Nevada iSense: Development of Plant Biosensors using *Nicotiana tabacum* as Transgenic Plant Model

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Goals

iGEM 2010 Goals:

- **Register iGEM-compatible Plant Part**
- **Characterize Tobacco BY2 Plant Transformation System**
- **Model *In silico* Plant Promoters of Abiotic Stress Signaling Pathway**

Long Term Goal:

- **Develop Plant Biosensors**

Background

According to the USDA, approximately 400 million acres of land in the U.S. are used in crop agriculture. In the U.S. alone, greater than $14 billion is lost each year due to stress-related crop damages. More importantly, as the global population increases and the amount of arable land decreases, protecting our agriculture is vital to the well-being of everyone. These issues are expected to get even worse in light of global climate change.

Ensuring the health and safety of those crops from environmental and biological hazards such as drought, poison, and pathogens is a critical task of the modern farmer. The Nevada iGEM team believes one viable, reliable, and unique solution is the design and implementation of plant biosensors. **Our goal is to design plant biosensors that fluoresce under various stress conditions including: cold, salt, and drought.** Having these plant biosensors controlled by stress-inducible promoters provides easy, fast, and accurate measurement of plant stress.

This year we took the first steps by developing several iGEM parts that are plant compatible and include promoters, reporters, and some progress on a plant vector.

We also would like to introduce the iGEM community to the use of *Nicotiania tabacum* BY-2 cells, which have several advantages worth considering: they model plant systems well, they are cheap and safe to handle, and as we show, they can express iGEM compatible parts.

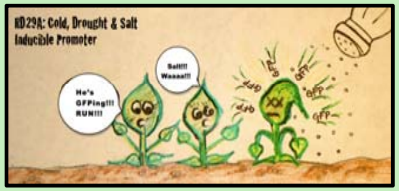
Our approach of attaching fluorescent reporters to promoters marks a real-time measuring possibility for relating expression in gene families. We have begun work on modeling these plant promoters using Boolean networks.

Promoters

Part Name	Inducer	Source	Part #
CAM-35s	Constitutive	*CaMV*	BBa_K414006
rd29A	Cold, Drought and Salinity	*A. thaliana*	BBa_K414008
DREB1C	Cold	*A. thaliana*	BBa_K414007

Reporter + Plant RBS (Kozak Sequence)

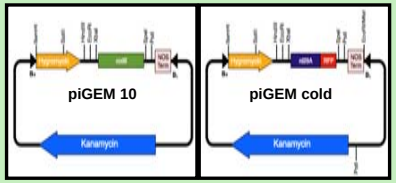
Part Name	Part #
Yellow Fluorescent Protein + Kozak	BBa_K414004
Green Fluorescent Protein + Kozak	BBa_K414001
mCherry + Kozak	BBa_K414003



Composites

Part Name	Part #
35s + Kozak + Green Fluorescent Protein	BBa_K414006
rd29A + Kozak + Red Fluorescent Protein	BBa_K414000

Part Name	Function	Part #
ccdB	Minimal selectable marker gene	BBa_K414005
piGEM	Vector for efficient plant transformation.	In Progress



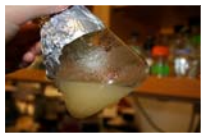
Tobacco BY-2 Cell Transformations

WHAT ARE BY-2 CELLS?

- Non-green rapidly growing plant cell line derived from *Nicotiana tabacum.*
- Undifferentiated cells that can be grown as suspension cells in liquid medium or as callus on solid medium
- Cells proliferate mitotically and can be maintained in culture indefinitely
- Model plant system to study metabolism, cell division and signal transduction pathways.
- Compared to HeLa cells for Humans

WHY IS THIS A GREAT MODEL SYSTEM?

