Today’s Menu

Appetizers
- The Citadel
- Our Team
- Project Overview

Entrees
- PYY
- Population Control

Desserts
- “Downtown DNA”
- Closing Remarks
In the Kitchen…

Undergraduates
Patrick Sullivan – 1st year, Electrical Engineering
Brian Burnley – 4th year, Biology & Literature
Hunter Matthews – 2nd year, Biology

Professors
Claudia Rocha – Microbiologist
David Donnell - Geneticist
The Citadel
The Military College of South Carolina
The Brain-Gut Axis

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Obesity – A Growing Problem?

2008 Survey
32.2% among adult men 35.5% among adult women
Peptide Tyrosine Tyrosine (3-36)

- Endogenous hormone of ileum & colon
- Released proportionally to caloric intake
- Traverses BBB to drive energy homeostasis
- Agonist of Y2-receptor: Inhibits gastric motility & causes decrease in appetite
PYY^{3-36}
Design Goals

1. Construct Biobrick encoding \( \text{PYY}_{3-36} \) for \( E.\text{coli} \)
2. Work towards integrating \( \text{PYY}_{3-36} \) into a system for an intestine-based chassis to serve as a novel, safe, and potent means for influencing neurological processes such as appetite from across the brain-gut axis.
A PYY<sub>3-36</sub> Biobrick

Parts submitted

- K373000
  - PYY3-36 protein coding region

- K373001
  - PYY3-36 translational unit

- K373002
  - PYY3-36 within autotransport system
Controlling Expression

• System needs a hard limit to peptide production
• Population control system contains three main components:
  – Quorum sensing allows cells to communicate with one another and initiate cell death when density has grown too large.
  – Sensitivity tuners from Cambridge 2009 allow for adjustment of the population density threshold at which cell death begins.
  – Poison protein ccdB is responsible for cell death.
Population Control Circuit
Human Practices

Aided fellow iGEMers performing surveys!

- Mexico-UNAM-CINESTAV (Legal issues within synthetic biology)
- METU_TURKEY_SOFTWARE (Improving the Registry of Standard Biological Parts)

Hosted a community event to educate and entertain!
DNA Extraction Protocol

Materials:
- 220mL of distilled water (distilled water works well)
- 3 tbsp boiling water
- 1% NaCl
- 3 tsp of baking soda
- 2 tbsp of 70% isopropanol
- 1 tsp of 95% ethanol
- 1 drop of phenol
- 1 drop of chloroform
- 3 strawberries (or the DNA source of your choice - try bananas, peaches, etc.)
- 3 mmol/L NaCl (if you prefer)

Step 1: Prepare the Buffer
- In a clean glass container, combine 130mL of distilled water, 10% of NaCl, and 1 tsp of boiling water.
- Stir until all of the ingredients have dissolved.
- Stir in 1 tsp of phenol.
- Add 2 or 3 drops of contact lens solution (or similar) to the mixture.

Step 2: Isolate the DNA
- In a 250mL beaker, mix the ingredients and a small amount of distilled water into it.
- Place 2 drops of the NaCl solution into a clean container.
- Mix 2 drops of the phenol solution from Step 1 into the NaCl solution.
- Gently swirl the mixture for 2 minutes.

Step 3: Filter the DNA
- Pour the mixture into a clean container.
- Pour the fluid and buffer mixture from Step 2 into the filter.
- The buffer should be clear and free of any pulp from the fruit.

Step 4: Isolate the DNA
- Pour 1 tsp of the liquid from Step 1 into another clean glass container.
- Carefully pour 1 tsp of distillation alcohol into the narrow glass container.
- The solution will float on top of the buffer liquid.
- A material will appear between the two layers of alcohol and the buffer solution.
- This material is DNA.

What is happening?
- The liquid prepared in Step 1 looks like a “buffer.” The chemicals contained in the buffer will break apart the outer structure of cells and allow the DNA inside to be released into the liquid.

What is happening?
- Mashing the strawberries and placing them in the liquid from Step 1 allows the buffer to reach all of the cells, destroy them, and release the DNA.

What is happening?
- Pouring the mixture from Step 2 through the coffee filter separates out all of the unwanted fruit pulp and solids, leaving only the DNA in the final solution.

What is happening?
- Because the alcohol weighs less than the buffer liquid, it will float on top of the buffer. As the two liquids mix, the ethanol will reduce the salt concentration of the buffer and allow the DNA to separate into the chloroform.

The “Downtown DNA” Event
Accomplishments

Foundational
• Founded a new iGem team
• Established a building point for future iGem teams from our school

BioBrick Parts Designed
• We designed a new genetic part, a protein coding sequence that encodes Peptide Tyrosine Tyrosine (3-36).
• We synthesized this part, we've entered the part data into the Registry, and we will provide the part DNA to iGEM HQ.

Systems Designed
• We designed a new genetic circuit for the expression of PYY\textsubscript{3-36} and the regulation of expression via AHL-mediated population control.

Human Practices
• We completed a community outreach event in which our students and professors engaged local residents and visitors to Charleston, South Carolina.
• We aided the following teams with their own Human Practice projects:
  - Mexico-UNAM-CINVESTAV
  - METU_Turkey
A Warm Thanks to

The Advisors
Claudia Rocha, Ph.D.
David Donnell, Ph.D.

The Benefactors and Supporters
Brigadier General Sam Hines
Michael Livingston, Ph.D.
Lance Braye
Adam Akerman
References


Population Density
AHL Concentration
Poison Production

Steady State Population
Natural Carrying Capacity
AHL Threshold

OFF  ON
Accomplishments

1. Basics
2. Parts Added to the Registry
3. Human Practices
4. Established a synthetic biology research group at our school. We founded a new iGEM team, composed of three undergrad students and two professors.
5. We built a new genetic part, a protein coding sequence that encodes Peptide Tyrosine Tyrosine (3-36). We synthesized this part, we've entered the part into the Registry website, and we are providing the part DNA to iGEM HQ.
6. We designed a new genetic circuit for the expression of PYY3-36 and the regulation of expression via AHL-mediated population control.
7. We completed a community outreach event in which our students and professors engaged local residents and visitors to Charleston, South Carolina. We informed the people about synthetic biology, iGEM, and our team. We demonstrated a DNA extraction using household products, and we encouraged science in everyday life. We gave away handouts showing the method for perform the experiment at home (we also gave away cookies and Jell-O).
8. We aided the following teams with their own Human Practice projects:
   1. Edinburgh
   2. Mexico-UNAM-CINVESTAV
   3. METU_Turkey