Notebook Week 7 (July 10-14)

Project: iGEM 2017
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Dates: 2017-07-10 to 2017-07-14

MONDAY, 7/10

Plan:

- Team Meeting at 10-12
  Go through updated presentation and each chair present what is done and what needs to be done later

Meeting Notes with Farny 71017.docx

To do based on meeting:

- Update lead assay protocol with specifics
  - Send update to BU, ask if they have the same type of plate reader
  - Figure out date to go
- Upload programing for linking Instagram and Twitter (trying to also to link to Facebook page)
- Take boomerang of gold nanoparticles
- Make Jamboree banner (look at old ones, get input for ideas, find dimensions)
- Make “proof of principle” (pbrR with GFP) & hopeful final design (pbrR with chromoproteins)
- Look into coming up with a google form to have other teams indicate whether their country has lead contaminated water
  - Collaboration with Germany inspired us to do this
- Research the multiple ways that we can implement our project
  - Prophylactic: talk to government in Flint (especially water testing companies)
  - Cost benefit anlaysis will depend on how the project is being implemented
  - Tablet idea (just add water to detect lead)
- Continue working on Case Study
- Research lead detection take home kits that are currently available (to complement Cat's lead detection company outreach)
- Make PDFs for each week titled “supplemental material for week X” that contains all the attachments (word docs, excel files, etc.) for that week
- Make lead stock (1000ppm)
- Run sequence alignment for Pbr gene and pbrR that Brown used

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- Restreak B.subtilis Plates
- Check electroporated cells w/ IPTG
- Cat- finish making the MRS dilutions & label MRS container for lead assay
  - Completed
- Call remaining water companies in Mass, talk about if ones from other states should be contacted
  - Con-Test Analytical
  - Envirotech
  - Call Geo Labs back
  - Northeast
  - SCILAB
  - Mass. Department of Public Health, new number?
- Got individual colonies from serial dilutions of Lactobacillus on MRS Agar!

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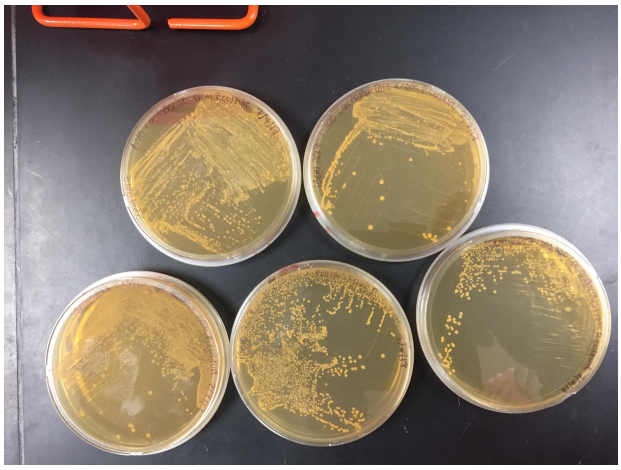

- Analyzed Lead Growth Curve Optimizing Data from (7/7/17)

LeadGCOptimizing.xlsx

- Research about salt water for Germany
    - https://water.usgs.gov/edu/drinkseawater.html -desalination in the US is important in Florida and California
    - http://www.pnas.org/content/102/38/13517.long
    - https://www.theguardian.com/sustainable-business/2014/aug/21/geological-survey-salinity-pure-water-shortage-chemical-runoff-agribusiness-watershed
- Make parent overnight cultures for Lead Growth Curves tomorrow in 20 mL cultures in flasks
    - Picked 3 colonies from each plate and put in 20 mL of media of choice (LB & MRS)
    - Place flasks in shaking incubator at 250 rpm at 37 degrees
    - Plan for tomorrows growth curves:
        - Adding lead at lag phase altering altering by dilution the OD to 0.1 in the am due to best results from trial performed on 7/7/17
        - Lactobacillus -> 3 subcultures from parent culture with starting OD of 0.1
            - Control - 0 ppm
            - 50 ppm
            - 100 ppm
            - Redoing this again because we are no longer using the Culturelle pill in our parent culture since we got single colonies when plating
        - B.subtilis -> 3 subcultures from parent culture with starting OD of 0.1
            - Control- 0 ppm
            - 100 ppm
            - 200 ppm
    - Made new lead stock of 1,000 pp. & How much lead from that stock to add to obtain ppm desired..

