

Fluorescent Detection of Cadmium in Water Supplies

- Gaston Day School
- 2013 iGEM Team

Our Team

- Advisor: Ms. Byford
- Seniors:
 - Steven Allen (Team Co-Leader)
 - Parth Patel (Team Co-Leader)
 - Gordon Ellison
- Juniors:
 - Will Reiber
 - Jewel Wasson
 - Paige Brown
 - India Mackinson
 - Elizabeth England
- Sophomores:
 - Claire Lamadrid
 - Heather Cheung
- Associate Members:
 - Misbah Ahmad
 - Nana Boateng

Project Choice

- BioSafety Level 1
- High School Classroom Environment
- Parts available in the registry
- Local Funding
 - Relevant to our area
 - Agriculture
 - Industry

Original Intentions

- Two teams
- Multiple Projects
 - Alternative Energy
 - Heavy Metal Detection
- Time and resources

Original Intentions

- Biofuels
 - AceE, AceF, Ipd genes
 - 1-butanol
 - *E. Coli* genes → BioBricks
 - Primers
 - Assemble one large operon
 - Overexpression?
 - Efficiency?

ATSDR List

- Agency for Toxic Substances and Disease Registry
 - Division of CDC Substance Priority List

1. Arsenic	6. Benzene
2. Lead	7. Cadmium
3. Mercury	8. Benzo (a) pyrene
4. Vinyl Chloride	9. Polycyclic Aromatic Hydrocarbons
5. Polychlorinated Biphenyls	10. Benzo (b) Fluoranthene

Cadmium

- Sources
 - Industry
 - Mining / Smelting
 - Zinc, Lead, Copper
 - Water System
 - Soil / Rock Erosion
 - Nickel-Cadmium Batteries
 - Metal-Coatings
- Federal Limit = 0.005 mg/L

Cadmium

- Health Effects:
 - Affected Systems:
 - Cardiovascular
 - Respiratory
 - Gastrointestinal
 - Known Carcinogen
 - Can cause cancer in any affected system
 - Stronger effects in children

Safety

- Is our number one concern!
- High school environment
 - High traffic
- Protective Measures
- Safety Training
 - Standard Safety Procedures
 - Sterile Technique

