Heavy Metal Detectors

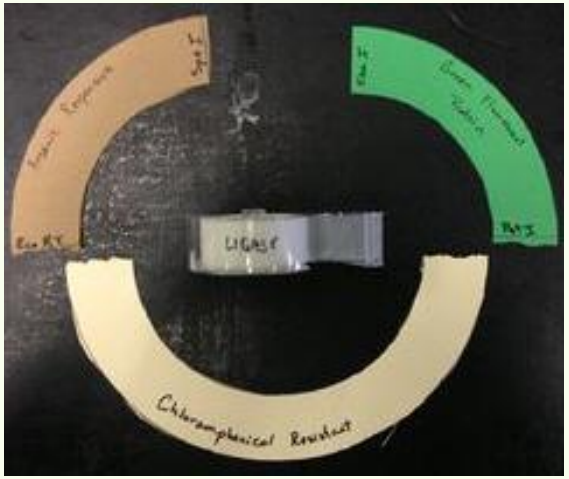
Gaston Day School iGEM Team

Our Team

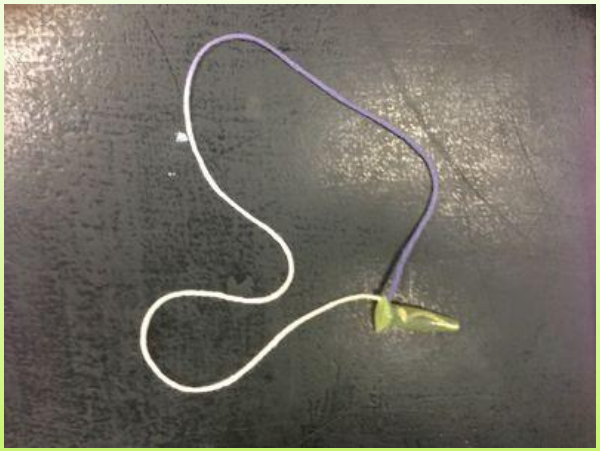
- **Seniors**
- **Juniors**
  - Steven Allen (Team Leader)
  - Parth Patel (Team Co-Leader)
  - Samuel DuBois
  - Gordon Ellison
- **Sophomores**
  - Will Reiber
  - Audrey Weiss

Outreach

- BioBrick “Building”



- Wheat Germ DNA Necklaces



Our Lab

Our New PCR Machine

It's As Old As We Are!

Project Choice

- BSL 1
- High School Classroom Environment
- Local Funding
  - Relevant problems in our area (& around the world)
    - Agriculture
    - Industry
    - Natural occurrence
- Parts available in Registry

ATSDR List

- Agency for Toxic Substances and Disease Registry
  - Subdivision of CDC
  - Most Recent 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

Lead

- Lead Pipes
  - Soldering
- Ammunition & Fishing Equipment
- Industrial Sector
  - 2.5 million tons annually
- Paint (Lead-Based)
- MCL — 0.015 mg/L
  - Maximum (Allowable) Contaminant Level

Lead

- Lead poisoning primarily affects:
  - Brain
  - Cardiovascular System
- In adults, High Lead Levels → High Blood Pressure
- In children, lead can affect development
  - Inhibits brain development
  - Symptoms: Migraines, shortened of attention span, etc.

Arsenic

- Ground water
  - Common in wells
- Smelting and Construction Industries
- Paint pigments
- Wood Preservative
- MCL — 0.010 mg / L

Arsenic

- Linked to cancer of urinary, respiratory, and dermal systems
- Other effects: skin discoloration, stomach problems, paralysis, and loss of sight
- Prolonged exposure may cause death

Cadmium

- Industrial Workplaces
- Nickel-Cadmium Batteries
- Metal-Coating
  - Does not easily corrode
- MCL — 0.005 mg / L

Cadmium

- Affected systems:
  - Cardiovascular
  - Respiratory
  - Gastrointestinal
- Known carcinogen
  - Will cause cancer in all affected systems in ingested
- Stronger affects in children

