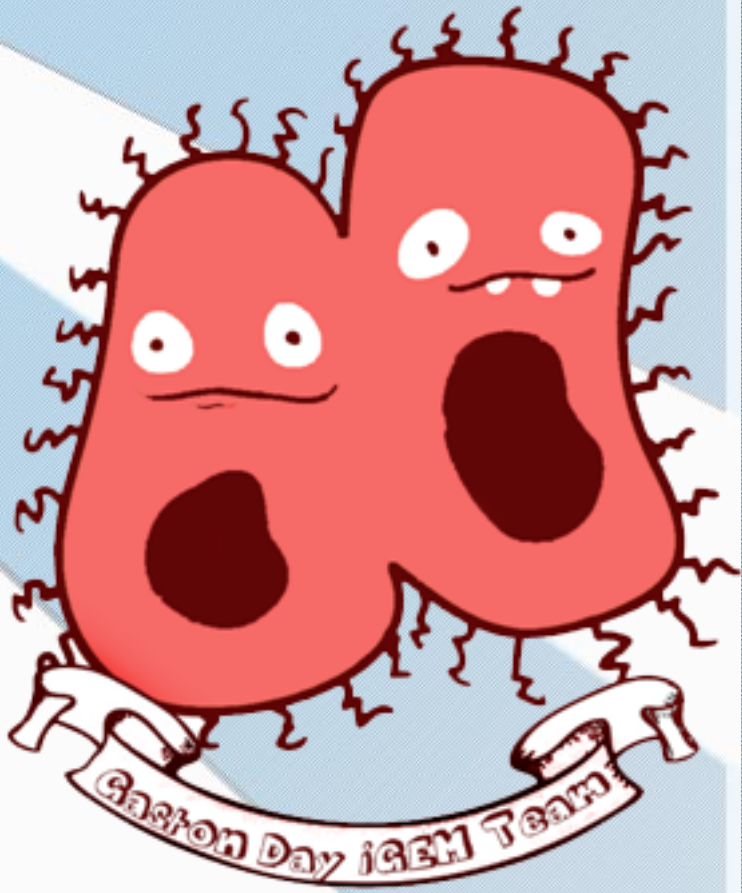
Red Fluorescent Nitrate Detector

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

Advisor

- Ms. Byford

- **Juniors**
  - Al Hall
  - Daniel Thompson
  - Will Rudisill
- **Sophomores**
  - Rosemary Dunning
  - Ivana Chan
  - Amir Feinburg

The Team

Gaston Gazette

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

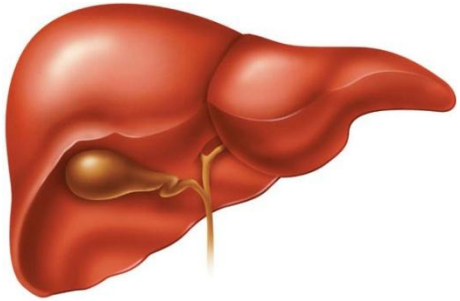
- Animal effects
  - Most dangerous in ruminants (cows and sheep)
  - Labored breathing
  - Vomiting
  - Still births
  - Death

Nitrate Dangers

- Human effects
  - Spontaneous abortion
  - Cancers resulting from chronic consumption
  - Methemoglobinemia or "Blue baby syndrome"