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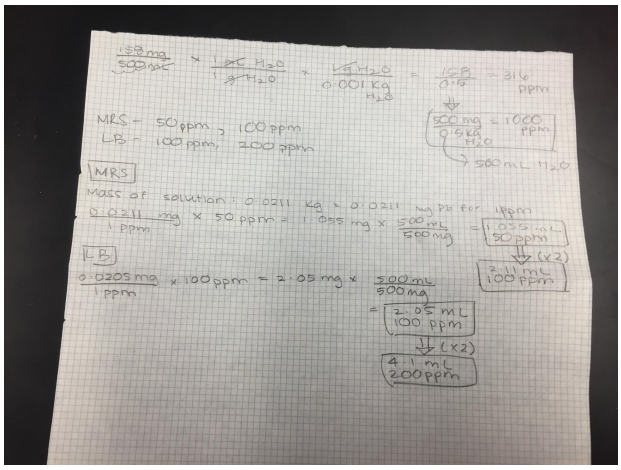
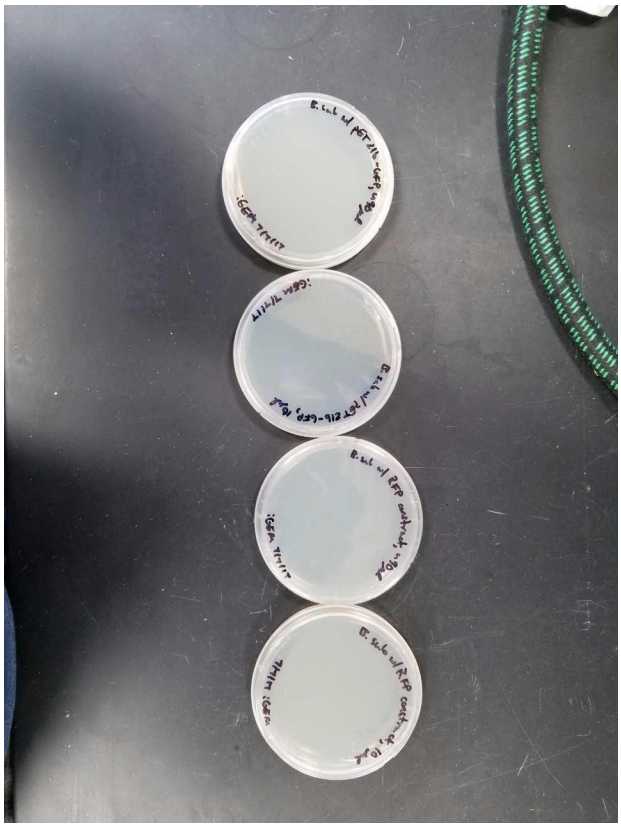


Must make all volumes consistent within each single bacteria experiment so will add DI Water to control and DI water to the smaller ppm to reach the larger volume of the largest ppm.

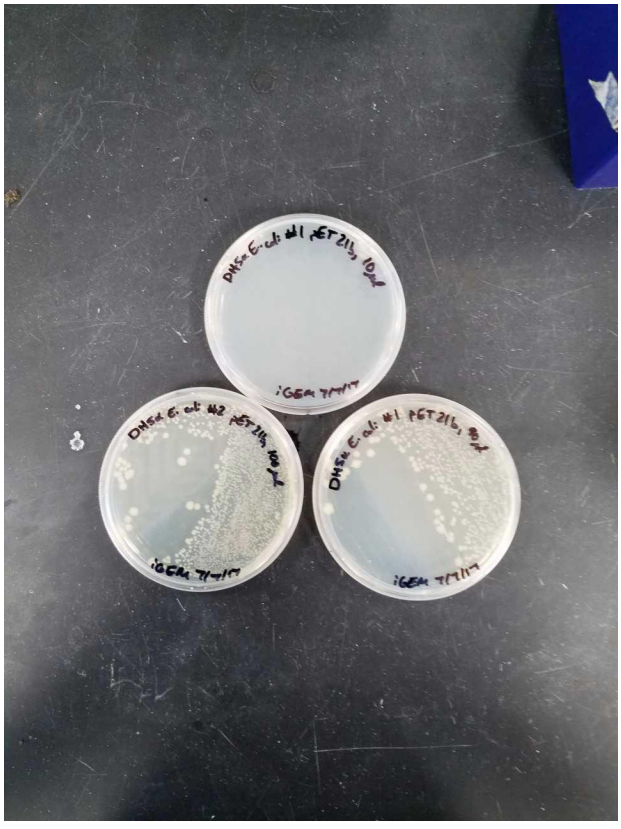
- Lactobacillus
  - Control- Add 2.11 mL of DI h20
  - 50ppm- Add 1.055 mL lead 1000 ppb stock and 1.055 mL DI h20
  - 100 ppm- Add 2.11 mL of lead 1000 ppb stock
- B.subtilis
  - control- Add 4.1 mL of DI h2O
  - 100 ppm- Add 2.05 mL of DI h2O and 2.05 mL lead 1000 ppb stock
  - 200 ppm- Add 4.1 mL lead 1000 ppb stock