Safety

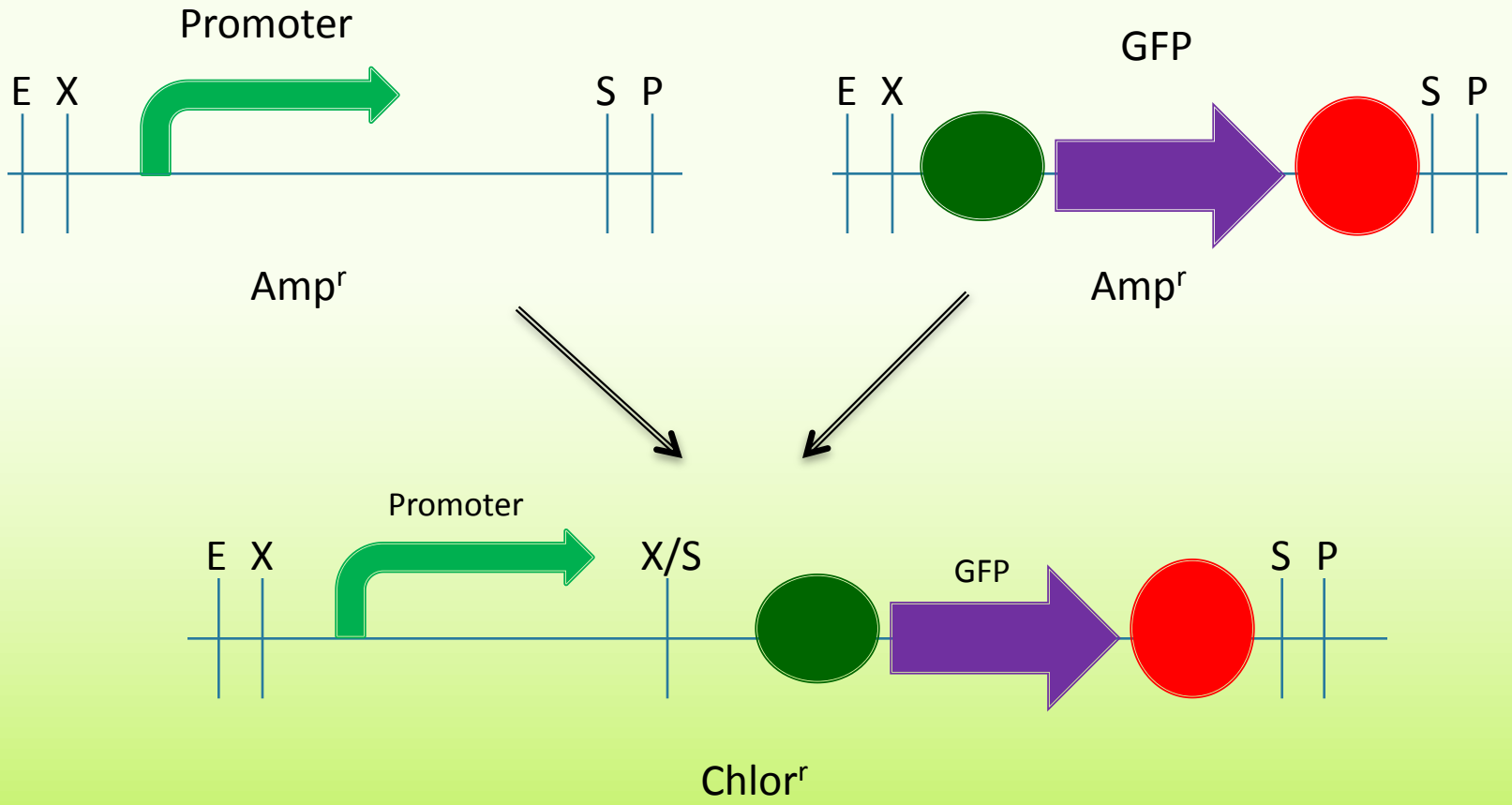
- Is a Priority!
- Training
  - Proper Sterile Technique
  - Standard Safety Procedures
- Secure Facilities
  - Keep Lab Secure
  - High Traffic Environment
- BioSafety Level 1
  - Restricts materials and chemicals available

Our BioBricks

- **Cadmium**
  - BBa_K824008
  - Cadmium promoter (BBa_K174015) and GFP coding region (BBa_I13401)
- **Arsenic**
  - BBa_K824009
  - Arsenic promoter (BBa_J33201) and GFP coding region (BBa_I13401)
- **Lead**
  - BBa_K824012
  - Lead promoter (BBa_I721001) and GFP coding region (BBa_I13401)

Our BioBricks

- 3 Parts
  - Cadmium, Arsenic, and Lead detectors



Initial Testing Protocol

- 4 mL LB cultures with varying concentrations of Cadmium Nitrate or Lead Acetate
  - Cadmium and Arsenic Promoters
    - 0 – 100 mM Cadmium Nitrate
  - Lead Promoter
    - 0 – 20 mM Lead Acetate

