

Red Fluorescent Nitrate Detector

Gaston Day School iGEM Team

Abstract: Increasing levels of fertilizer required for mechanized farming can result in elevated nitrate levels in soil and groundwater. Due to contaminated food and water, humans are at risk for methemoglobinemia caused by enterohepatic metabolism of nitrates into ammonia. This process also oxidizes the iron in hemoglobin, rendering it unable to carry oxygen. Infants in particular are susceptible to methemoglobinemia, also known as “blue baby syndrome”, when formula is reconstituted using water contaminated with nitrates. By combining the red fluorescent protein coding region with a nitrate sensitive promoter, we are developing an inexpensive, simple, visual test for nitrate contaminated water. Use of this detector in agricultural areas could alert families to the presence of nitrates in groundwater and prevent blue baby syndrome.

Nitrates

- NO_3
- Federal Standard = 10mg/L
 - May be little safety factor
- Problems occur when NO_3 is converted to NO_2 (Nitrite)
- Possible for Nitrites to combine with amines to form nitrosamines
 - Known carcinogen

Nitrate Dangers

- Human effects
 - Spontaneous abortion
 - Cancers resulting from chronic consumption
 - Methemoglobinemia or “Blue Baby Syndrome”
 - 10-20% methemoglobin causes cyanosis, respiratory, and digestive problems
 - 20-30% methemoglobin causes anoxia in tissues due to reduced oxygen carrying capacity
 - Over 30% can cause brain damage or death

Nitrate Dangers

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

Sources of Nitrates in North Carolina

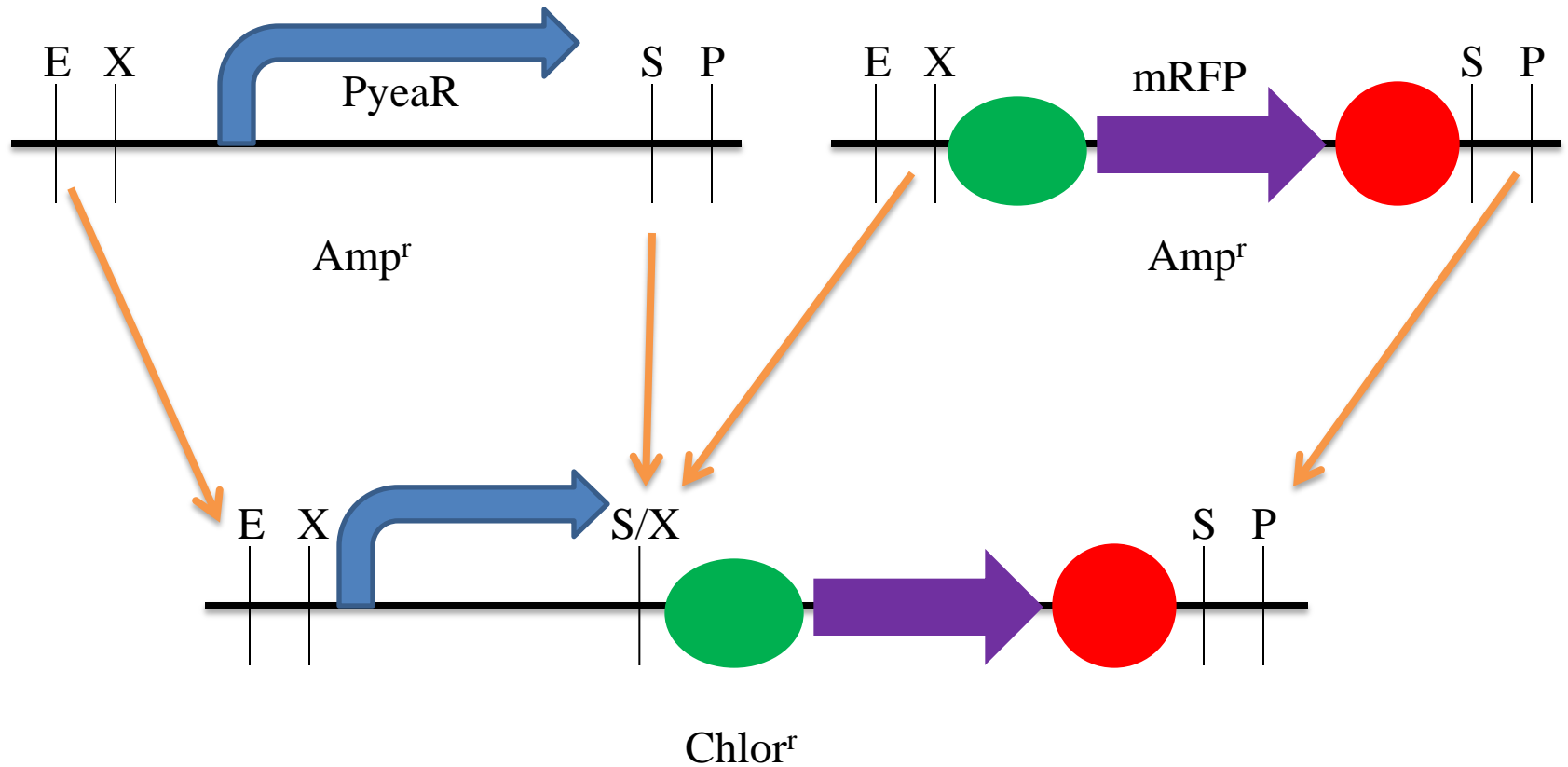
- Human waste
 - Septic tanks or defective sewage systems
- Urban areas
 - Combustion engines
- Mechanized farming
 - Fertilizer use and run-off
 - Livestock waste
 - Leaking lagoons



