iGEM Project Updates!

7/13/2018

Heavy Metal Detection Using Paper-Based Assay

- Levels of toxic lead and chromium(VI) in Lake Michigan have been found to be 10x higher than the California deemed maximum
- Utilizing a cell-free biosensor to detect if chromium/lead is present in water (two separate assays)
- Each test has two logic gates
    - One circuit constitutively produces a repressor for its respective metal
    - Repressor is used in the second circuit to inhibit the promoter from producing a colorimetric output unless metal is present
- Created based on Adam's ADS088 and ADS094 plasmids

Updates

- Successfully completed CFPS control experiment with Adam's pADS071 & pADS072
- Completed our project description (http://2018.igem.org/Team:Northwestern/Description)
- Spoke with the Purdue Team regarding collaboration, Interlab & our projects
- Started working in the old iGEM lab
- Ordered DNA
    - Backbones and primers
- Contacted potential sponsors
- Solidified sponsorship from Arbor Biosciences, received 96 myTXTL reactions
- Continued project funding through experiment.com
- Worked with Adam to learn how to create a cell-free extract, midi-prep
- Met with Professor Jewett and modified project method

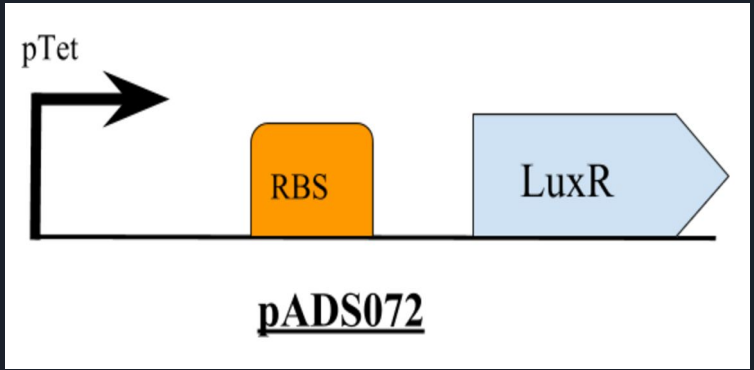
Project Goals and Timeline

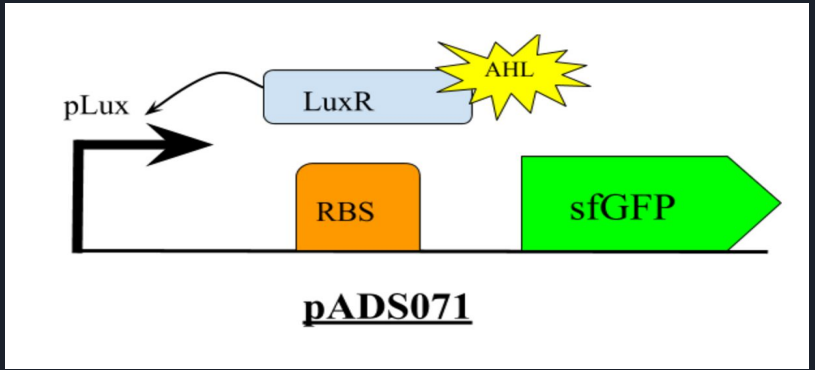
Opportunity for iGEM Part =

1	2	3	4	5
Prove LuxR/pLux activator-promoter system reliably produces sfGFP in pADS071 and pADS072 plasmids, testing set control and variable groups. Affirming plasmids work as expected in presence/absence of certain molecules.	Clone LuxR system into pADS088 and pADS094 plasmids optimized for CFPS. We will similarly test set control and variable plasmids, affirming sfGFP is produced when expected.	In the pADS088 and pADS094 LuxR plasmids, change reporter from sfGFP to colorimetric output (as recommended by Prof. Jewett). Current enzymes we are considering include β-Glucuronidase (GusA) and β-Galactosidase (LacZ).	Replace the LuxR system with chromium and lead promoters and repressors (chrB/chrP, pbrR/pbrAP). Test this metal detection system in the presence of lead and chromium for colorimetric output.	Blot lead and chromium systems onto paper; test to see if this paper-based assay produces colorimetric output reliably, attempt to optimize system.

