EDINBURGH: A WEAPON OF MASS NUTRITION
THE PRODUCTION OF STARCH AND β-CAROTENE FROM CELLULOSIC BIOMASS


Global problems
These are a number of global problems which it is up to our generation to change:

- Global warming
- Depletion of fossil fuel reserves
- Deforestation
- Increasing population
- Biofuel crops replacing traditional agriculture

These problems are interlinked and require sustainable and increasingly urgent solutions which will not harm the national or global economies.

MicroMaize: a single bacterial solution
We envisage using bacteria and eventually yeast to:
1. Convert waste biomass into starch
2. Produce β-carotene

Why starch?
Starch is a high density, easily transportable and verifiable product. It could be:

- sold to the biofuels industry for further processing (into ethanol, butanol, biodegradable plastics etc.)
- used as feed for livestock
- used as a starch supplement in the human diet

Why β-carotene?
β-carotene is the precursor to vitamin A. Vitamin A deficiency results in:

- Half of these children die within a year as a result of anemia and immune deficiencies.

Cellulose degradation
We used the following genes:

- cex (endoglucanase) from Cytophaga hutchinsonii
- cstA (exoglucanase) from C. finni
- bgL (β-glucosidase) from Cellulomonas fimi

Together these break down cellulose into D-glucose

Starch synthesis
This involved:

- glgC16 (an up-regulated mutation of the ADP-glucose pyrophosphorylase gene, glgC) from E. coli
- SU7 (amylase 1) from Z. mays
- IS02 (amylase 2) from Z. mays
- GB55 (granule-bound starch synthase) from Arabidopsis thaliana

Together these genes upregulate the native glycogen synthesis pathway in E. coli and cause the conversion of glycogen into starch.

β-carotene synthesis
We exploited:

- dxs (1-deoxyxylulose-5-phosphate synthase) from E. coli
- xylE (Geranylglycerol-3-phosphate synthase) from Pantotoca ananatis
- cstA (phosphate synthase) from P. ananatis
- bgL (phosphate desaturase) from P. ananatis
- xylE (Geranylglycerol-3-phosphate synthase) from Pantotoca ananatis
- appY (encodes an anaerobic transcriptional regulator related to anaerobic energy metabolism) from E. coli

The presence of these genes encode convert products of glycolysis into β-carotene.

Other aspects
We considered two approaches to getting the cellulases out of the cells:

- Option 1: induced cell lyses by E from phage qX174
- Option 2: Adding the type I secretion system of E. coli O157 and tagging each protein with the target molecule of this system.

In both cases, cellulase gene transcription is under the control of the glucose-repressible promoter PstA.

We added a limonene synthesis (Citrus limon) component from Edible 07 for taste.

We planned to use the RNAse system of UC Berkeley ‘07 to degrade the RNA and render cells safe for consumption.

Chassis
We used Esherichia coli as a chassis for the iGEM competition.

Overview

Cellulase degradation
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Software

Main features
- Easier to use than similar tools: one only needs to tell which genes and promoters are assembled in order to form their gene circuit. A set of reactions and kinetic equations will be automatically generated.
- Link with the MIT registry: users can directly type in the regulatory part IDs used in their circuit design to build a model.

Web interface:

The Team

Registered BioBricks™

Cellulase Degradation

- Coding parts
- Promoters and Measurement

Starch synthesis

- Coding parts
- Measurement

β-Carotene Synthesis

- Coding parts
- Measurement

Limonene Synthesis

- Coding parts
- Measurement

Other parts

Cells transformed with dxs+crtB1 produced a reddish hue, indicative of lycopene production. Lycopene is the penultimate step in the synthesis of β-carotene.