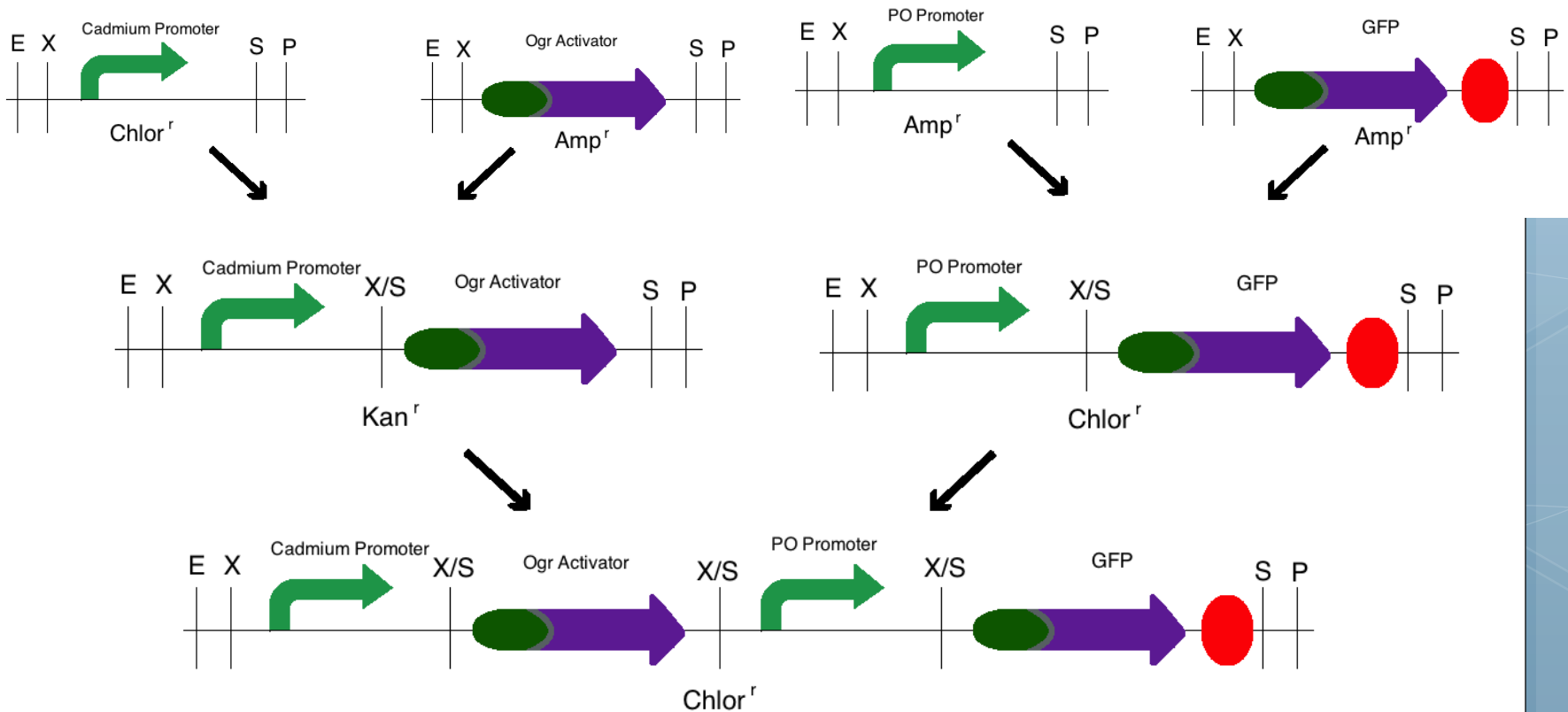
Cadmium Sensor

- Mutagenic PCR
 - U.C. Davis 2010 Protocol
 - Random Mutations through varied concentrations, buffers, and polymerase specificity
 - Possibility of increased sensitivity in promoter or reduced function
- No improvement in sensitivity