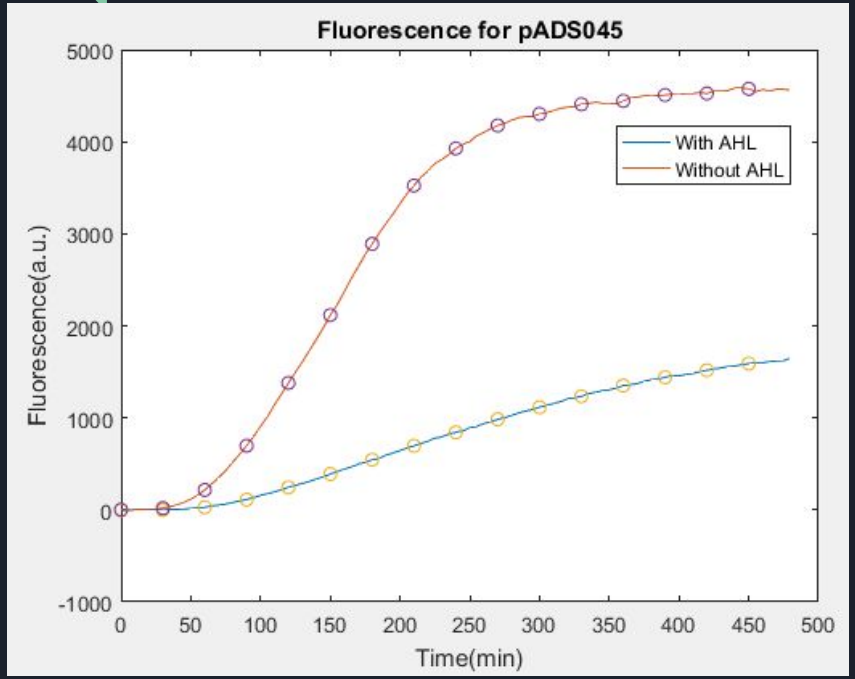
Results from pADS071, pADS072 LuxR sfGFP Experiments

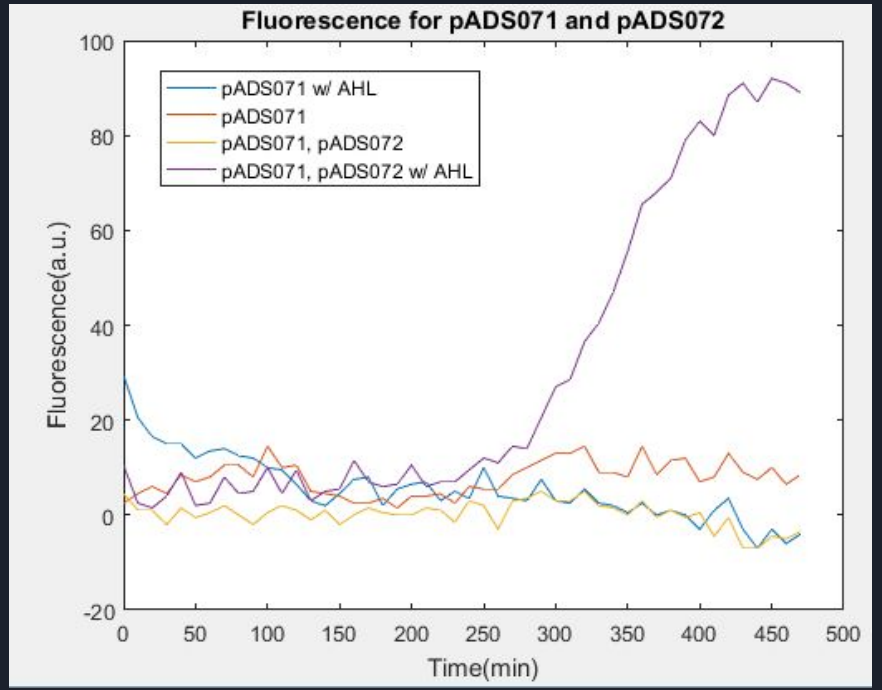
- Goal of Experiment:
  - The assay consisting of pADS071 and pADS072 have been shown to facilitate CFPS of sfGFP.
  - LuxR activator is synthesized in pADS072; in the presence of AHL, LuxR binds to the pLux promoter in pADS071, which promotes sfGFP production.
  - However, since this system was not well optimized, sfGPF expression was low and the system could not be blotted on paper.