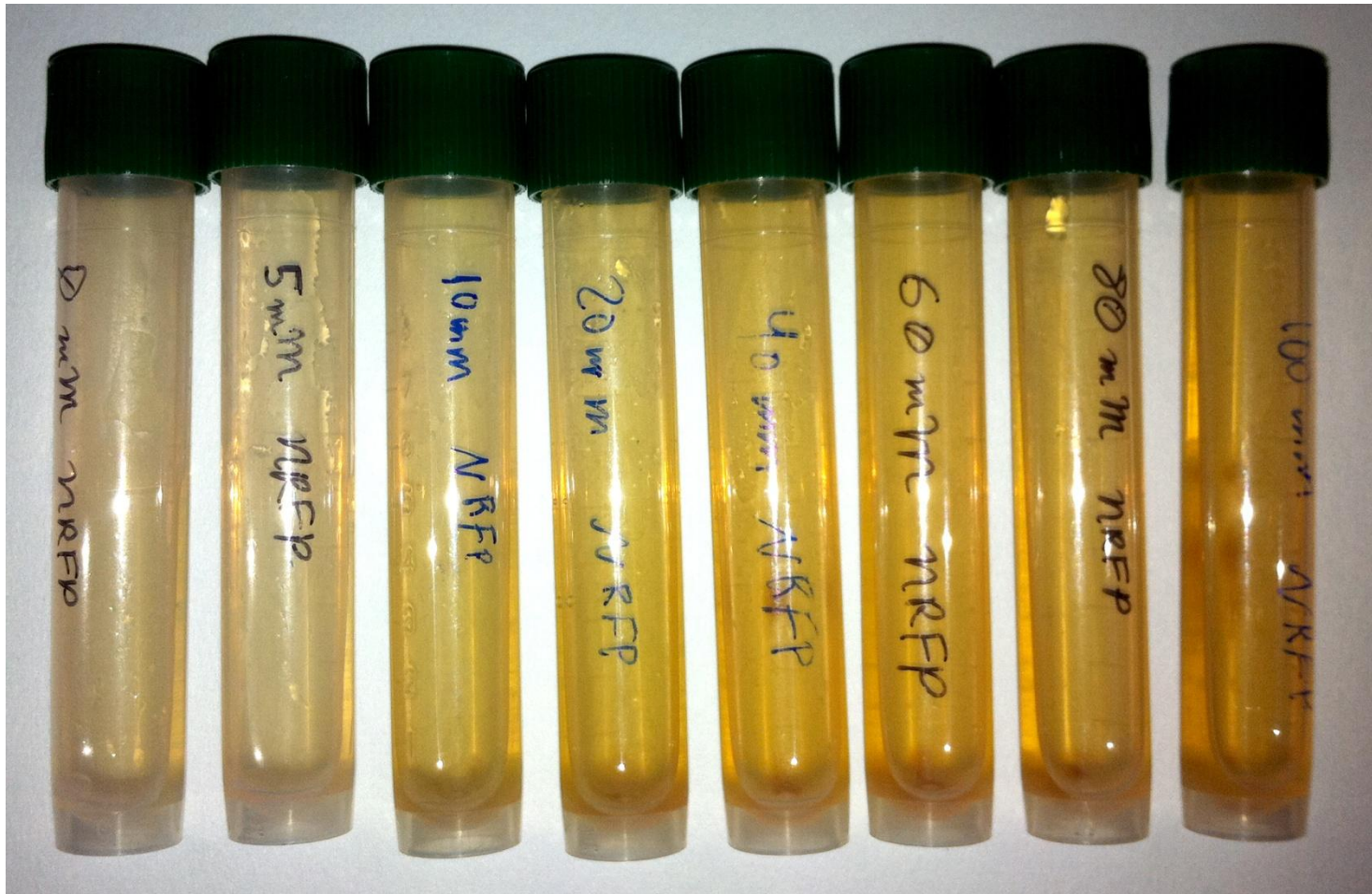
Construction Process

- Combine nitrate sensitive promoter with RFP to produce *E. coli* that turn red in the presence of elevated nitrate levels
 - PyeaR promoter (K216005)
 - RFP coding region (K081014)
 - BioBrick collection
 - Gingko/NEB BioBrick Assembly Kit

Construction Diagram



Working Construct

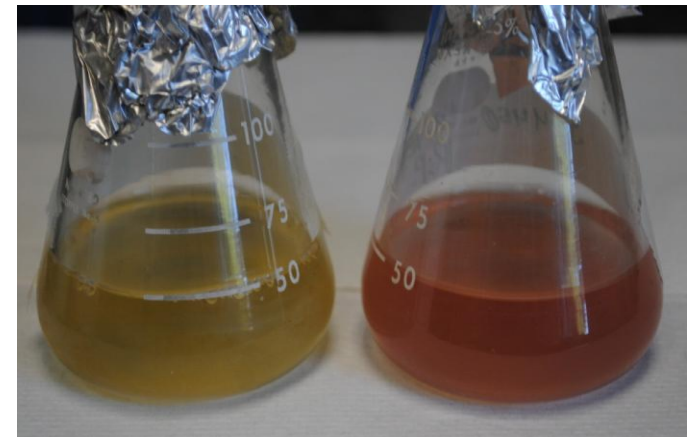


Potential Marketing

- Small farmer or less developed areas
 - Testing for runoff from their farms
 - After fertilization
 - Downstream of hog lagoons
- Easy to use
- Simple and safe use and disposal

Simple and Safe

- Kit is self-contained
- Results easy to interpret
- Household bleach used for decontamination
- Recommend 15 minute treatment with 10% bleach



