Penn State iGEM 2013

Plants as Plants: Natural Factories

Cellulose Synthase Project

Our Goal: to increase of cellulose production within the primary cell wall of vascularized plants.

Figure 1 CesA Construct Design

Method: introduce secondary cell wall cellulose synthase complexes into the primary cell wall by using a primary cell wall promoter. More specifically, expressing CesA 4, CesA7, and CesA8 under the CesA1 promoter.

Outcome: Isolated all components of our construct excluding CesA4

Parts submitted: BBa_K1043005, BBa_K1043006

Further Study: Successfully assembly of construct, followed by transformation into Agrobacterium then into a plant of choice. Testing would be conducted through florescence and/or a biomass analysis

Cas9 Project

Our Goal: to create a regulatory circuit in plants by introducing the Cas9 Protein

Method: Testing by using a ratio of GFP:RFP expression

Outcome: Due to time constraints and a refocus on our projects we decided to put this project on hold.

Further Study: All of the components have been isolated and the vector backbone has the CMV35s promoter as a left border and the Nos terminator as the right border. With the insertion of the middle components, and further testing it should be a working circuit.

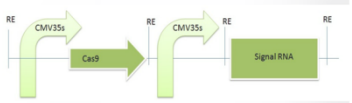
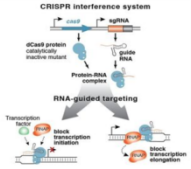


Figure 3 Cas9 Construct



Vanillin Project

Our Goal: To convert naturally abundant phenolic compounds to vanillin in novel plants.

Method: Introduction of the HCHL enzyme from Pseudomonas putida to convert feruloyl-CoA to vanillin in Nicotiana.

Outcome: Isolation of all the component parts, construct assembly and bacterial transformation.

Further Study: Stabilize bacterial transformation, followed by transformation in plants and testing.

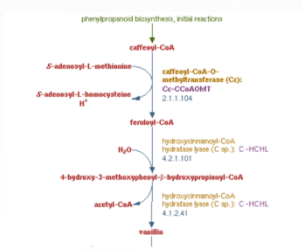


Figure 2 Vanillin Pathway

Promoter Project

Our Goal: to increase of cellulose production within the primary cell wall of vascularized plants.

Method: introduce secondary cell wall cellulose synthase complexes into the primary cell wall by using a primary cell wall promoter. More specifically, expressing CesA 4, CesA7, and CesA8 under the CesA1 promoter.

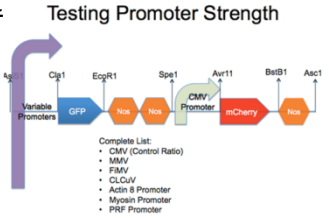


Figure 4 Promoter Project Construct

Outcome: Isolated all components of our construct excluding CesA 4

Parts submitted: BBa_K1043005, BBa_K1043006

Further Study: Successfully assembly of construct, followed by transformation into Agrobacterium then into a plant of choice. Testing would be conducted through florescence and/or a biomass analysis

Human Practices

Our Goal: To reach out to high school students and teachers to portray the importance of synthetic biology and the ethics behind it.

Method: We presented a small lecture on the fundamentals and ethics of synthetic biology to both students involved with Penn State's ScienceU program and a group of teachers who felt the need to learn more about the growing field of synthetic biology.

Outcome: Lesson plans were created with the help of the education majors on our team for those teachers to implement in their classrooms. This came with an activity for students to learn how to design constructs, an instructional video that covers the basics of synthetic biology, an interactive presentation for the teachers to introduce the topic, and an activity for the students to discuss the ethics of synthetic biology.

Butanol Project

Our Goal: To produce butanol within physcomitrella.

Method: Synthetic production of the enzymes that make up the University of California's cyanobacteria pathway to produce n-butanol within physcomitrella via 3-(R)-hydroxybutyryl-CoA intermediate.

Outcome: Although the pathway to create butanol was not finished, we were able to complete a intermediate pathway which seems to have successfully produced Polyhydroxybutyrate (PHB).

Parts Submitted: BBa_K1043007, BBa_K1043008 and BBa_K1043009

Further Study: To continue testing constructs in Nicotiana, and expand to testing in Physcomitrella. As well as work on getting clearer microscopy results and GCMS results.

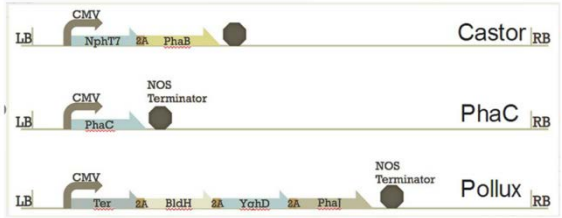


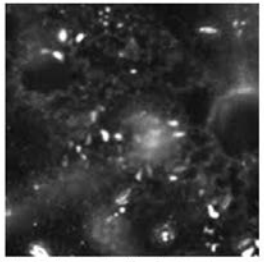
Figure 5 Butanol Construct

Butanol Project Results

The samples tested appear to have granules that are not present in the negative control sample. These may be a result of the transformation with Agrobacterium carrying the Castor and PhaC constructs. We are currently waiting on GC-MS results to confirm the production of PHB in Nicotiana tabacum.

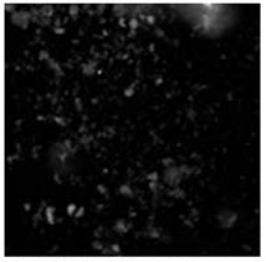
Microscopy Images:

Negative Control:



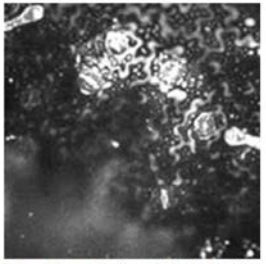
Nicotiana tabacum stained for 5 minutes in a saturated aqueous solution of BODIPY493/503 and examined via spinning disk confocal microscopy with GFP filter set.

Positive Control:



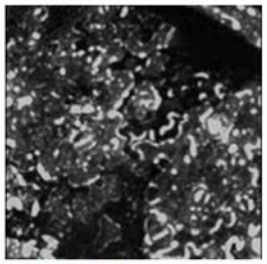
Powdered PHB stained with saturated aqueous solution of BODIPY493/503 and examined via spinning disk confocal microscopy with GFP filter set.

Transgenic Sample 3 IMG:Z1



Nicotiana tabacum transformed with Agrobacterium tumefaciens carrying the Castor and PhaC constructs. Stained for 5 minutes in a saturated aqueous solution of BODIPY493/503 and examined via spinning disk confocal microscopy with GFP filter set.

Transgenic Sample 3 IMG:Z4



Nicotiana tabacum transformed with Agrobacterium tumefaciens carrying the Castor and PhaC constructs. Stained for 5 minutes in a saturated aqueous solution of BODIPY493/503 and examined via spinning disk confocal microscopy with GFP filter set. (Different Image of sample)