Results from pADS071, pADS072 LuxR sfGFP Experiments





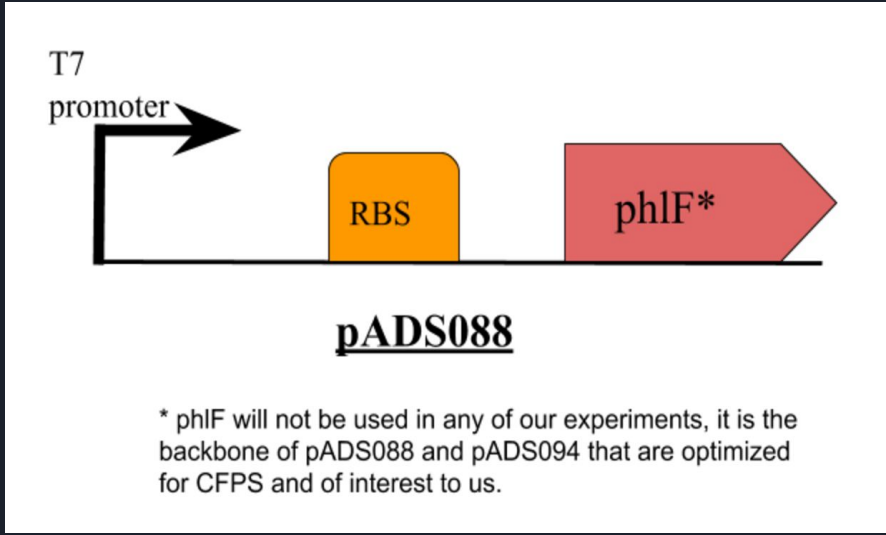
Results from pADS071, pADS072 LuxR sfGFP Experiments

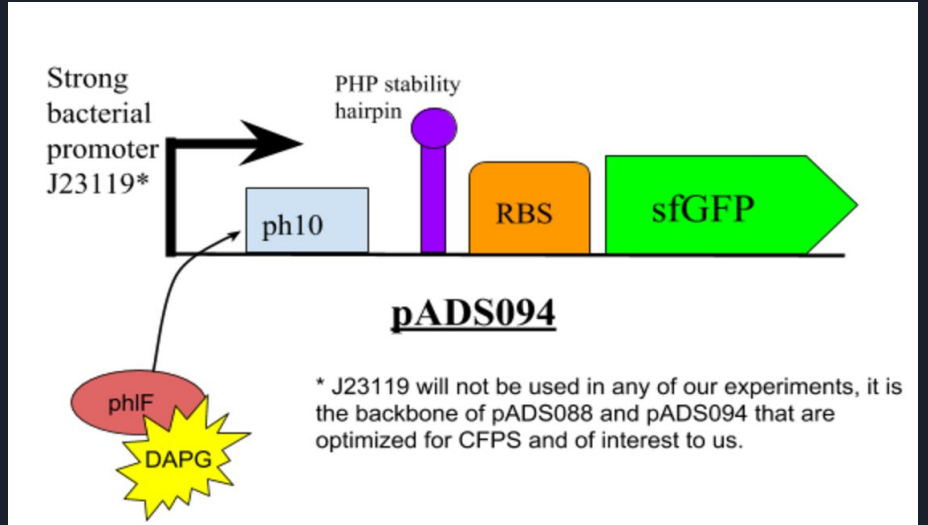
Demonstrated that:

- pADS045 constitutively produces ample sfGFP regardless of AHL presence (positive controls)
- pADS071 (reporter) requires both LuxR produced by pADS072 and AHL to generate fluorescence
  - Absence of either results in little to no fluorescence
- This was our first biosensor!