Negative

Positive

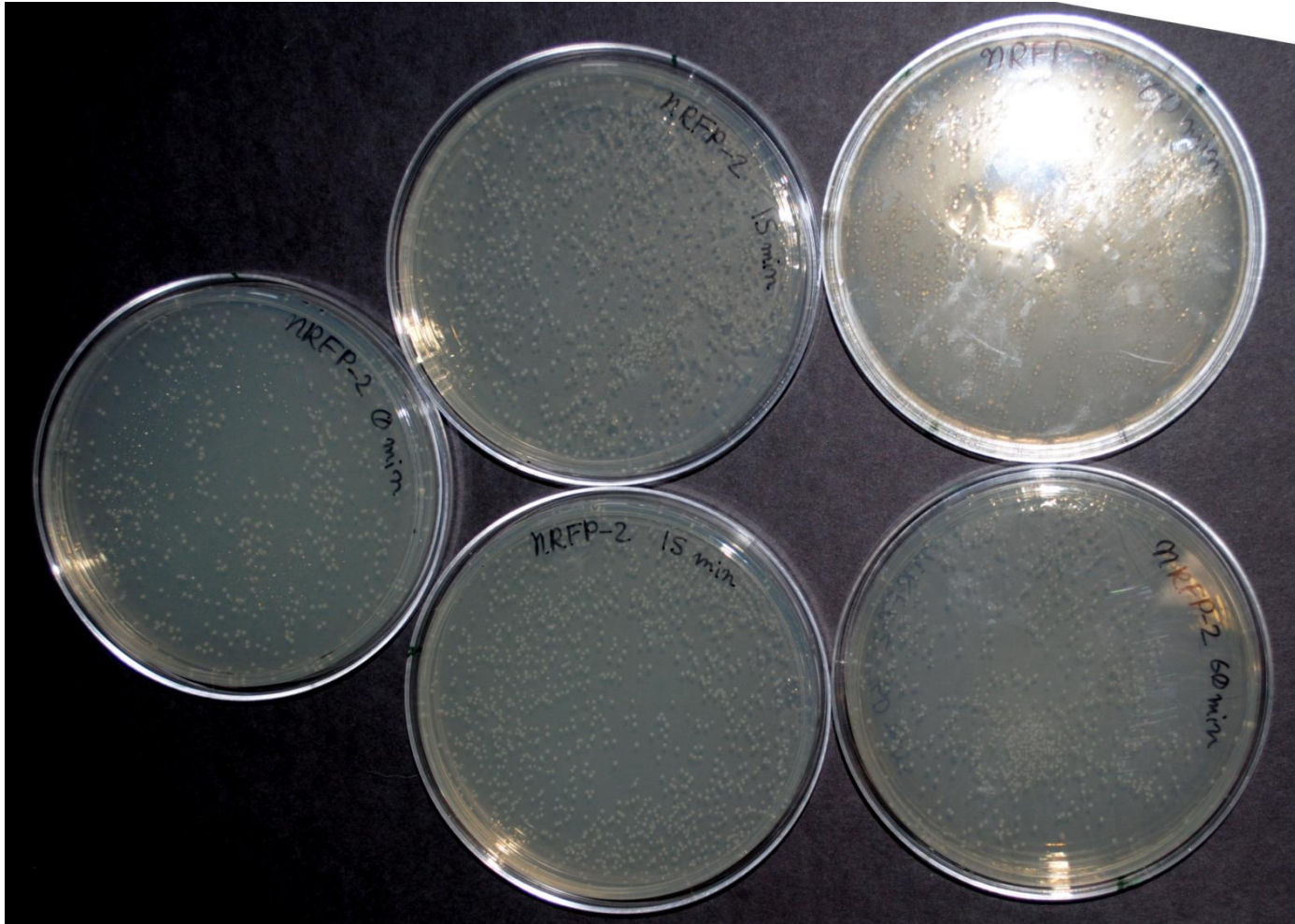
Me? Read Directions?

- But what if I don't...
 - Treat with bleach for 15 minutes?
 - Our tests showed complete elimination of viable bacteria by 5 minutes
 - Treat with bleach at all?
 - There is no guarantee of safely disposing it
 - Best case scenario: Municipal water supply
 - Worst case scenario: Local pond/stream

Dumping Down the Drain

- What we thought:
 - The Municipal water supply is chlorinated
 - Osmotic pressure and chlorine should destroy it
 - The bacteria should die over time
- What Happened:
 - After 60 minutes in tap water, it was plated and produced viable cultures with no large difference between the number of colonies for the 0 and 60 minute plates

Tap Water Treatment



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
- Gift of Dr. Steven Lindow at UC Berkeley

Future Plans

- Produce *fnr*L28H-*narG* BioBrick
- Increase sensitivity of the Nitrate Detector.
 - As it is, it is 10-fold less sensitive than needed.
- Add cell suicide gene to increase safety

Sponsors

Sandra and Bill Hall
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Gaston Day School
New England BioLabs
Teknova
USA Scientific

