# Notebook Week 3 (June 12-16)

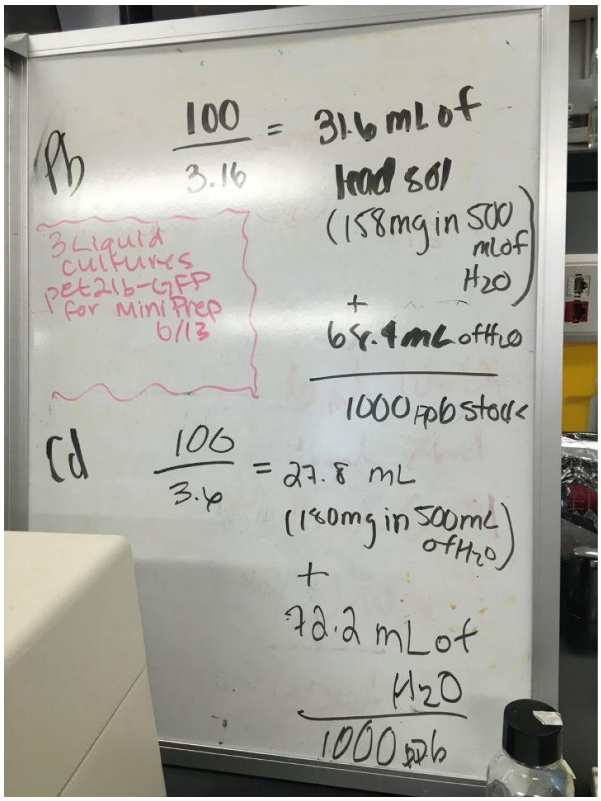
**Project:** iGEM 2017
**Authors:** Locke Bonomo, Haylea Northcott, Mike Savioe, Edith Sawyer, Aylin Padir, Catherine Sherman
**Dates:** 2017-06-12 to 2017-06-16

MONDAY, 6/12

Lead Assay
SDS Sheet: https://beta-static.fishersci.com/content/dam/fishersci/en_US/documents/programs/education/regulatory-documents/sds/chemicals/chemicals-l/S25383A.pdf
Followed DIY Lead Assay Protocol
https://benchling.com/s/prt-nazcaLrYltYKbAN8XSch

- Made Lead Spiked Water different than in protocol
    - hard to measure out mg on scale safely
    - For lead -> weighed out 158 mg of lead nitrate in 500 mL of water
    - For cadmium -> weighed out  180 mg of cadmium in 500 mL of water
- Make the dilution to get 1000 ppb of both Pb & Cd solution for stock

IMG_3088.JPG




31.6 of Pb solution + 68.4 mL of H20 = 1000 ppb of Pb stock
27.8 mL of Cd solution + 72.2 mL of H20 = 1000 ppb of Pb stock
Continued to follow DIY Lead Assay Protocol

Lactobacillus Prep

1. Aseptically transformed 50mL of MrS media into 50mL conical tube. Add contents of 1 capsule of lactobacillus. Vortex to mix.
2. Transfer 1 mL from 50 culture into three 15-mL tubes along with 9 mL of MRS media
3. Grow overnight
4. Check OD in the morning and dilute to desired starting OD, start growth curve experiment

Streaked Lactobacillus on 3 MRS plates, put 150µL of master mix on each of the prepared MRS plates. Let sit for ~25 min to dry, then placed in incubator

Updated flyer and pamphlet:

Lead Forum.docx

Lead Pollution in Our Drinking Water.docx

Mike & Edith- set up liquid cultures for interlab study trial 2 for overnight growth

Lactobacillus growth curves (exp't prep): OD of 0.05 - 0.10, replicates, check growth every 2 hours then 24 hours after overnight. Should streak on plates.

Prepare reagents to make chemo-competent B. subtilis. Transformants ready by Thurs-Fri hopefully.

## TUESDAY, 6/13

Edith & Aylin- Lead Assay Trials
Mike, Haylea, Locke- Lactobacillus Growth Curve, NEGEM PP presentation
Cat- Labeled tubes for interlab, Miniprep of pet21b-GFP, streaked plates with 4 colonies of each of the devices and the positive and negative controls for interlab study

Make more LB Broth and LB Agar for plates for the growth of Baccillus.

Lead Assay:
The lead assay was set up several times to test different variables and optimize the assay, especially in how to detect the smaller amounts of lead essential to the project.

