Red Fluorescent Nitrate Detector

Gaston Day School iGEM Team
Red Fluorescent Nitrate Detector

- The Team
- The Project
- The Problem
- The Process
- The Lab
- The Plans
The Team

• **Seniors**
  – Sheran Hussain
  – William Farmer
  – Ryan Kane
  – Brian Elgort
  – Lauren Toole

• **Juniors**
  – Al Hall
  – Daniel Thompson
  – Will Rudisill

• **Sophomores**
  – Rosemary Dunning
  – Ivana Chan
  – Amir Feinburg

**Advisor**
• Ms. Byford
Nitrates in North Carolina

- Sources of Nitrate pollution
  - Mechanized farming
    - Fertilizer use and run-off
    - Livestock waste
      - Leaking lagoons
  - Human waste
    - Septic tanks or defective sewage systems
  - Urban areas
    - Combustion engines

- Approximately 5,240,569 North Carolinians drank nitrate-polluted water in 1997-2003
Nitrate Dangers

• Animal effects
  – Most dangerous in ruminants (cows and sheep)
  – Labored breathing
  – Vomiting
  – Still births
  – Death
Nitrate Dangers

• Human effects
  – Spontaneous abortion
  – Cancers resulting from chronic consumption
  – Methemoglobinemia or “Blue baby syndrome”
The Metabolism of Nitrates

$\text{NO}_3^-$

$\text{Fe}^{2+}$
The Project

• Biological Nitrate Detector
  – Nitrate sensitive promoter linked to Red Fluorescent Protein reporter

• Relatively easy to detect and quantitate

• Cost-effective alternative method

• Self-replicating
What Happens with the E. coli
The Process

• Combine nitrate sensitive promoter with RFP to produce *E. coli* that turn red in the presence of high nitrate levels

• pNICE with nitrate sensitive promoter (*narG*) donated by Dr. Steven Lindow at UC Berkeley

• RFP from BioBrick collection
**narG/L28H-fnr Promoter**

- **narG promoter**
  - Regulates nitrate reductase gene in *E. coli*
  - Expression only under anaerobic conditions
  - Secondary regulation by transcription factor *fnr*

- **L28H-fnr**
  - Mutant *fnr* provided to allow aerobic expression of *narG* promoter
What We Wanted to Do

Diagram:

- RFP
- inaz
- Fnr Narg
- B
- E
- X
- N
- S
- P
- N
Actual Initial Construction

- Map of *narG/L28H-fnr*

- Cut with BamHI/EcoRI
- Cut RFP BioBrick vector with EcoRI/PstI
- Ligate into pUC19
- Later steps to convert to BioBrick standard
The Lab

Gaston Day School iGEM lab
Centrifuge

- Clinical centrifuge vs. lab centrifuge
Creating Our UV Light Box

• Could not purchase a professionally-made UV light box
  – Necessity for Ethidium Bromide gels
• Constructed our own UV light box.
• Built from:
  – A donated, old art light box (11 ¾ inch bulbs)
  – An 8 inch UV bulb.
  – A soldering iron
  – A roll of tape
  – A few hours work
iGEM in the High School

• Most high schools unaffiliated with university or hospital
  – Necessary equipment may not be on hand
    • -80°C freezer, PCR machine, adjustable spectrophotometer, autoclave
• Work space shared with classroom space
• Initial level of knowledge is much less
• Enthusiasm may be greater!
• No stipends to pay – team members live at home
  – Significantly reduces cost of project
The Plans

• Begin before the end of this school year
  – Create fliers and posters to generate interest around school
  – Go around the community, looking for donations and funds

• Produce a summer schedule
  – Outline each member’s position and job
  – Identify when they will be working
The Goals

• Continue this year’s project
• Produce $fnrL28H-narG$ BioBrick
• Produce functional Red Fluorescent Nitrate Detector BioBrick
• Establish GDS team as annual competitors
  – Possibly in conjunction with research class
• Be a resource for other high schools interested in competing
Individual Sponsors

• Gold Level
  – Hussain Family
  – Scott Olson
  – Suzanne and Bill Duncan

• Silver Level
  – Farrah Bui
  – Bill and Audrey Page
  – Wendy Philbeck
  – Jim Green
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