- Make MRS plates with lead concentrations & make more LB Broth
- Redo PCR of gene 3 = efored, following Chromoprotein PCR
  - Mike completed - PCR reaction was completed, and 1% gels were set for tomorrow to do purification and cleanup
- B. Subtilis did not grow on selective plates from electroporation experiment. E. Coli grew on two of the selective plates from electroporation. Competent cells will most likely have to be remade for further testing.

plates1.jpg



plates2.jpg



- Vector prep of pET21a and pET42b - digest and gel purification
    - Mike completed
- Brown 2007 Team to look at: http://2007.igem.org/wiki/index.php/Lead

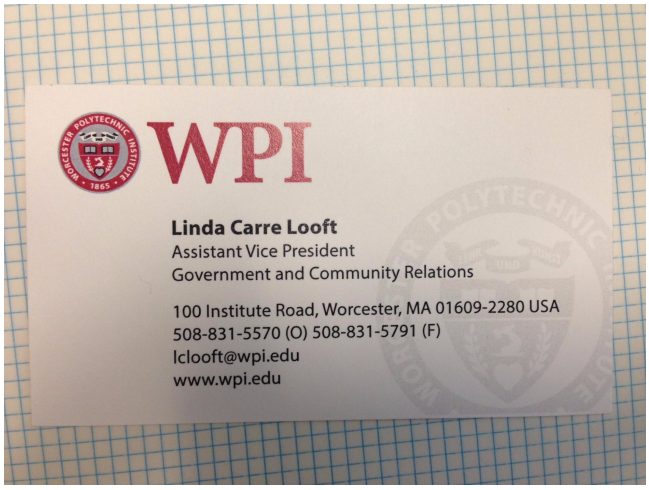
- Peking 2010 Team to look at: http://2010.igem.org/Team:Peking/Project

TUESDAY, 7/11

Plan:

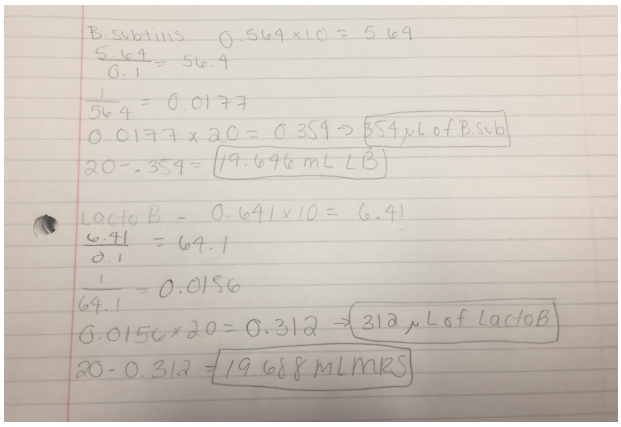
- Meeting with Linda Looft @ 9am
  - Cat & Aylin meet at 8am to brainstorm list of questions about how to engage the WPI community in our project and come up with a brief elevator pitch for our project (see "Questions for Linda Looft" document in Community Outreach folder)
  - Google form asking community for input about where lead contamination is present?
  - See Questions for Linda Looft page in Community Outreach folder for the notes from the meeting

linda looft contact.jpeg



- Email Timothy from water company
  - Completed, emailed back asking about meeting
- Lead Growth Curve Optimization Trial 2
  - Grew really well overnight in flasks shaking at 250 rpm at 37 degrees
  - Overnight OD
    - Lactobacillus- 0.641 (1:10) = 6.42
    - B.subtilis- 0.564 (1:10) = 5.64
  - Dilutions made to get to an OD of 0.1