Problems

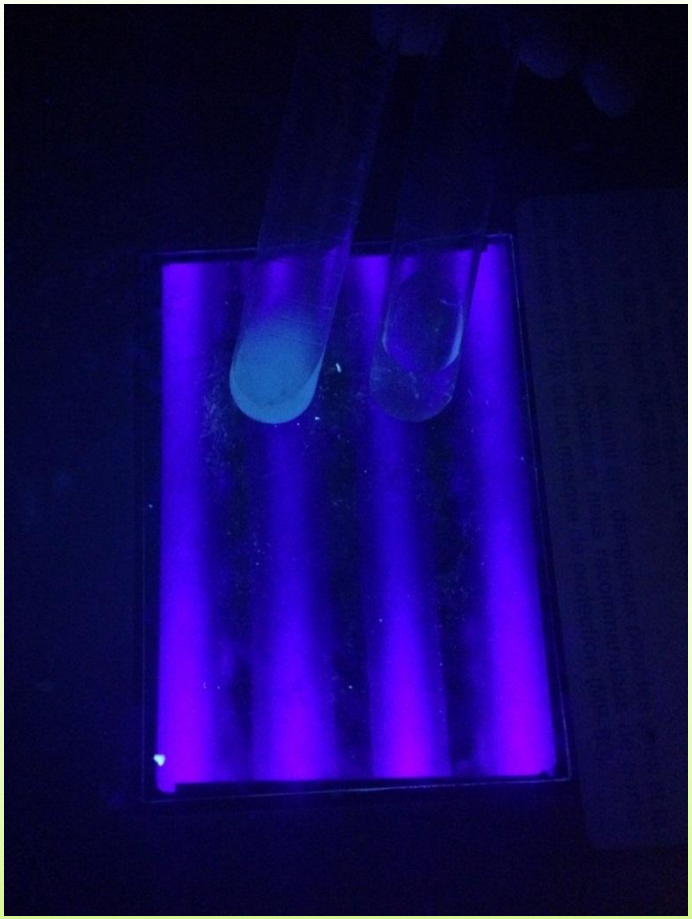
- Control (left) & 50 mM Cadmium Nitrate (right)

- LB Fluoresces Naturally!

Problems & Solutions

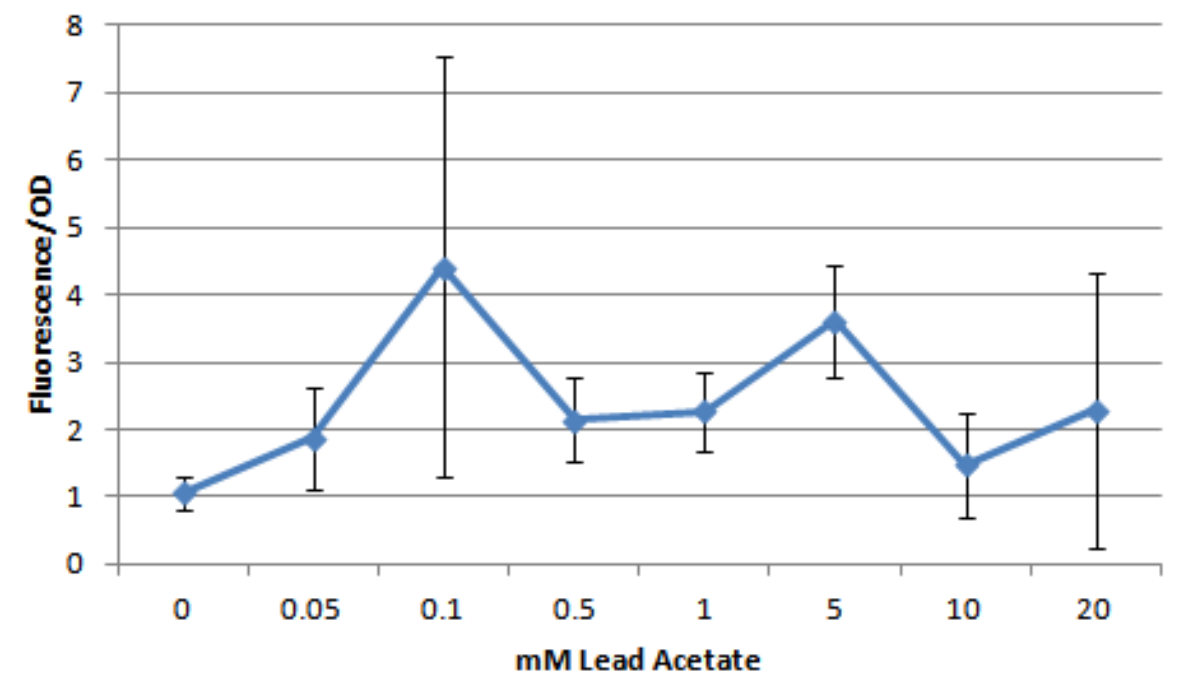
- M9 Medium?
- PBS
  - Phosphate Buffered Saline
    - Inexpensive
- Grow in LB (Same Protocol)
  - Spin Cells
  - Resuspend in PBS
- Fluorescence per O.D.