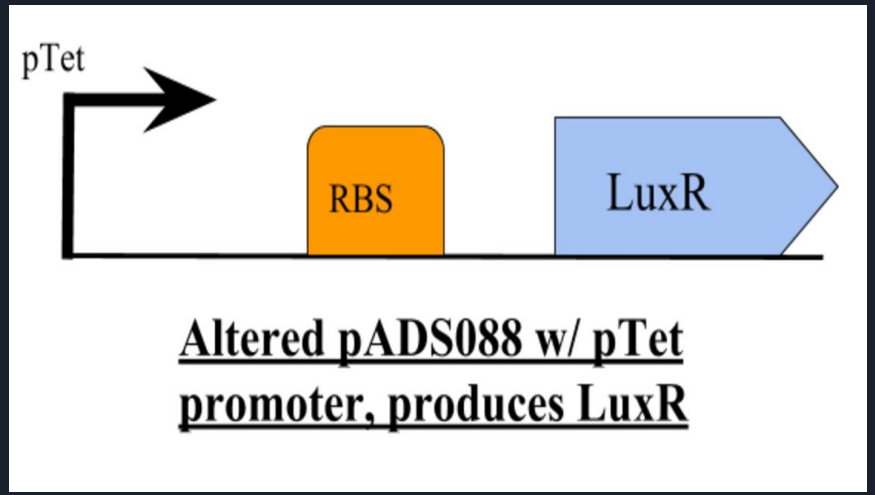
Step 2: Clone LuxR/pLux system into pADS088 and pADS094

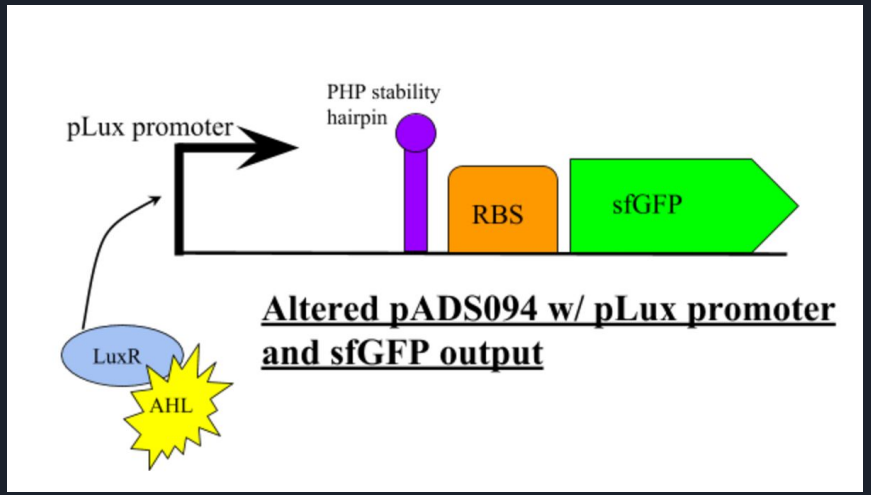
- Experiment Reasoning:
  - pADS088 and pADS094, unlike pADS071 and pADS072, are optimized for CFPS due largely in part to the presence of a PHP stability hairpin.





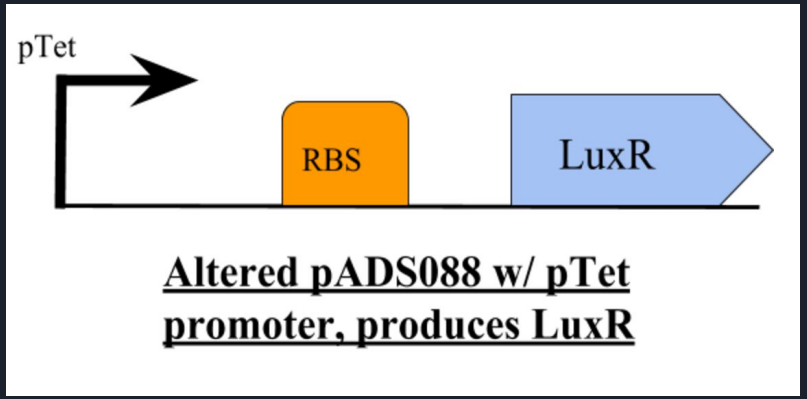
Step 2: Clone LuxR/pLux system into pADS088 and pADS094

