Notebook Week 10 (July 31-August 4)

Project: iGEM 2017
Authors: Locke Bonomo
Dates: 2017-07-31 to 2017-08-04

MONDAY, 7/31

- Team Meeting @ 10am
- Autoclave flasks for growth curves Tuesday
- Make overnight cultures for Growth Curves
    - 2 Lactobacillus
    - 1 B.subtilis
- Lead Assay:

LB Outline:
For LB, there have been many trials, but a lot of variation in results. There is a very clear difference between the control and 1000ppb, but there is a problem with specificity. In trying to solve this, we have adjusted the pH of the phosphate buffer, vortexed the samples directly before putting them in the plate, made new samples each day, made new GSH each day, and considered if temperature of the gold particles could effect efficiency. We found that the phosphate buffer pH is optimized for LB, vortexing the samples right before adding them doesn't effect the assay, and that GSH needs to be made fresh each day. Making the samples fresh each day ensures that they are not contaminated, and the absorbance will not differ because of outside sources. In addition, all of the math for the dilutions was double checked, and different combinations of concentrations of gold, GSH, and sample were tried.

Water Plan:
Water was tested with the updated protocol, and was found that there is a very stark difference between the control and 1000ppb. The greatest difference was shown at 10 minutes, but there was a steady difference throughout the experiment. The data looked like it did in the papers about the lead assay which is definitely an important start. The next steps for this would be to see if we can replicate all of the results from the paper to see if we have a low specificity. The first steps would be to try a wider variety of lower concentrations of lead, and eventually develop a standard curve. After this, the samples that were collected for various areas could be tested. Another thing to consider is how else this can be applied to our project.

- Human Practices update

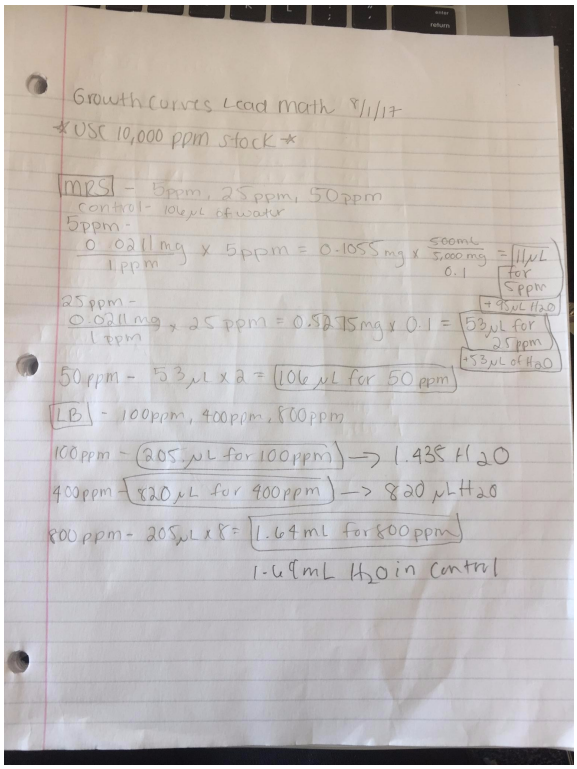
Human Practices.docx

- From iGEM website: "How to conduct a valid and legitimate survey"
    - https://www.nsf.gov/news/special_reports/survey/index.jsp?id=overview
    - https://academic.oup.com/intqhc/article/15/3/261/1856193/Good-practice-in-the-conduct-and-reporting-of
    - https://oqi.wisc.edu/resourcelibrary/uploads/resources/Survey_Guide.pdf
    - http://www.aapor.org/Standards-Ethics/Best-Practices.aspx#best10
- Water Reports to check out:
    - Link to EWG Water Resources
    - Tap Water Resource
    - Problems with Bottled Water
- Idea: For Community Outreach/Human Practices Page on the Wiki, Interactive map of where we reached out to, different markers for if a location was used/integrated into project. This would make it easy to see and ideally pop up with a link to where the information would be found. The map would need to include the northeast (at least, but we also had one outreach point in Colorado, but that could stand alone)

TUESDAY, 8/1

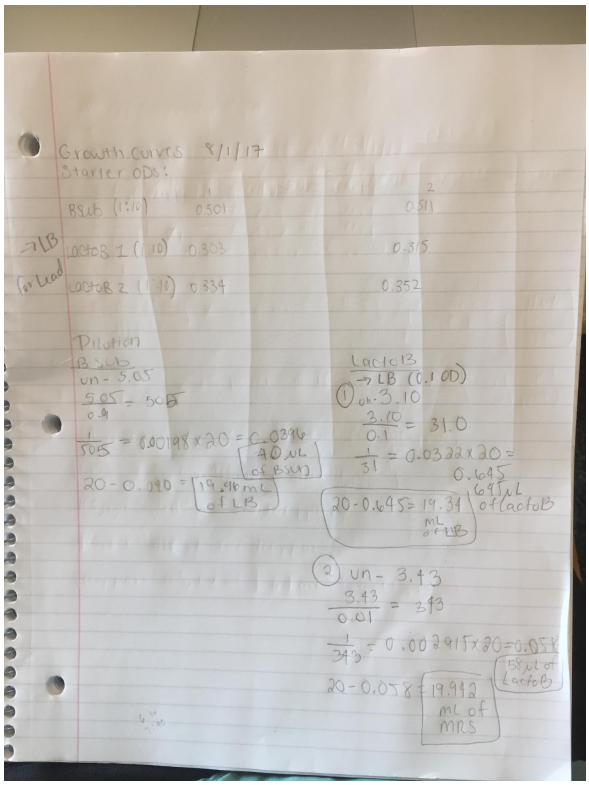
- Do growth curves of B.subtilis and Lactobacillus - 20mL cultures in flasks
    - Dilute back to a starting OD of 0.01 then add lead during lag phase
    - Continue Wednesday AM for overnight culture OD and continue for a few hours in the AM to see either a plateau or decrease
        - lead from 10,000 ppm stock
        - Math for concentrations below