The Metabolism of Nitrates

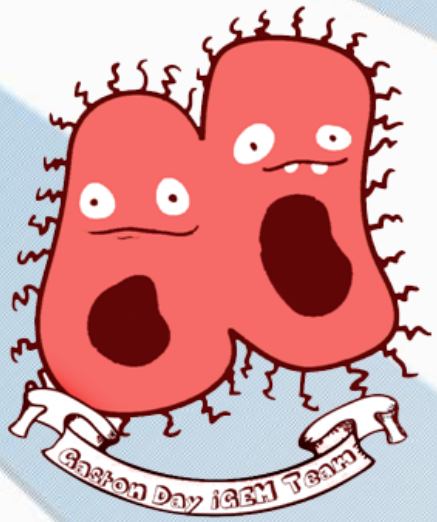
NO_3^-



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

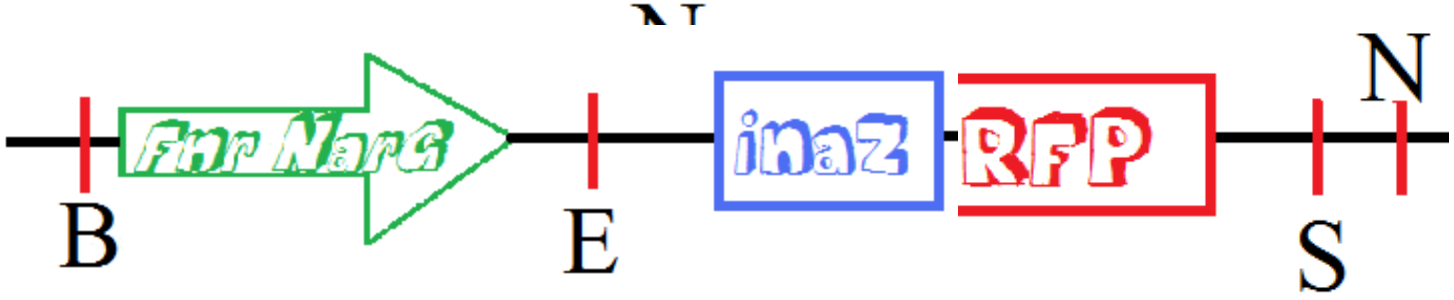
- Combine nitrate sensitive promoter with RFP to produce *E. coli* that turn red in the presence of high nitrate levels

- pNICE with nitrate sensitive promoter (*narG*) donated by Dr. Steven Lindow at UC Berkeley
- RFP from BioBrick collection

narG/L28H-*fnr* Promoter

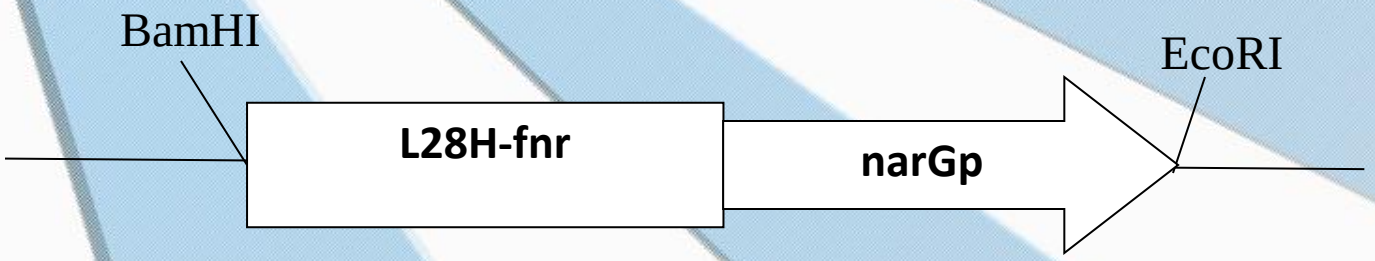
- *narG* promoter
  - Regulates nitrate reductase gene in *E. coli*
  - Expression only under anaerobic conditions
  - Secondary regulation by transcription factor *fnr*
- L28H-*fnr*
  - Mutant *fnr* provided to allow aerobic expression of *narG* promoter