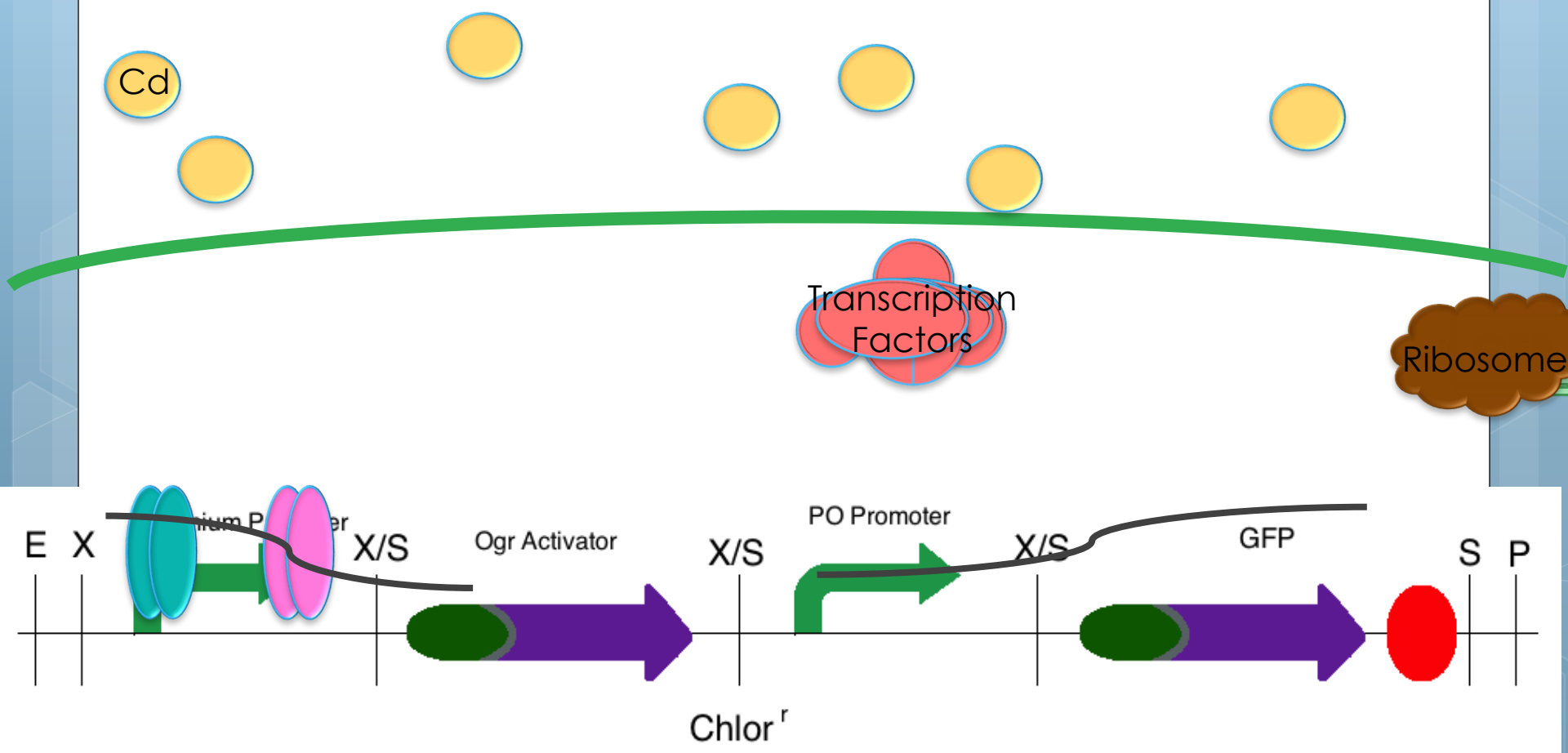
Cadmium Sensor

- Cambridge 2007 Sensitivity Tuners
 - Bacteriophage Activators and Promoters
 - Should not affect cell activity
 - 2 of 15 combinations chosen
 - Ogr and Delta Activators combined with PO Promoter
 - Hoped for 30 – 40 fold sensitivity

Construction



Sensor Model



Testing Protocol

- 4 mL LB Culture in Plastic Tubes
 - Positive Control
 - Last year's detector
 - Negative Control
 - DH5a
 - Concentrations
 - CADMIUM CHLORIDE?? 1m???
 - 1 mM
 - 5 mM
 - 10 mM
 - 50mM

Complication One

- Control (left) & 50 mM Cadmium Chloride (right)



- LB Fluoresces Naturally!