IMG_3545.JPG



    - B.subtilis
        - control, 100ppm, 400 ppm, 800 ppm
        - Parent culture starter OD - 0.505 (1:10)
    - Lactobacillus
        - control, 5ppm, 25 ppm, 50ppm
        - Parent culture starter OD- 0.343 (1:10)
- Growth curves of lactobacillus in LB
    - Grow Lactobacillus overnight in MRS
    - Dilute to OD of 0.1 and then put in LB
    - Starter OD- 0.310 (1:10)
- Math for dilutions to obtain both a 0.01, and 0.1

IMG_3544.JPG



Final growth curve data & analysis- Plan to redo both growth curves with starter OD at 0.01 again next week

StarterOD0.01GrowthCurves.xlsx

LactobacillusinLB.xlsx

- Do Gibson Assembly again of pBr insert into RFP plasmid backbone
- Lead Assay:
    - Try one LB Assay with lower concentrations (50, 100, 250, 500, 1000ppb), see if there is a difference

LB Trial 1 with new cold AuNPs.xlsx

LB Trial 2 with new cold AuNPs.xlsx

    - Start standard curve with water, need to decide on exact concentrations, look at protocol to try to replicate results (results from LB assay look promising, so we are putting this off until we are done with LB)
- Performed miniprep for pet21a and pet42a and gel purified samples
    - Yield was too low to use for Gibson assembly (Pet21a: 9.2 ng/uL and Pet42a: 8.3 ng/uL)

gelforPet21aand 42a.jpg

WEDNESDAY, 8/2

- Remaining ODs for growth curves from Tuesday, 8/1

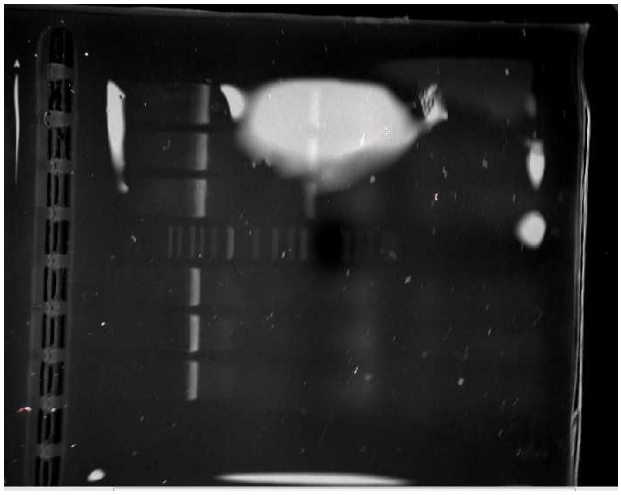
THURSDAY, 8/3

- Team meeting at 10 am: discussion of where we are at in the project so far with respect to the lead assay, the probiotic, and the biosensor. Also presented on the progress of our individual roles (human practices, community outreach, collaborations, social media, graphic design, and wiki)
    - Next steps: lead assay- meet with Professor Matthews for help with data analysis; after completing 2 more trials of LB standard, work on standard with H2O
- Gibson Assembly (transformation of competent cells and plate)
    - Did not work

FRIDAY, 8/4

- pet21a and pet42a mini-preps, vector digest, gel purification

vectorprep.jpg



The bands appear to be of the correct size for both vectors. pET21a is the three lanes on the bottom of the gel; pET42a is the top 3 lanes, with a 2-log ladder separating them. The fuzziness below the pET21a lanes is the smaller restriction fragment, which was <100bp so was not able to be resolved by our 1% gel. The small restriction fragment of the pET42a is much larger and can be seen as the lower bands. The top bands were cut out and separately melted, but purified and eluted together in 25ul of NE into one tube each for pET21a and pET42a.

Restriction Digest and Gel Purification Data 8.4.17

	A	Tube #	pET21a	pET42a
1	Weight of each gel fragment (g)	1	0.725	0.787
2		2	0.860	0.788
3		3	0.594	0.581
4	NTI buffer added to each tube (ul)	1	1450	1570
5		2	1720	1570
6		3	1190	1160
7	Final yield (ng/ul)	-	27.4	31.3

The yield is somewhat low, but is better than our previous miniprep (~10 ng/ul). We will be able to do Gibson assembly with these products.

- Lead Assay (everyone)
    - The lead assay will include 1-2 trials of LB, to try to obtain a standard curve. The curve might be logarithmic as opposed to linear. The new AuNPs should be used, and kept cold in the fridge. The samples should be vortexed before putting each one in the plate.
    - LB Trial 3 with cold new AuNPs

LB Trial 3 with cold new AuNPs.xlsx

    - LB Trial 4 with cold new AuNPs and tested "unknowns" (unknown 1 200 ppb; unknown 2 2000 ppb)

LB standard plus unknowns.xlsx

    - The standard curve may need to be done with each sample because there is so much variability between trials
- Cleanup of aeBlue and amilCP PCR products (PCR done a couple weeks ago)
    - ~30 ul left of each, so added 60 ul NTI. Eluted in 25 ul NE.
    - Concentrations:
        - aeBlue: 128.3+ ng/ul
            - checked twice, first time was even higher (~243 ng/ul)
        - amilCP: 35.4 ng/ul