Working Constructs



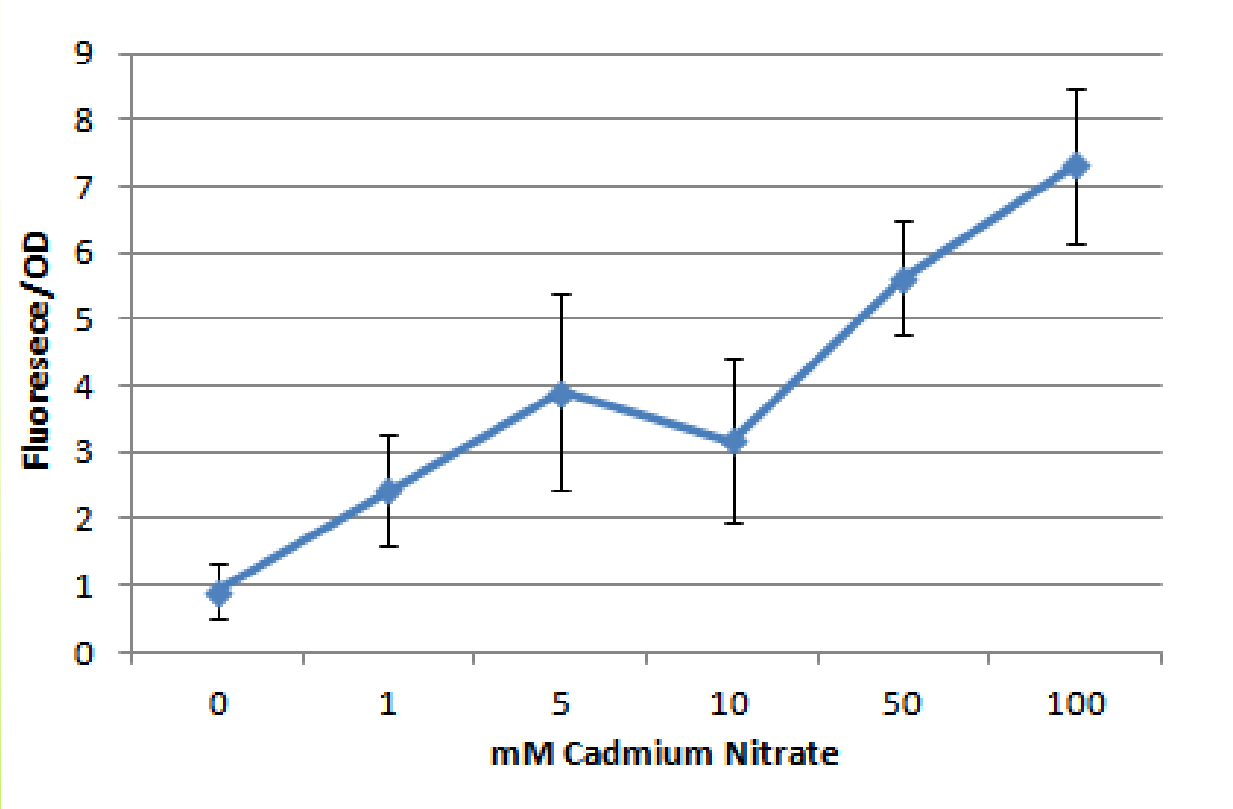
Results: Lead Detector

- Inconsistent
- Increase at 0.05 mM
- Sharp increase for low concentrations



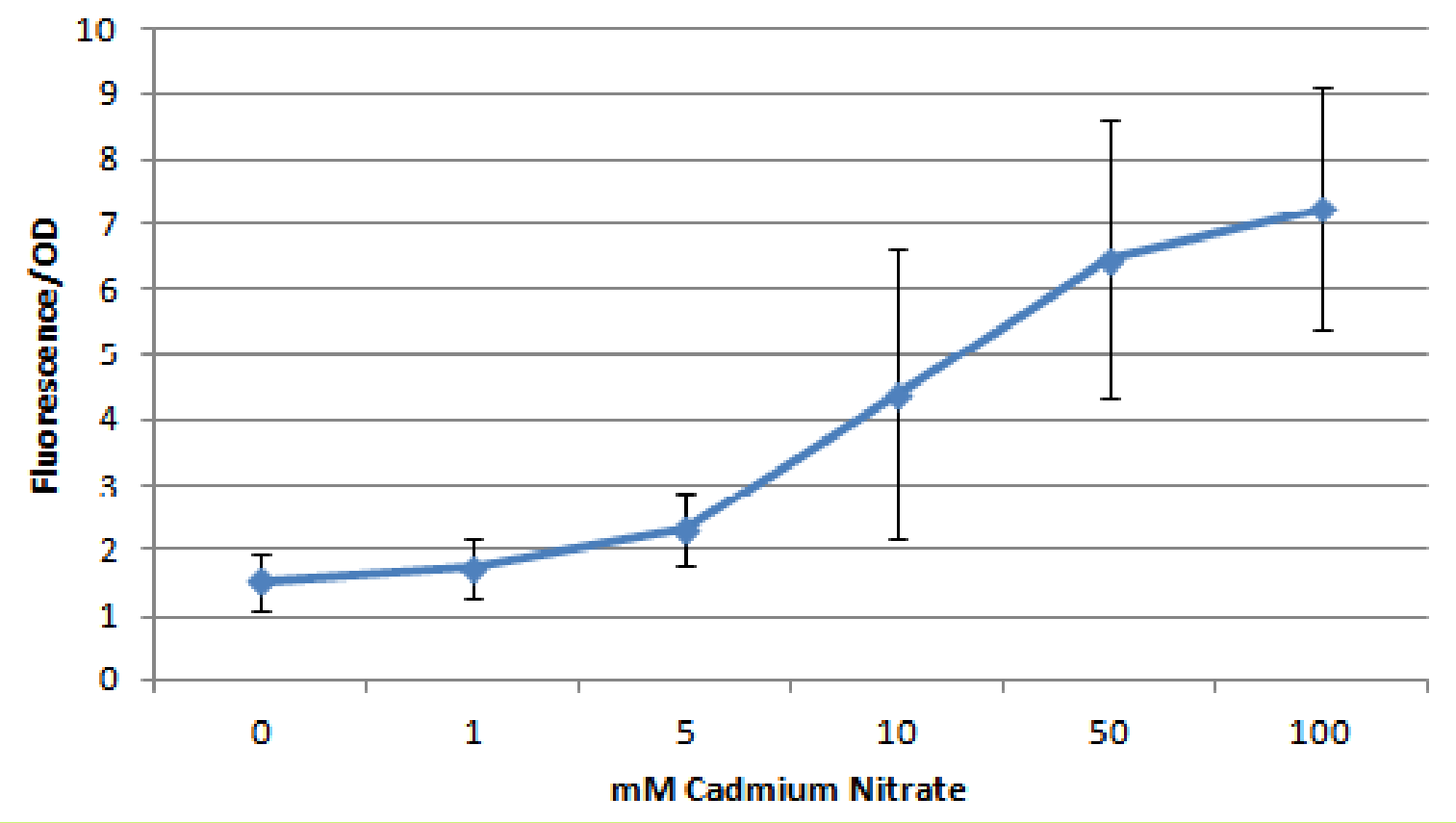
Results: Arsenic Detector

- Tested using Cadmium Nitrate
  - Safety (BSL 1)
- Minimum Detectable level is below 1 mM



Results: Cadmium Detector

- Detection Level is between 1 & 5 mM



Sensitivity?

Contaminant	Federal Limit (mg/L)	Federal Limit (ppm)	Our Detectable Level (ppm)
Lead	**0.015 mg/L**	**.015**	**10**
Arsenic	**0.010 mg/L**	**.010**	**236.4**
Cadmium	**0.005 mg/L**	**.005**	**1182**

Future Plans

- Increase Sensitivity
  - Mutagenic PCR
    - 2011 UC Davis iGEM Team
  - Sensitivity Tuners
    - 2007 Cambridge iGEM Team

Future Plans

- Fluorescent Proteins
  - Which is the most sensitive?
- Multiplex
  - Multiple promoters & reporters
  - Identify mixing of colors
- “Cell Suicide Gene”
  - 2010 University of Hong Kong 2012 iGEM Team
- Research Lead Promoter

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