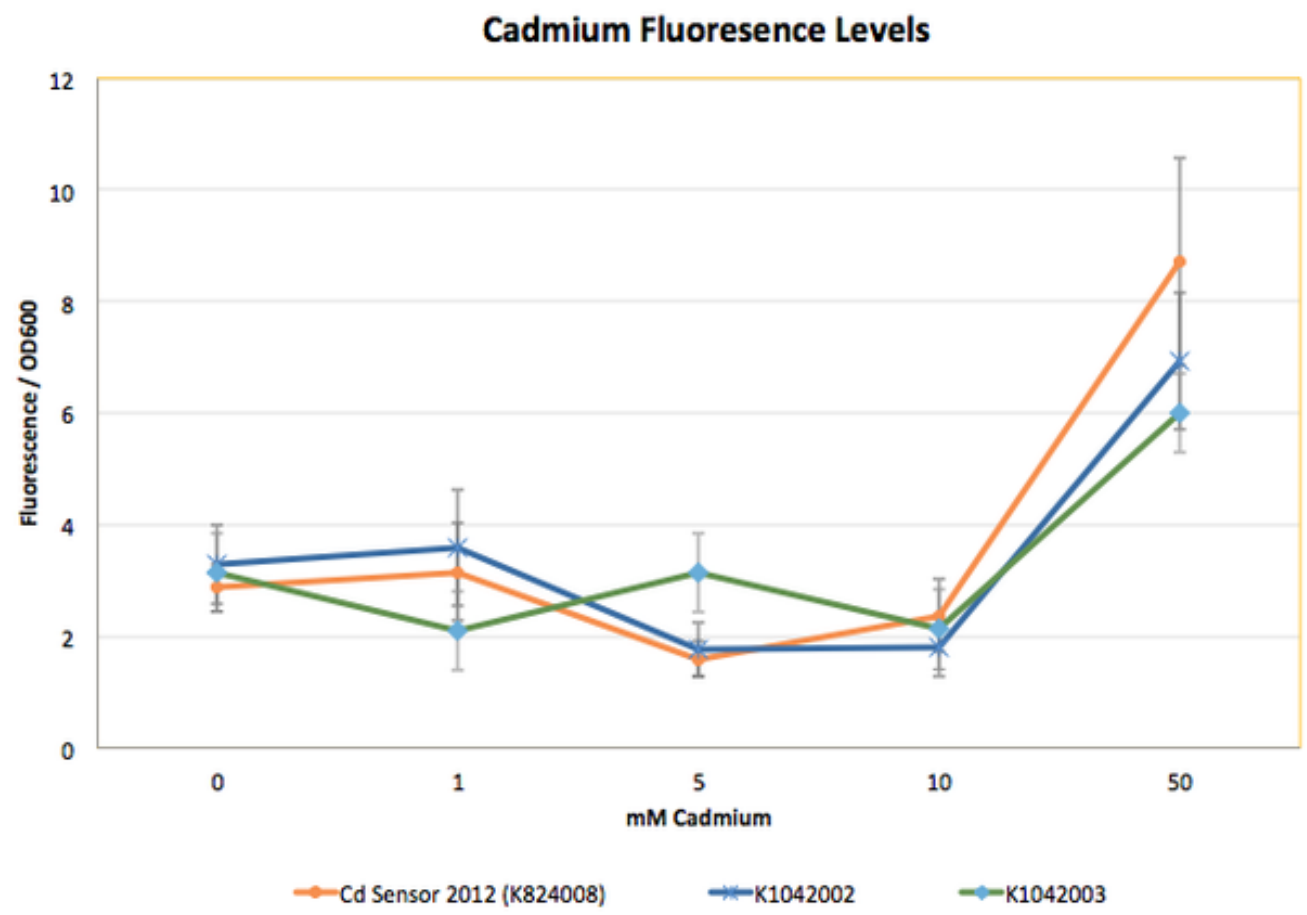
Results

- Luria Broth Fluoresces naturally
 - Account for growth & GFP
- Protocol
 - Spin cells down for 5 minutes
 - Resuspend in 1 mL PBS (1X)
 - Measure in Fluorescence per OD 600

Results



Our Results



Interpreting Our Results

- Initial construct is not more sensitive despite promising initial results
- Less growth at 5 mM but increased at 50 mM
- Why did this happen?
 - Possible solubility issue at 50 mM
 - Cambridge also saw reduced growth