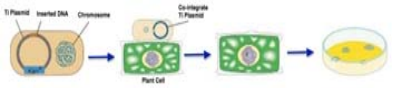
- Rapidly reproducing
- Easily transformed in short amount of time (~6 weeks to 1° transformants)
- Organism is relatively simple and predictable
- High homogeneity
- Exhibits the general behavior of plant cells
- Single cell representation of multi-cellular organism aids in investigation of cell-cell interactions
- Ability to undergo fluorometry assays due to colorless nature
- Only proliferate *in vitro*, eliminating detrimental environmental side effects



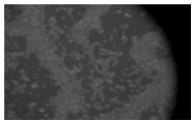
Liquid culture of BY-2 cells

AGROBACTERIUM TRANSFORMATION

- Gram-negative bacteria, *Agrobacterium tumefaciens*
- Horizontal gene transfer vector for plant systems
- Although it integrates into the genome randomly, there is a relatively high transformation efficiency



COLD-INDUCED EXPRESSION OF RFP



Plated, transformed BY-2 cells after 3 weeks of cell growth

piGEM cold (rd29A+RFP) Transformed BY-2 Cells at 25°C (Left); piGEM cold (rd29A+RFP) Transformed BY-2 Cells after 4hrs at at 4°C (Right)

Future Directions

- Generate more stress promoter-reporter fusions.
- Develop fluorescent plate reader assay to rapidly assess induction time course in BY-2 cells.
- Use subsequent data to validate model and establish network relationships between promoters.
- Identify and make promoters inducible by plant fungal, bacterial, insects, and viral challenges.

Summary

- The Nevada iGEM team successfully created nine parts that are now in the iGEM registry.
- We demonstrated that BY-2 cells can serve as a model plant system for future iGEM competitions.
- We believe further development of our Boolean model will allow us to better characterize abiotic stress networks in plants.

Modeling

We utilized Boolean networking to begin our investigation into the cold-response pathway of *Arabidopsis.* Ideally, continuous fluorometry assays would be used for collecting data on expression levels at any number of infinite time points. However, there was not enough time to create parts, perform transformations, and collect data. Therefore, microarray data for eight transcription factors of the pathway, at four time points, was collected from the Gene Expression Omnibus database. The data was normalized, interpolated, and Boolean values were set based on a threshold of 3.5. This Booleanized data was then fed through a program written by Dr. Karen Schlauch. The program checked all possible inputs, for each gene, generating text files for inputs that held true at all time points. From these files, a model of the pathway could be developed and compared with what was known about the genes in this pathway.

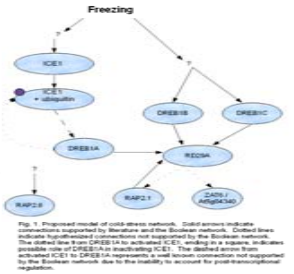


Fig. 1. Proposed model of cold-stress network. Solid arrows indicate connections supported by literature and the Boolean network. Dotted lines indicate hypothesized connections not supported by the Boolean network. The dotted line from CAT3 (A to activated ICE1, ending in a square, indicates possible role of DREB1A in inactivating ICE1. The dashed arrow from activated ICE1 to DREB1A represents a well known connection not supported by the Boolean network due to the inability to account for post-transcriptional regulation.

Safety

For teams who are unable to manage risks posed to crops and humans, but still wish to make valuable contributions to iGEM and the scientific community abroad, we have demonstrated the benefits of NT BY-2 cells for experimentation. NT BY-2 cells are contained to their media and can be easily exterminated with bleach and other protocols similar to bacteria disposal.

To those teams ready to make the leap into plant transformation, we wish to recognize the efforts of this year's Harvard team to create a genetic fence to help with plant containment. One additional idea we wish to put forward would be for iGEM to encourage a recognizable genetic 'tag' for all iGEM plant parts to be transformed into plants. This tag could be a constitutively expressed fluorescent protein to flank the inserted iGEM part. A glowing plant would be an easy way of identifying an iGEM plant that has escaped containment.

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