FullSizeRender.jpg



  - Experiment will last 10 hours 6:45am - 3:45 pm

Lead Optimization Analysis -

LeadGCOptimizationTrial2.xlsx

    - Looking at the growth curves you can see that B.subtilis is not affected by neither 100ppm or 200 ppm lead. So the next steps would be to increase the lead concentration. At some points in the graph you can see that the control even grew less than the 2 cultures with lead but eventually caught up to the same OD and started to grow better within two hours.
    - For lactobacillus, we were expecting to see an affect on both 50 ppm and 100ppm throughout the 10 hours of measurements the two with lead always lagged behind the control culture but there wasn't a difference until about the last 3 hours and you can see a large difference overnight. So the next steps with lactobacillus would be to continue the growth curves after taking the overnight OD, so that we can get the largest time span possible. For example, growth of an overnight culture tonight and dilute back in the AM to an OD of 0.1 and take the ODs from 8-5 and then leave overnight and continue taking the OD Friday morning with the same lead concentrations to see if the larger difference stays between the three that is seen in the growth curve above in Excel file.
- Lead assay: LB row by row trials 1 and 2
    - WITHOUT the extra 2/4 ul (respectively) of control media
    - LB appeared to have contamination, but it was tried anyway (because when completing actual assay tests there will be bacteria present). The points were very scattered. A second lead assay was done with the same LB lead dilutions, but they were spun down in the centrifuge first. This made them appear to be all the same color and have the same clarity. These trails are being discounted because the bacteria present may have absorbed an unknown concentration of lead, altering our expected values and skewing data. The second trial will be redone tomorrow.

LB (2).xlsx

- Email BU (plate reader, tenative date for visit: 7/26)
    - Completed, still need to send updated protocol
- Update protocol ***
        - Look at L-glutathione stock to see whether the instructions for making a solution are already listed
        - Clarify how to make the phosphate buffer (i.e. where did the original protocol come from?)
        - Is making an NaCl solution considered "common knowledge"
        - GSH solution: preliminary step of lead assay (needs to be made every time the lead assay is run)
        - Remove lead spiked water stock (idea is that we are checking to see whether there is lead present in the water)
        - Make protocol for how to make media containing various concentrations of lead
            - Started
        - Well preparation and plate reading needs to be updated to fit the "row by row format"
- Call remaining water companies in Mass, talk about if ones from other states should be contacted
    - Con-Test Analytical
    - Envirotech
    - Call Geo Labs back
    - Northeast
    - SCILAB
    - Mass. Department of Public Health, new number?
- Research for Germany
    - Completed, see Germany Information page in Community outreach folder
- Emailed Team Aachen to schedule a second Skype call
- Flint Response Notes:
    - Fact Sheet issues by White House 2016
    - NPR Flint Timeline 2012-2016
    - Flint Water Action Website
    - Governor's Goals to Strengthen Flint 75 Point Plan
    - Flint News, Cost Break Downs
    - Filter Specifics
    - EPA Response to Flint

In down time, refer to the To-Do list based off of meeting from 7/10.

- PCR gel analysis of chromoprotein efoRed
    - Results are inconclusive as band did not appear on gel of correct size.