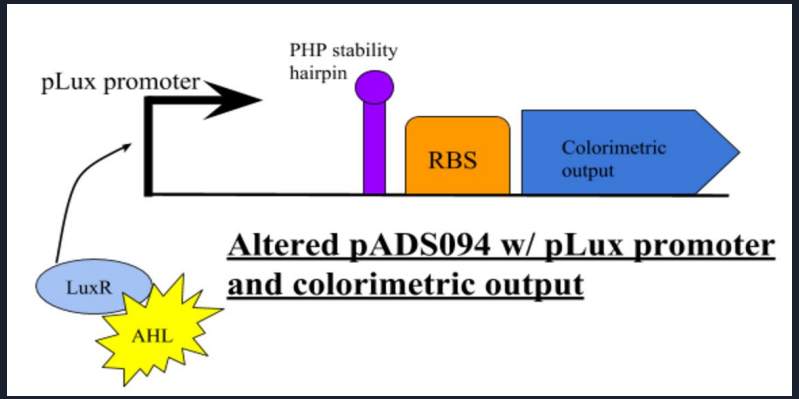




Step 3: Change sfGFP to colorimetric output

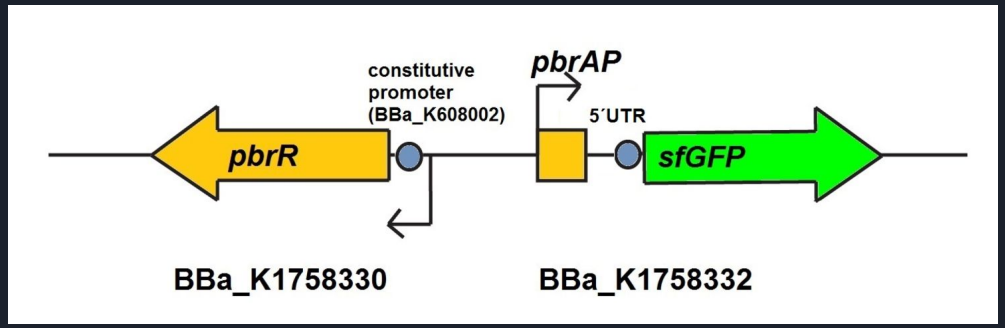
- We met with Professor Jewett, who suggested we move from sfGFP to a colorimetric output.
- This is because colorimetric output is easier to identify and measure, especially in the context of ‘citizen science’, as we ultimately hope to make our paper sensor available to the public.
- Current colorimetric outputs we are considering are β-Glucuronidase (GusA) and β-Galactosidase (LacZ).

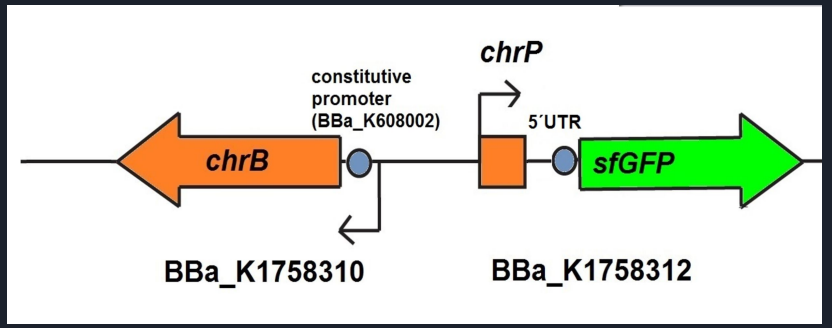




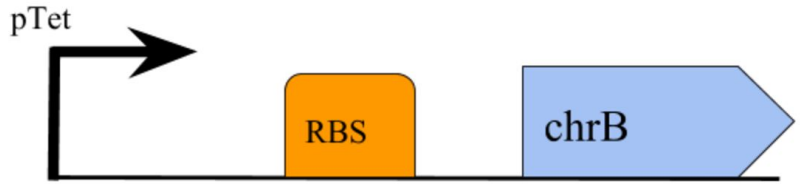
Step 4: Replace LuxR with chrB/pbrR, replace pLux with chrP/pbrAP

- Replacing LuxR/pLux with the chromium and lead detecting systems, the crux of our project.
- Anticipate doing so using Gibson Assembly, incorporating Biobrick prefix/suffix during this step.
- Repressor is constitutively being made and binding to operator sequence; when present, toxic metal binds to repressor and sfGFP/colorimetric output is produced.

