Notebook Week 1 (May 31-June 2)

Project: iGEM 2017
Authors: Locke Bonomo, Haylea Northcott, Mike Savoie, Edith Sawyer, Aylin Padir, Catherine Sherman
Dates: 2017-05-31 to 2017-06-02

WEDNESDAY, 5/31

Based on Discussion with Farny:
Researched old iGem projects invovling the detection of heavy-metals
https://benchling.com/s/etr-7CTFaqeHaW6aZdGxBGpl

- What were their experimental designs? (sensors? outputs?)
- Were they successful? (How far was the team able to go? Were the results reproducible?)

Idea for design

- constitutive promoter for ArsR (for us this will be PbR); ArsR bound to promoter (pArs) that governs the expression of GFP, for example; when Ars is present, it will bing to ArsR, causing it to unbind pArs, allowing GFP expression; GFP expression dependent on the amount of Ars present
- Could the sensor be pH dependent?
  https://benchling.com/s/etr-fXbHILalfI8kryPNiwIR
- Use of chromoproteins?
  https://benchling.com/s/etr-0nyXdACW0NAysRdViz8h
  - E. Chromi iGEM team (colored E. Choli): http://2009.igem.org/Team:Cambridge
    - low Ars —> red (maybe this is the color you have just to know that the test is working)
    - moderate Ars —> yellow (in combo with red this will turn orange)
    - high Ars —> blue (in combo with red, yellow, this will turn brown)
    - danger Ars —> purple (dominant= black)
  - Maybe start light so as Ars increases, shade darkens? (helps with color blind people)
  - Build final design with constitutive promoters to see the result of the color combinations (so that you can see low->med->high lead levels will change the color in the final design)
- Has anyone dealt with mutations to Ars promoters that deals with different sensitivities to Ars?
  - Promoter: error prone PCR to make modifications to yield different selectivities to lead (intermediate step to identify promotors that will fulfill the requirements for our final design)
- Start in E. Coli, but goal is to have it in a completely safe organism (probiotic with metal binding properties)
  - How do we grow probiotics in the lab? (label with genus and strain, then literature search on how they grow)
  - Initial testing: on their own, how much lead can they bind? how much causes them to die?
    - Harvard bacterial plate with increasing antibiotic concentration experiment instead with increasing amounts of lead (Kishony)
      - Has a paper been published?
- Lead quantitative test kit
  - Something that will work with a plate reader
- Touch Tomorrow activities

pH Research:
How to change pH using methods other than H+ pump/Things that are responsive to pH:

- Gram-positive bacteria possess a myriad of acid resistance systems that can help them to overcome the challenge posed by different acidic environments. In this review the most common mechanisms are described: i.e., the use of proton pumps, the protection or repair of macromolecules, cell membrane changes, production of alkali, induction of pathways by transcriptional regulators, alteration of metabolism, and the role of cell density and cell signaling.
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC193868/figure/f1/?report=objectonly

Nagahama (2014)
Project description:
Engineered *E. Coli* to bind to cadmium in water. Also developed *E. Coli* that have a positive chemotaxis to *E. Coli* that have bound to the cadmium "gathering them up".
Circuit Info:

Utilized the ASpA circuit modified from several parts BBa_K896008(promoter),(RBS),BBa_C0083(Protein coding sequences),(Double terminator). BBa_K896008(zinTp).

- Did not finish whole experiementbut were able to establish a chemotaxis relationship between bacteria.

NEFU China (2014)

Project description:

Engineered *E. Coli as* Nanocrystal *E. Coli* Flocculation Units. Used the bacteria in three functions. First function is to detect for presence of Cadmium ions. The second system is a recycling system that synthesises crystals. The last system is a floculating system to increase the ability of capturing bacteria to remove secondary pollution.

Circuit info:

Utilized the smtB-OP-smtA device (a resistance system against many metals). Fucntions as a repressor in absence of metal contamination. Presence of metal converts sub-optimal promoter to a potent promoter. Also encouded a pigment encouding gene. It was used a reporter gene. Exchanged biobrick part SmtA with smtB-OP-smtaA.

- The project was successful.

Penn (2014)

Project descrition:

Used AMB-1 bacteria as a system of detecting cadmium in polluted water sources.

Circuit info:

Used the PYMB essentials biobrick master protocol. AMB-1 plasmid. They had great difficulty in transforming the plasmid into the bacteria.

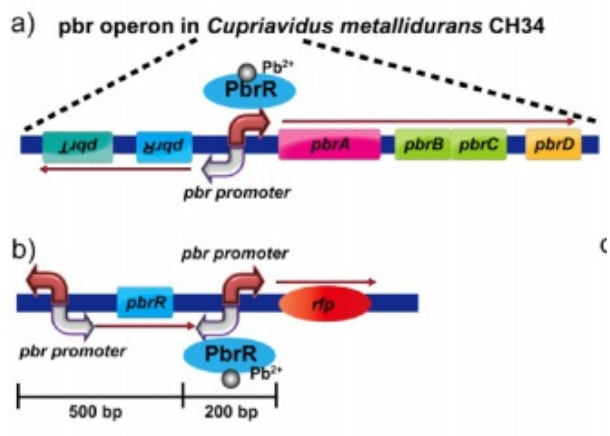
- This project was very confusing and the magnetic sensing did not seem to be very aligned with our project goals.