- First trial: familiarization with multi-channel pipettors; plate set up as listed in DIY Lead Assay Protocol; GSH was added to the wells first, then AuNP were added, and finally the lead-spiked water was add; no observed change in absorbance over timer
    - Maybe the GSH & AuNP are reacting before the lead is added, try adding the reagents in a different order
- Second trial: plate set up as follows
    - D1: GSH --> AuNP --> H2O (E1, F1, G1 replicates)
    - D2: GSH --> AuNP --> Pb (1000 ppb) (E2, F2, G2 replicates)
    - D3: H2O --> AuNP --> H2O (E3, F3, G3 replicates)
    - D4: H2O --> GSH --> AuNP (E4, F4, G4 replicates)
    - D5: Pb --> GSH --> AuNP (E5, F5, G5 replicates)
    - D6: H2O --> H2O --> AuNP (E6, F6, G6 replicates)
    - D7: H2O --> AuNP --> GSH (E7, F7, G7 replicates)
    - D8: Pb --> AuNP --> GSH (E8, F8, G8 replicates)
    - D9: H2O --> AuNP --> H2O (E9, F9, G9 replicates)
- Best results occured when the lead and gold particles were added first, and then the GSH was added
    - Results:

61317-2.xlsx

Lactobacillus Growth Curve:
The OD was measured for each of the 3 replicates of liquid culture every hour from 8:30-5.
After the T7 (3:40PM) timepoint, we did a 1:10 dilution of C1 to double-check the OD and the dilution didn't reflect the undiluted OD, where the undiluted OD was 1.6 and the diluted OD was 0.334, reflecting a true OD of 3.34. We're concerned we have gone outside of the linear range of the instrument, so will be performing undiluted and diluted ODs for all measurements for tomorrow's replicates.

Lactobacillus rham. Growth Curve Experiment 1

| | A | Culture 1 | Culture 2 | Culture 3 |
|---|---|---|---|---|
| 1 | Starter cultures | 6.17 | 6.57 | 5.95 |
| 2 | T0 (8:30) | 0.1 | 0.1 | 0.1 |
| 3 | T1 (9:40) | 0.15 | 0.127 | 0.137 |
| 4 | T2 (10:50) | 0.325 | 0.283 | 0.312 |
| 5 | T3 (11:50) | 0.590 | 0.533 | 0.579 |
| 6 | T4 (12:50) | 0.901 | 0.857 | 0.913 |
| 7 | T5 (1:50) | 1.247 | 1.196 | 1.278 |
| 8 | T6 (2:45) | 1.504 | 1.475 | 1.528 |
| 9 | T7 (3:50) | 1.657 | 1.635 | 1.660 |
| 10 | T8 (4:50 PM) | 1.747 | 1.730 | 1.732 |
| 11 | T9 (8:20 AM) | 2.018 | 2.008 | 2.012 |
| 12 | T9, 1:10 dilution | 6.92 | 6.78 | 6.75 |

1. diluted starting cultures 1:10 w MRS after ~16-20 hours of incubation
    a. .617*10= 6.17
    b. .657 -> 6.57
    c. .595 -> 5.95
2. Pipetted the appropriate amount of culture to achieve an OD of 0.1 in 15 mL MRS of each culture, respectively labeled 1, 2, and 3
3. Took the OD every hour, using 1 mL of each culture and replacing the 1 mL after the OD was taken

Math for calculating dilution for culture 1 to achieve 0.1 OD600:
6.17/.1 = 61.7
In 1 mL of culture: 1/61.7 = 0.0162
For 15 mL total culture, use: .0162 * 15mL = 0.243 mL of culture
14.757 mL MRS

Next time: 250 mL flask (autoclaved) w/ 50 mL culture so don't have to replace culture from cuvettes.

Contains Lactobacillus First Growth Curve analyzed with moving average

Growth CurvesTrial1.xlsx

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Bacillus Subtilis:
Made liquid cultures (6) from three colonies on each of two plates. Allowed to grow overnight in shaking incubator.

The interlab study liquid cultures did not all grow correctly so new cultures will be set up to complete interlab study tomorrow 6/14/17. Four colonies from each device and the positive and negative controls were picked and streaked on new CAM plates to make sure there would be active colonies to pick from if the liquid cultures for tomorrow did not grow correctly. Everything for the interlab study was set up today (tubes were labeled with time periods, device, and which culture).

The mini prep was analyzed with the nano drop, and 106 ng/μL of DNA was collected from the first culture (~80 and 74ng/μL were collected from the other two). The first culture will be used for future testing with pet21b-GFP.
Miniprep Protocol:

WEDNESDAY, 6/14

Plan:
Aylin, Cat, & Edith- Interlab Study, Lead Assay (scale back GSH solution by 2μL, try with 570nm/450nm & look into ratios, if GSH changes are not enough- try altering NaCl) See Aylin Excel Sheet 6/13/17-2 GSH dilution

- Updated DIY Lead Assay protocol in accordance with observations from 6/13/17

Locke, Mike, & Haylea- Growth Curve for Lactobacillus Trial 2, Growth Curve for Bacillus Subtillis

Interlab study cultures did not all grow correctly. Streaked plates for interlab study grew and colonies from those plates will be picked for next set of liquid cultures.