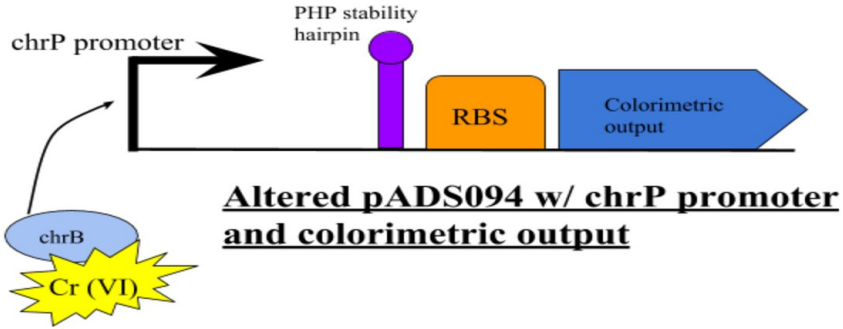




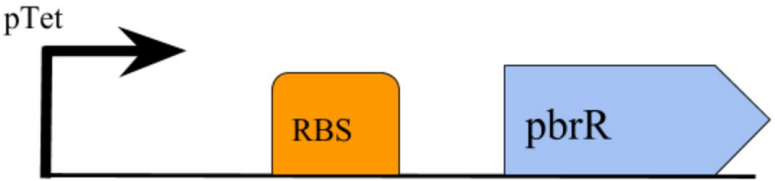
Step 4: Replace LuxR with chrB/pbrR, replace pLux with chrP/pbrAP



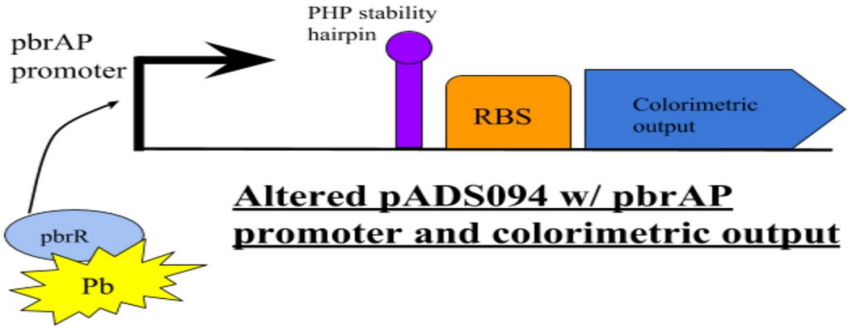
Altered pADS088 w/ pTet promoter: produces chromium repressor chrB



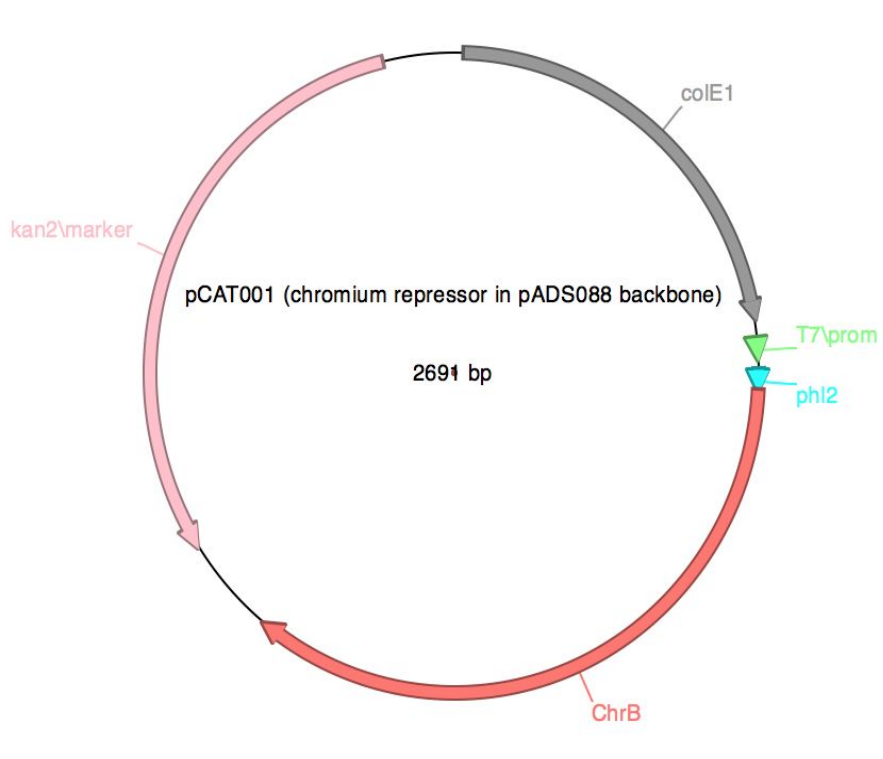
Altered pADS094 w/ chrP promoter and colorimetric output