Old iGEM team

- Plasmid used was pSB1A3 (Wei et al, 2014)
- Used OmpA for external lead recognition (available from bio bricks database) (N terminus of OmpA was added before Pba gene)
- *E. Coli*
- Constitutive operator Pbla

Circuit used by Wei et al:

image.png



- 6 genes (pbrA-D and pbrR and T) pbrR and pbrT are regulatory and before promoter.
- pbrT responsible for lead transport. pbrR possibly inhibitory.
- Operator is pbr, sensitive to lead
- Focused on human stomach proton pumps (kdpA, B, C, F) (https://biocyc.org/ecoli/new-image?object=ATPASE-1-CPLX)

THURSDAY, 6/1

Research on plasmids, assays, colormetric tests, probiotics, and retrieved specimens for probiotic research from local Walmart

- Probiotic with high lead-binding capability?
  https://benchling.com/s/etr-6n1I7EyJRNtLmNz1Pr6P

zam6397.pdf

- Lactobacillus growth media and transformation:
  - MRS agar info
  - List of transformation protocols

FRIDAY, 6/2

Made Fluorescence Friday plates
Haylea & Mike - Made touch tomorrow poster with iGEM info
Edith & Aylin - research for lead map across USA
https://benchling.com/s/etr-SIjsYXjpE3nrrvZANIGu
Cat & Locke- Research non-pathogenic strains for biosensor -> picked bacillus subtilis
Ordered broth for MRS agar (probiotic agar)
Ordered lead nitrate, L-glutathione, & gold nanoparticles (lead assay)

http://aem.asm.org/content/78/18/6397.full

- Bacteria have net neg charge, many metals have cationic/pos charge. Theory: nucleation sites on cell surface have ability to bind pos-charged metals. Once bound, many more metals could bind and precipitate on cell wall.
  - theory supported by changing pH -> alter cell surface charge -> reduced metal-binding ability (Fein et al.)
- Diff optimum pH for metal-binding of diff bacteria
- These unique microbes have the ability to cope with metals through a variety of mechanisms - precipitation of metal particles and active efflux.
- Can bind metals intra- or extracellularly
- Resistance mechanisms are often plasmid encoded, but in some instances, the genes are found on the chromosome, suggesting an important evolutionary pressure to keep these genes; examples include mercury (Hg^{2+}) resistance in Bacillus, cadmium (Cd^{2+}) efflux in Bacillus, and arsenic efflux in *E. coli* (**16**, **96**).
- gut microbiota has key roles in regulating digestion by providing enzymes required for metabolic breakdown by processing and metabolizing compounds as they enter the host through normal diet (**61**, **93**). It is therefore likely that microbes are presented with metals in water and food and may play a role in protecting the host from their adsorption.
- One of the more accepted and newer models of bioavailability is the simulator of the human intestinal microbial ecosystem (SHIME), an *in vitro* GI model that is unique because it incorporates the activity of the human GI microbiota (**21**, **115**). No other tests or standard models take into account the effect of the human GI microbiota in altering the bioavailability of metals.
  - We could try to get access to the SHIME to see how effective probiotic is predicted to be, in context of the gut microbiota.
- Only 40 to 60% of ingested metals are absorbed across the intestinal barrier into the body, but there is a variance in bioaccessibility that is unique for each metal and depends on the route of entry, the foodstuff consumed, and the type of host microbiota
- Three main mechanisms for the binding of metals to bacterial cell walls are known: (i) ion exchange reactions with peptidoglycan and teichoic acid, (ii) precipitation through nucleation reactions, and (iii) complexation with nitrogen and oxygen ligands (**10**, **11**, **67**). Gram-positive bacteria, particularly ***Bacillus* spp., have high adsorptive capacity** due to high peptidoglycan and teichoic acid content in their cell walls.
- **Lactobacillus**:
  - Can be used for reducing metal toxicity bc they:
    - have resistance mechanisms that effectively protect their cells
    - can bind and sequester heavy metals to their cell surfaces, and remove them thru subsequent defecation
  - For lactobacilli in the gut microbiome, they possess heavy-metal and antibiotic resistance genes on their plasmid
    - can we find out what these genes are and their sequences?
  - reduce oxidative stressess caused by metal toxiticity in vitro

    - The ability of lactobacilli to bind and sequester metals depends on the strain's resistance mechanisms.
        - mer and ars operons cause efflux of mercury/arsenic from cell cytosol, but isn't ideal for detox of GI tract bc results in cycling of metals.
        - Possibly the ideal species for detoxification are those which lack the genes encoding metal transporters and thus only bind and sequester heavy metals.
    - Arsenic: neg charge, unlike most metals (lead is pos). Although anionic carboxylic and phosphate groups are the most-abundant ionic groups and give lactobacilli their net negative charge, peptidoglycan layer and surface proteins, such as S-layer proteins, are known to contain positively charged groups. *Lactobacillus acidophilus* strains and *Lactobacillus crispatus* DSM20584 are known to produce S-layer proteins, which may explain their activity against arsenic (**91**).
        - We could engineer the cells to produce more of neg-charge proteins/groups on their cell surface to more effictively bind/sequester lead
- studies on lead and cadmium are often conducted together, as the elements seem to react with bacterial species in similar ways.
    - maybe we should do cadmium as well as lead?
    - Halttunen et al. (**39**) showed that *Lactobacillus* and *Bifidobacterium* species can bind lead and cadmium in solution. They observed a rapid binding phenomenon across all studied species, with the largest amounts of both lead and cadmium bound within 5 min to 1 h (**39**, **106**). Most importantly, the metal remained strongly sequestered by the cell and did not disassociate, even 48 h after testing.