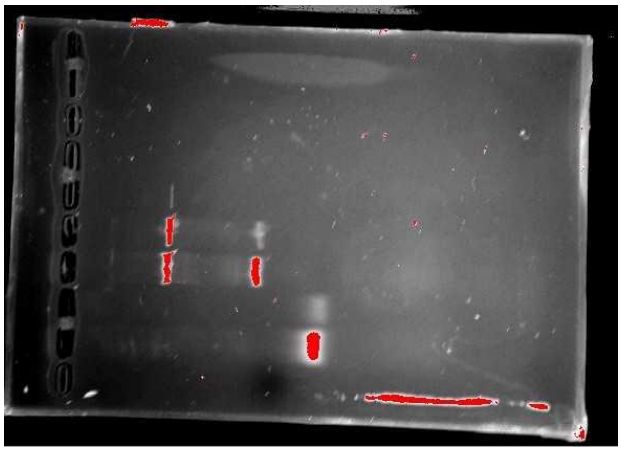
gel for notebook.jpg

- Performed gel purification and cleanup of vectors Pet21a (17.6 ng/μL) and Pet42a (18.6 ng/μL)
    - An undiluted A3 buffer was accidentally used for a wash step, resulting in the DNA being possibly less clean than it should be, further testing is necessary
- PCR purification of chromoprotein PCR samples were completed. Concentration are shown below:

PCR Cleanup Results

	Sample number	Chromoprotein	DNA Concentration in ng/ L
1	Sample 1	fwYellow	77.3
2	Sample 2	aeBlue	72.7
3	Sample 4	amilGFP	18
4	Sample 5	amilCP	51.5
5	Sample 6	tsPurple	25.1

gelgelgelgelgel.jpg



WEDNESDAY, 7/12

- TEAM MEETING!
  - Completed
- Make Overnight Culture of Lactobacillus and B.subtilis for Growth Curves
  - Plan for tomorrow:
    - Parent culture from B.subtilis and Lactobacillus dilute back to OD of 0.1
    - Shaking in flask covered in tinfoil at 250 rpm
      - Lactobacillus
        - control, 50ppm, 100ppm, 500ppm
      - B.subtilis
        - control, 300 ppm, 400 ppm, 500 ppm
- Make lead stock solution of 10,000 ppm
  - Math for stock solution below along with amount needed to make 20mL cultures desired ppm

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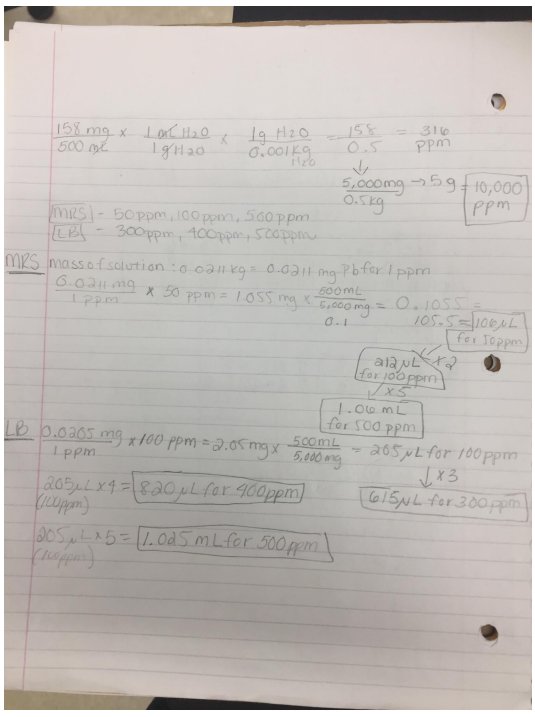


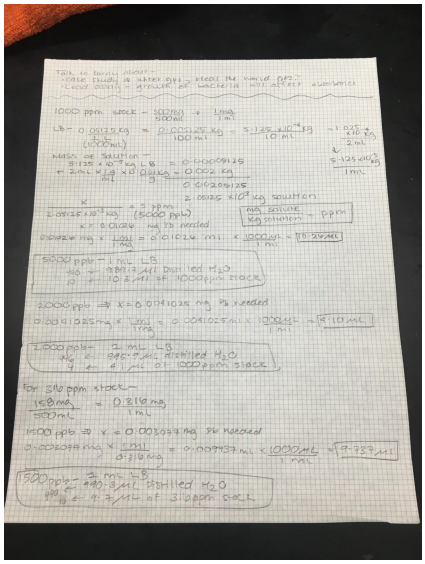
- Finish Lead Assay Protocol
  - Step --> Action; below contains "recipes"

