Convert to Boolean values

Set threshold

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**Program returns all possible Boolean inputs for each gene**

**Feed data to program**
### Results

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<th>RD29A(^5)</th>
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**Example of true input for RD29A:**

\[ 4 \, (-2 \mid 3) \, \& \, (4 \mid -6) \]

Holds true at all time points
Regulons involved in osmotic stress-responsive and cold stress-responsive gene expression in plants.

Future Development

- Fluorometry assays
- Determine if RAP2.1 is necessary for activity of the DREB1s
Human Practices
Students enter international genetic engineering competition

Friday, November 05, 2010
By Staff Report

Ten students from the University of Nevada, Reno will be presenting their work on plant biosensors at the International Genetically Engineered Machine Competition, iGem, Nov. 4-8.

Teams from top universities around the world choose a research project within the field of synthetic biology, and have little more than a summer to run experiments and prepare their data before presenting their research at the annual competition at the Massachusetts Institute of Technology.

University of Nevada, Reno undergraduate student Elaine Bersaba checks the synthetic biology experiment in preparation for the international competition this week at the Massachusetts Institute of Technology.
While both teams are busy with the final experiments on their projects as iGEM draws nearer, they have had a moment to reflect on the experience so far. “For students who have never done a research project, it’s a really great experience because you really need to come up with a whole project,” said Billerbeck. “[iGEM] is a good playground from the beginning of a project to its realization—although you also learn about the frustrations and sometimes you don’t feel so well.”

Despite the difficulties and the work left to do in the remaining few weeks, the teams are excited to share their projects with other like-minded undergraduates at the Jamboree. “I couldn’t have asked to work with a better group of people,” said Randy Pares, a biochemistry student on the UNR iGEM team. “Everyone has a different background and different strengths and everyone helps each other. We have Dr. Shintani and Dr. [Christie] Howard as our advisors, but really they handed the reins over to us.”

Shintani was impressed with the effort put forth by the team that he mentored. “It’s really their show,” said Shintani. “We’re just kind of there to make sure they don’t burn the place down.”

Keywords: iGEM competition synthetic biology genetic engineering

The UNR team has engineered plant cells to act as remote sensors of environmental stresses. Source: Nevada iGEM wiki.
Plant Summit

TODAY: 1:30-2:30pm

STATA 32-G449
Take home message

• iGEM has plant parts!

• Tobacco BY-2 cells are your friend.

• Models are awesome, like mining for digital gold.
Acknowledgements
Questions???

Don’t ask about principle investigator-induced stress. There is no chance of ever mitigating that biotic challenge.