Fluorescent Detection of Cadmium in Water Supplies

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Cadmium

- Released through mining and smelting
- Enters the food chain from uptake by plants from contaminated soil or water
- May enter aquatic systems through weathering and erosion of soils and bedrock
- Tobacco leaves naturally accumulate and concentrate relatively high levels of cadmium
- Health Affects
  - Cardiovascular, Respiratory, Gastrointestinal systems
  - Known carcinogen
  - Stronger affects in children
- Maximum Allowable level: 0.005 mg/L

Previous Work / Plans

- 2012 team constructed a functional cadmium detector
  - Detected Cd at 5-10 mM
  - Clearly NOT sensitive enough

- 2013 Goals:

1. Increase sensitivity to relevant level
   - Mutagenic PCR of promoter
     - UC-Davis 2011
   - Addition of sensitivity tuners
     - Cambridge 2007

2. Produce an operon with AceE, AceF, and Lpd as a precursor to Biofuels production in *E. coli*

Testing

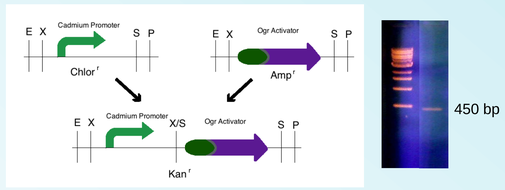
- 4 mL LB cultures with varying concentrations of Cadmium Chloride
- 0 – 50 mM Cadmium Chloride
- Spin down culture and resuspend in 1 ml PBS
- Measure OD_{600} and GFP Fluorescence

Increasing Sensitivity

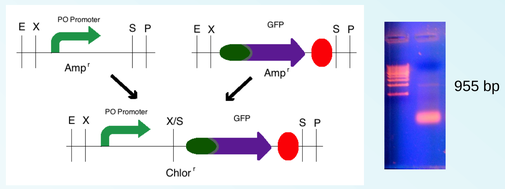
- Mutagenic PCR
  - Uses uneven dNTP concentrations, Mn in buffer, and low annealing temperatures
  - Reduces specificity of polymerase
  - Causes random mutations
  - Some mutations might increase sensitivity of promoter to Cd
  - Possibility of loss-of-function or reduced function as well

- Cambridge 2007 Sensitivity Tuners
  - Activators and Promoters are bacteriophage components
  - Less common and should not affect normal cell activity
  - Two combinations out of fifteen chosen due to limited resources and time
  - Ogr and Delta Activators combined with PO Promoter increase sensitivity the most
  - Expected 30 to 40 fold sensitivity increase

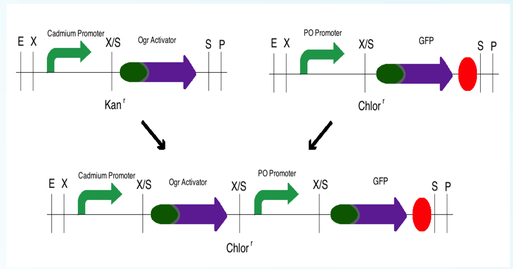
Construction



450 bp

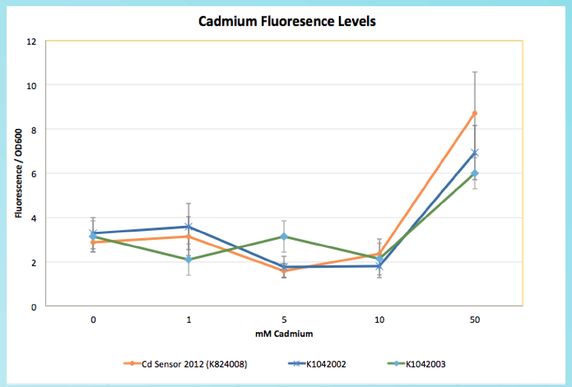


955 bp



The final construct should be 1.4 Kb.

Results



1. Mutagenic PCR
   - Multiple trials for both promoter and whole Cadmium Sensor
   - No improvement in sensitivity
   - Some actually worse or nonfunctional

2. Delta activator
   - K1042002 (not submitted)
   - No difference from original sensor (K824008)

3. Ogr activator
   - K1042003 (not submitted)
   - Marginal, but decreasing improvement at 5mM
   - Even if 5mM point is accurate, not biologically relevant

Issues Discovered

1. Mutagenic PCR was far more negative than expected.

2. 5mM Cadmium Chloride seemed to affect growth more than 50mM.
   - Solubility issues at 50mM?
   - Cambridge 2007 team also saw reduced growth at high induction levels

3. Positive preliminary tests are not always accurate

4. Correct assembly does not ensure anticipated function.

Project Alternate – BioFuel Production

- E. coli as a platform for Biofuel production
  - Rapid growth
  - Well known genome
  - Easy manipulation
  - Natural fermentation pathway
  - Well funded research avenue
- Pyruvate dehydrogenase complex converts pyruvate to acetyl-CoA
  - Operon contains pdhR, aceE, aceF, and lpd
  - pdhR is regulatory for the remainder of the operon

- Sequence data for aceE, aceF, and lpd from genbank
- Primer design using online design software within genbank
- Genomic DNA isolation using Promega kit
- PCR amplification with primers + leader sequence for cloning into BioBrick vector

- PCR failed to produce amplification
  - Poor primer design
  - Poor DNA quality/quantity

References:

Quail, MA, Haydon, DJ, and Guest, JR, (1994). Molec. Micro. 12(1), 95-104

Guest, JR, and Stephens, PE, (1980). J Gen. Micro. 12, 277-292.

Future Plans

- Adding part experience to 2007 Cambridge tuners once our part is built and fully tested.
- Try additional combinations of tuners and promoters
- Continue working with mutagenic PCR
  - Try mutating GFP for increased signal
  - Test additional promoter mutants for increased sensitivity
- Combine Cd detector with previous year's As and Pb detectors for multiplex function
- Clone AceE, AceF, and Lpd as individual genes and as an operon

Sponsors

Sandra & Bill Hall