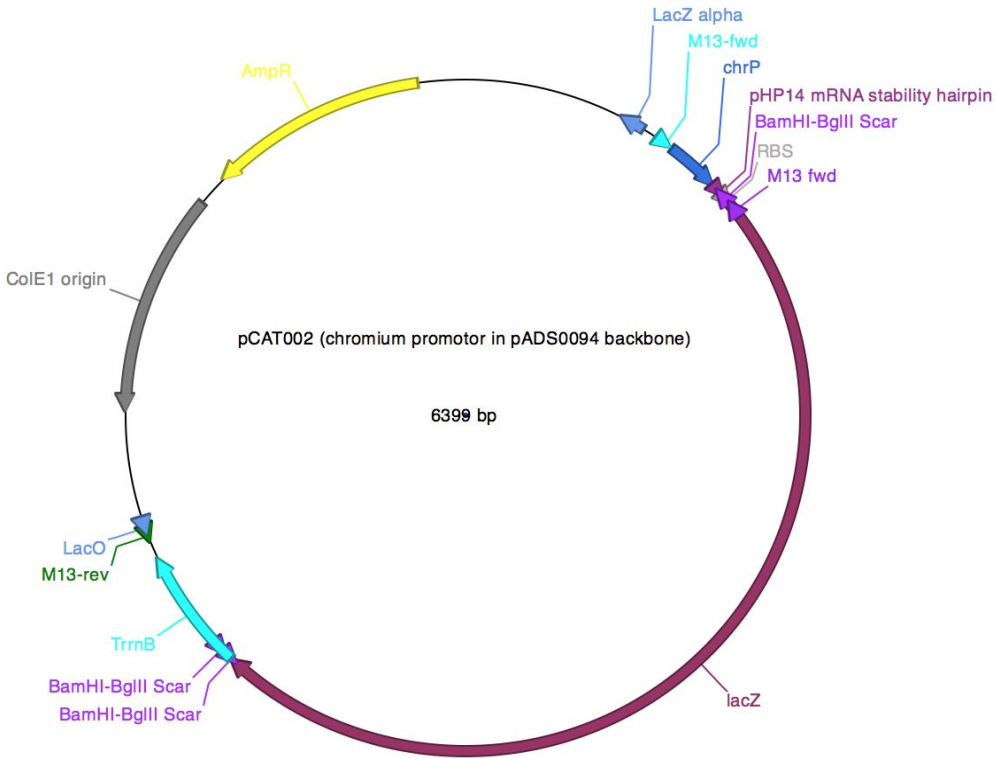
Altered pADS088 w/ pTet promoter: produces lead repressor pbrR