- Include procedure on how to make the standard curve

DIY Lead Assay Updated 7:12:17.pdf

- Lead Assay LB Trail 2 (maybe Trial 3, depending on how many gold particles are left)
  - New dilutions of LB made
  - Only Trial 2 was completed, but more gold particles were ordered

IMG_2026.JPG



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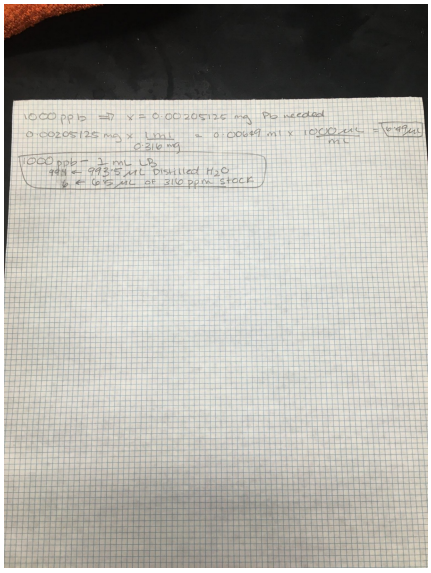


LB Trial 2c.xlsx

- Sequence alignment review
  - For gene: try translating and seeing if the proteins align
    - Alignments appear to be different
- Make progress on Google Form
  - Broad: Where are you from? Does your hometown have a problem with lead contamination in drinking water?
  - Ask other iGEM teams to distribute to students at their universities
- Call remaining water companies in Mass, talk about if ones from other states should be contacted

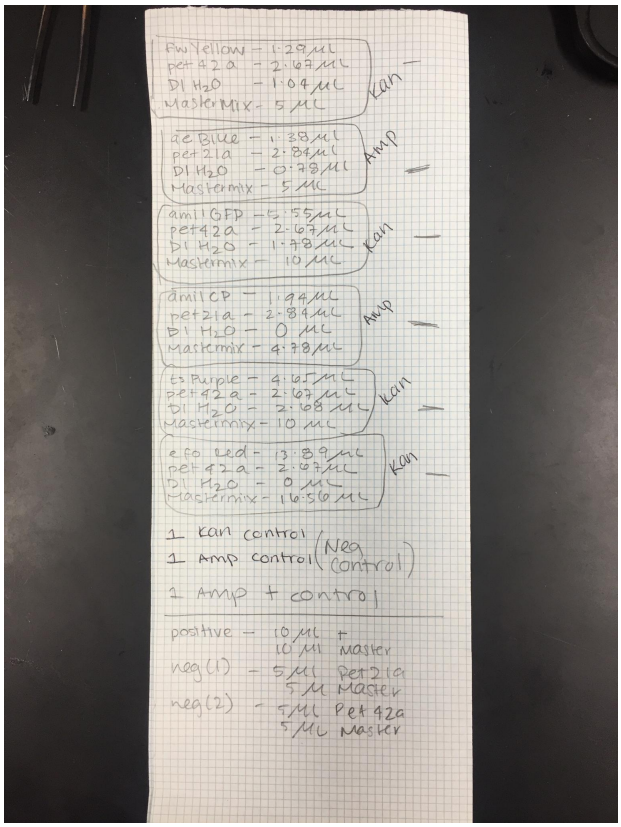
    - Con-Test Analytical
    - Envirotech
    - Call Geo Labs back
    - Northeast
    - SCILAB
    - Mass. Department of Public Health, new number?
- Research at home lead test kits
- Research cost benefit analysis math/format
    - Would need to identify Costs and Benefits, include time cost/gained
    - Subtract costs from benefits
- Respond to Frank from Mass DEP
    - Go through word docs from search page to see whether there are any further contacts

followup-fs.doc

- Research the multiple ways that we can implement our project
    - Prophylactic: talk to government in Flint (especially water testing companies)
    - Cost benefit anlaysis will depend on how the project is being implemented
    - Tablet idea (just add water to detect lead)
- Linda Looft Review (how to interact with contacts, ect)

Gibson Assembly round 2 completed. Will check for colonies tomorrow.