What We Wanted to Do



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

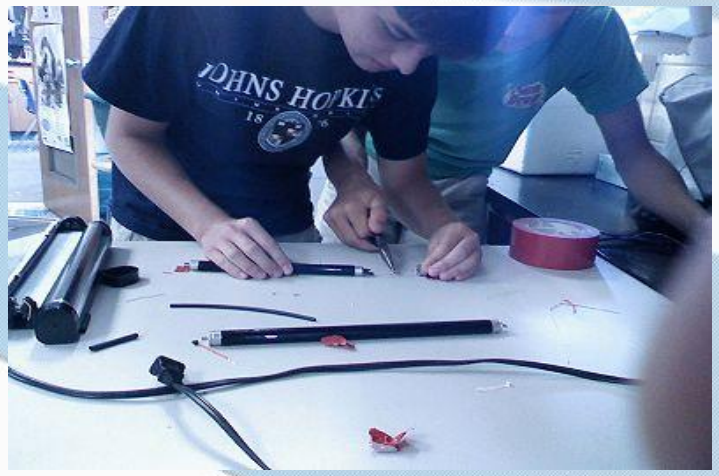
Gaston Day School iGEM lab

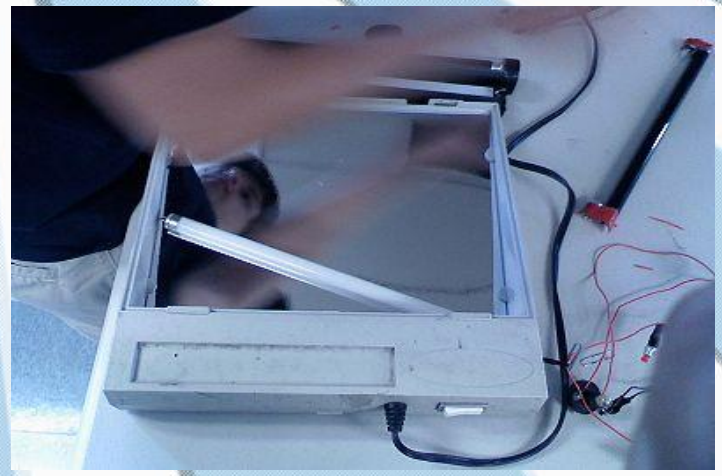
Centrifuge

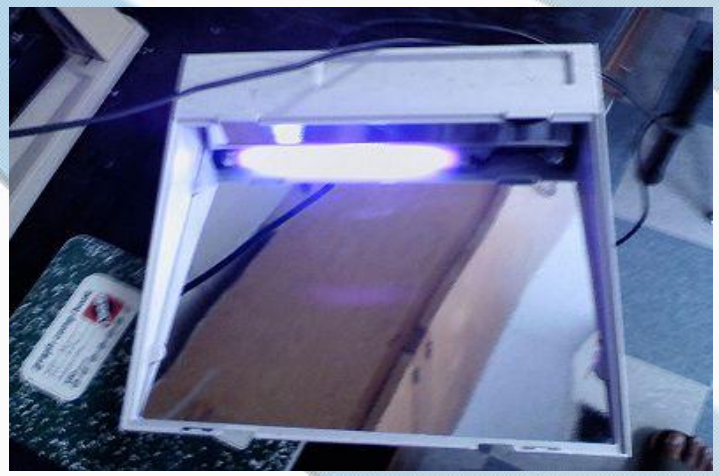
- Clinical centrifuge vs. lab centrifuge

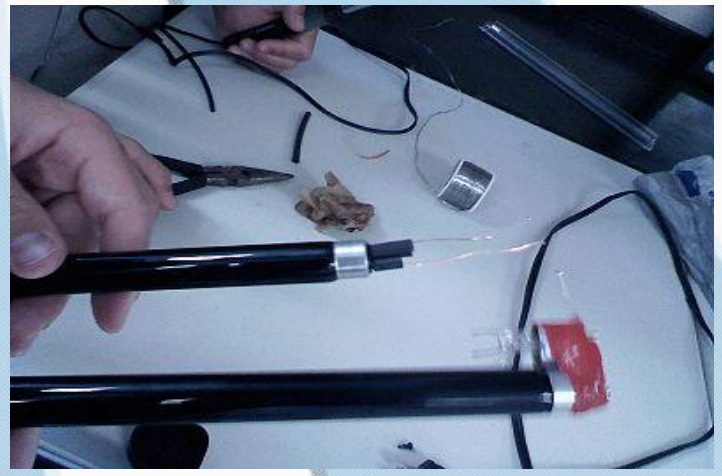
Creating Our UV Light Box

- 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 *fnr*L28H-*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
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