Altered pADS094 w/ pbrAP promoter and colorimetric output

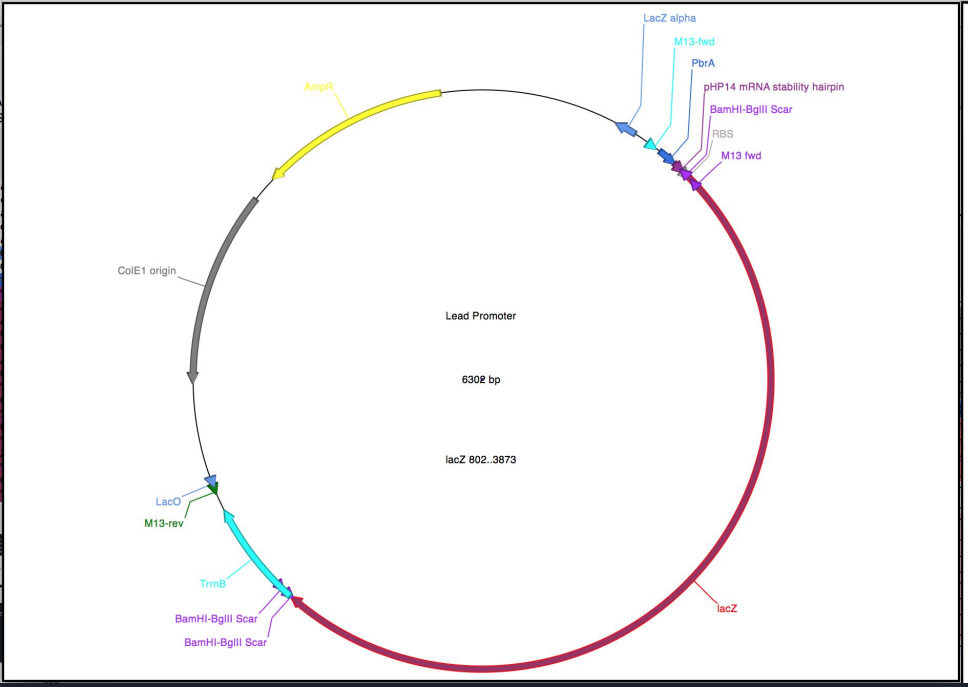


Chromium Repressor

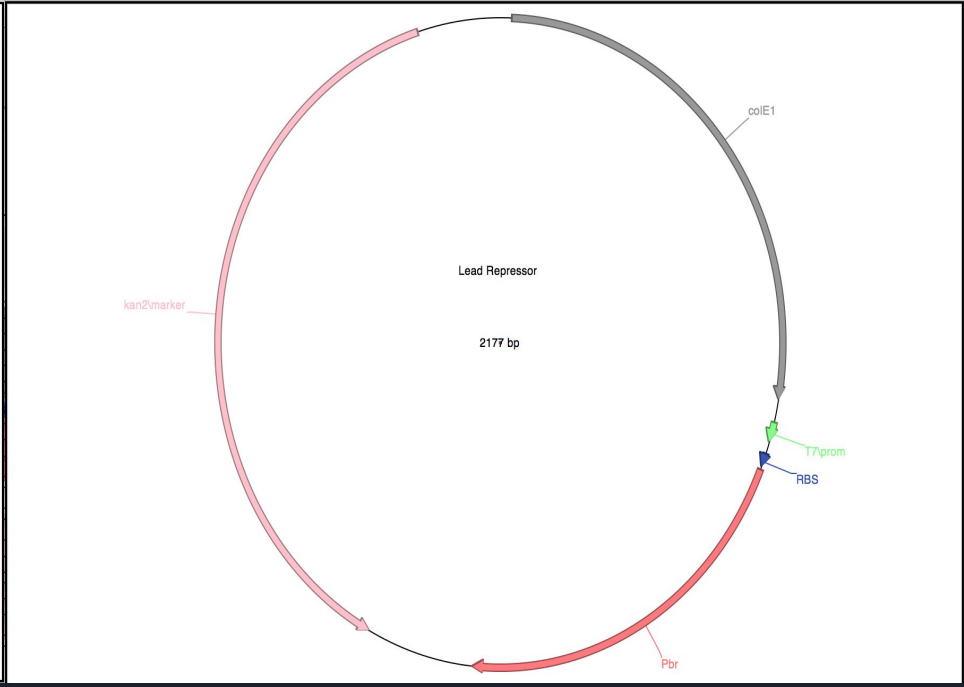


Chromium Promoter

ApE Representations of final plasmids



Lead Promoter



Lead Repressor

ApE Representations of final plasmids

Step 5: Blot lead and chromium-sensing systems onto paper

- Prof. Jewett recommended using wax circles to contain blotted assays.
- Purdue iGEM Team has wax printer, offered collaboration with transferring systems to paper.

Updated Human Practices

- Integrate what we learned from Professor Jewett and Adam into our project documentation
- Currently discussing a possible collaboration with environmental and conservation researchers at the Shedd Aquarium
- In contact with the “Evanston Chemist” from the Evanston water treatment center about touring the plant and learning more about their protocols for treating water after the spill

Shedd AQUARIUM

City of Evanston™

Travel

- Due to expenses/our academic calendar, we anticipate flying in Friday afternoon, staying Friday and Saturday nights in Boston (Oct 26 & 27), and leaving Sunday morning (Oct 28).
- Currently searching for most cost-effective flights and hotels or Airbnbs.
- Curious about being sponsored by our respective departments.

Questions

1. Scale/Lab Equipment
2. How will we obtain chromium and lead for testing?
3. Suggestions of other human practices contacts?
4. How can we effectively quantify the colorimetric outputs?
5. Will we be afforded the opportunity to have travel expenses sponsored by our respective departments?
6. Any last suggestions regarding our goals/next steps?