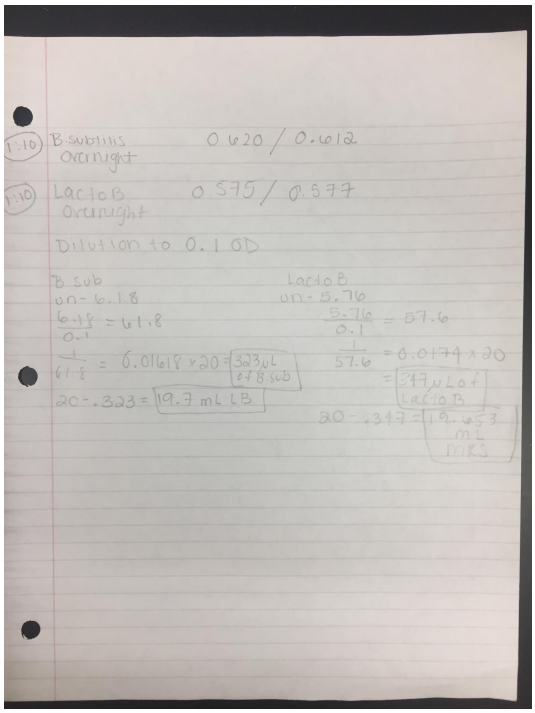
gibson.JPG



THURSDAY, 7/13

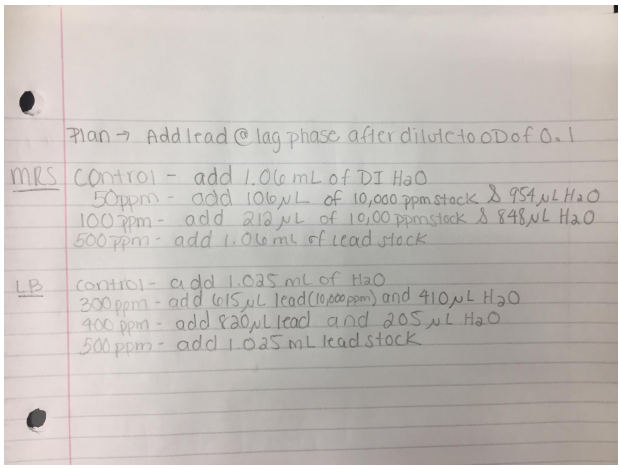
- Optimization of Growth Curves Trial 3
    - Overnight OD (two samples) & dilution math to get OD of 0.1 can be seen below in picture

IMG_3363.JPG



- Added lead as soon as diluted the OD back to 0.1
- Amount of lead and water put in each culture can be seen below in picture

IMG_3362.JPG



- Make lead MRS and LB plates for testing growth of Lactobacillus and B.subtilis
- Meeting with PBS at 1:30
- Blast Lead Sequences (BioBricks and Australia)

Australia pbrR Promotor Blast Result.pdf

Australia pbrR Blast Result.pdf

BBa_I721001 Promotor.pdf

BBa_I721002 Lead binding protein part 3 Blast Result.pdf

BBa_I721003 Lead binding and promoter part 8 version B Blast Result.pdf

BBa_I721004 Lead binding protein and promoter part 11 version a Blast Result.pdf

- Call remaining water companies in Mass, talk about if ones from other states should be contacted
    - Con-Test Analytical
    - Envirotech
    - Call Geo Labs back
    - Northeast
    - SCILAB
    - Mass. Department of Public Health, new number? 617-983-6201
- Make progress on Google Form
    - Broad: Where are you from? Does your hometown have a problem with lead contamination in drinking water?
    - Ask other iGEM teams to distribute to students at their universities
- Gibson Assembly was conducted for plasmids Pet21a and Pet21b using PCR'd chromoprotein inserts and original efoRed insert
- GPS Teachers:
    - The World's Water: Elisabeth Anne Stoddard (P), Derren Rosbach
    - Heal the World: Jill Rulfs (P), Helen G. Vassallo
- Lead Assay: LB Trial 3
    - Trial 3 results are below. We believe that the data from these results is off because we used left over GSH instead of fresh GSH which we have never done before. Therefore, the assay will be repeated for accuracy purposes.

LB Trial 3.xlsx

    - Trial "3a" results are below, as is the standard curve that uses data from the three trials (see 1:30 time point).