Complications, Complications, Complications

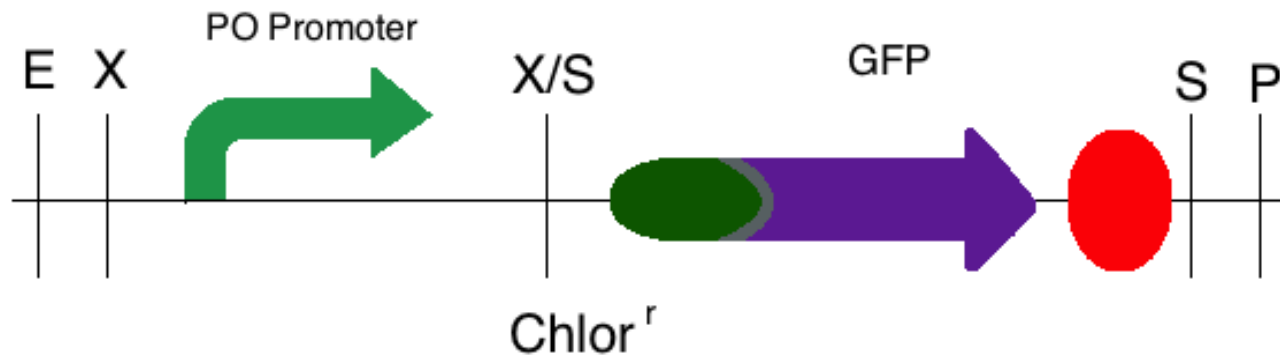
- Detector is not functioning properly
 - Test results
- Fungus over the summer
 - Complete decontamination
 - New solutions, antibiotics, cultures, etc.

Now What?

- Completely started over
 - Transform necessary parts
 - Repeat 3X
 - Digest
 - Size check
 - Ligation
 - Transformation
 - Digest
 - PCR

Our BioBrick

- BBa_K1042013
 - PO Promoter + GFP with Stops



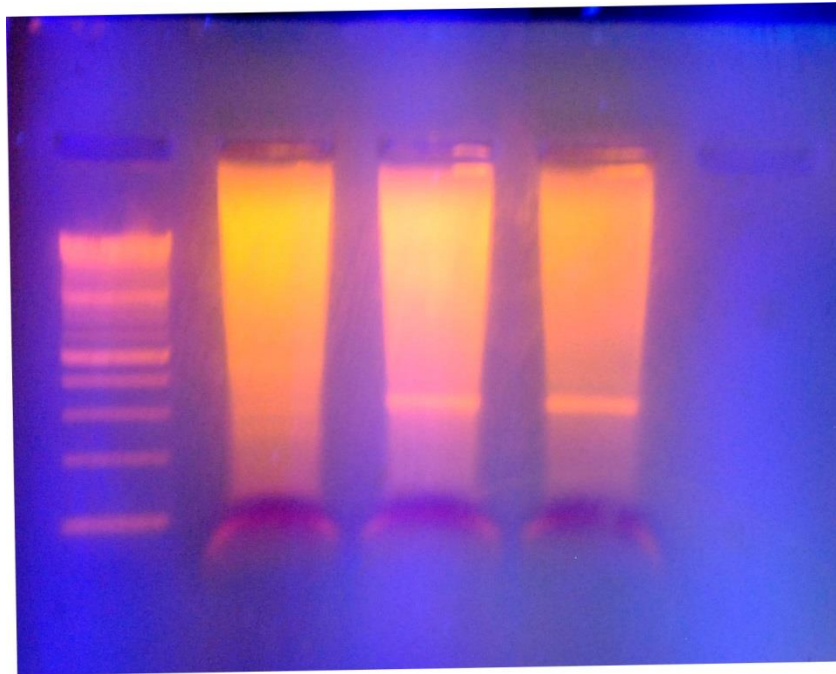
- Picture of PCR / Digest with Bands!
 - Accurate Size

Our New Parts

- Already submitted downstream part
 - K1042013
- Upstream part
 - Cadmium Promoter + P2 Ogr activator
- Entire construct
 - K1042015
 - Cadmium Responsive GFP + Ogr activator
 - Not yet tested

Bands!

- K1042015
 - 1413 base pairs



Solutions & Potential Use

- Ligate Cadmium Promoter + ogr activator with Bba_K1042013
 - Full detector
 - Appropriate antibiotic resistances
- Full detector
 - Easy to interpret results
 - Self-contained kit...
 - Marketing



Future Plans

- Sensitivity Tuners?
- Cell suicide gene
- Biofuels

Sponsors

Sandra and Bill Hall

New England Biolabs

SignSmart

Gaston Day School