LB Trial 3a.xlsx

LB Standard Curve.xlsx

- Info for Case Study
    - CDC Lead Home Page
    - CDC National Surveillance Data- State
    - CDC National Surveillance Data- County
    - CDC Fact Sheet for Parents

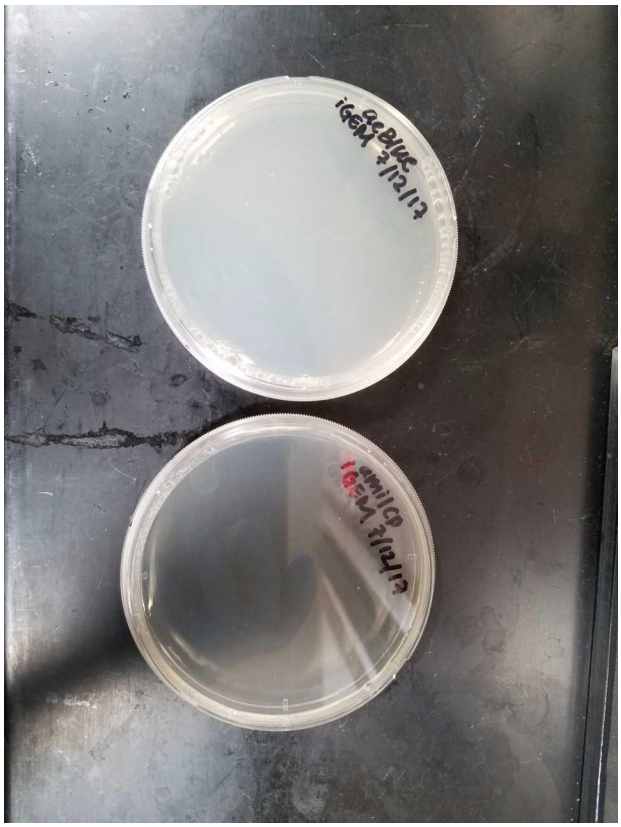
FRIDAY, 7/14

- Water Company Trip (Cat, Aylin) -Meet there @ 9:15
- Plates from Gibson Assembly were checked. Controls matched expected. with new positive control plates Thursday yielding 73 colonies. All experimental plates yielded no colonies except for the efoRed plate, which yielded 5 colonies.
- Continued Growth Curve
    - Final Analysis

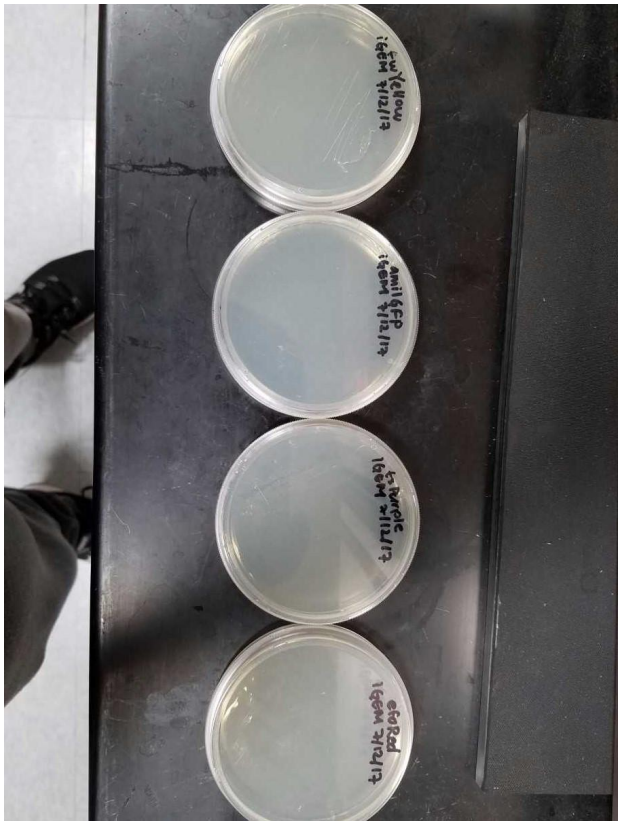
OptimizationLeadGCTrial3.xlsx

Gibson plates1.jpg

Gibson plates2.jpg



Gibson plates3.jpg



Gibson plates4.jpg