Lead Assay:
http://pubs.acs.org/doi/pdf/10.1021/ac60092a004

- The link above was found and used to help explain absorbance ratios

61417 GSH dilution.xlsx

Ran Two different Lead Assays, both with different GSH Dilutions. The first plate consisted of GSH concentration decreasing by 2μL from 12 to 2μL. It was run at 3 different wavelengths, 450, 570, and 595nm. The 2μL concentration seemed to be optimal. The two best observed wavelengths were 570nm and 595nm. The second plate was set up based on these results. The concentration started at 2μL and were decreased from there. This plate showed an optimal readings at 2 and 1.5μL. Another test will be done to better confirm the results. The results of the next test showed that the optimal GSH concentration is 1.5μL with 10.5μL of water.

GSH mini dilution.xlsx

GSH mini dilution (2).xlsx

B. subtilis growth curve data:

B. subtilis Growth Curve 1

| | A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|---|
| 1 | | | A1 | A2 | A3 | B1 | B2 |
| 2 | | Starter culture ODs | 0.486 | 0.467 | 0.549 | 0.433 | |
| 3 | | T0 (8:56) | 0.1 | 0.1 | 0.1 | 0.1 | |
| 4 | | T1 (10:00) | 0.185 | 0.177 | 0.182 | 0.126 | |
| 5 | waited 10 extra ten mins because change to 50ml tube | T2 (11:10) | 0.316 | 0.296 | 0.296 | 0.261 | |
| 6 | | T3 (12:10) | 0.656 | 0.573 | 0.596 | 0.433 | |
| 7 | | T4 (1:10) | 0.478 | 0.669 | 0.779 | 0.761 | |
| 8 | undiluted | T5 (2:10) | 0.710 | 1.09 | 1.07 | 1.74 | |
| 9 | diluted 1:4 | " | 0.68 | 1.224 | 1.104 | 1.088 | |
| 10 | | T6 (3:10 PM) | 1.116 | 1.492 | 1.428 | 1.96 | |
| 11 | | T7 (12:40 PM) | | | | | |

(Note about liquid cultures: today, we have 6 liquid cultures from 6 distinct colonies. This is useful for seeing how well the different colonies grow. We also picked another colony and started a large liquid culture from that, and we will perform 3 replicates from this tomorrow - this is useful for the replicates to see that they all grow the same, since they all branch from the same liquid culture).
to increase aeration, we moved the bacteria culture from 15mL conical tube to 50 mL

Lactobacillus growth curve 2 data:
The lactobacillus didn't grow correctly despite diluting the cultures correctly from a single starter culture. After a couple of hours of growth, it was evident that the cultures didn't grow correctly, so we decided to stop the experiment and try again next week.

Lactobacillus growth curve 2 data

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | | C1 | C2 | C3 | |
| 2 | T0 (9:20) | 0.1 | 0.1 | 0.1 | |
| 3 | T1 (10:20) | 0.276 | 0.153 | 0.068 | |
| 4 | T2 (11:25) | 0.510 | 0.276 | 0.106 | |
| 5 | | | | | |

Excel sheet with Bacillus Subtilis of individual growth curves of every colony and entire graph comparison all with moving average.

Growth CurvesTrial1.xlsx

B. subtilis growth curve

(Note about liquid cultures: today, we have 6 liquid cultures from 6 distinct colonies. This is useful for seeing how well the different colonies grow. We also picked another colony and started a large liquid culture from that, and we will perform 3 replicates from this tomorrow - this is useful for the replicates to see that they all grow the same, since they all branch from the same liquid culture).
to let the bacteria have air, we moved the bacteria culture from 15mL conical tube to 50 mL

Lactobacillus liq cultures

1. Accidentally dumped today's (6/14) starter culture,  so started 3 distinct 15-mL starter cultures from 3 culturelle pills. Let grow all day and overnight (6/14) for use tomorrow. For today, we're using a few-day-old liquid culture of lactobacillus that was in the fridge and made three 15-mL liquid culture replicates.
2. Took OD undiluted, 1:10 diluted, 1:4 diluted
3. didn't grow right (fucked up) so stopped taking ODs at T2

B. subtilis liquid cultures (purpose is to make competent cells from these cultures)

1. Acquired two plates of B. subtilis, labeled A and B respectively, and labeled 3 isolated colonies from each plate. Made 6 total liquid cultures, one from each colony, using 5 mL LB broth. (done on 6/13)

B. sub competency:
Need to take OD, grow 1-3 hours and check OD every 30 mins until reach OD 0.5-0.7
Step #8: Use white centrifuge @ 4,000 RPM

Prep to make B. sub competency cells

- Made and autoclaved the washing buffer, and compentency medium
- Made 20% glyceine solution

## THURSDAY, 6/15

- Work on NEiGEM presentation
- Skype with the UConn iGEM team
- Lead Assays with differing amounts of lead
    - Lead assay was performed with 1.5 uL of GSH and 10.5 of distilled H20 based on results from 6/14/17 for every sample other than the sample containing only AuNPs and H20. GSH was added after the lead then gold particles were added to the wells, in accordance with the results from 6/13/17. Lead concentrations were 1000 ppb, 750 ppb, 500 ppb, 250 ppb, 0 ppb, 0 ppb (H20 instead of GSH) by column from left to right.

high lead.xlsx

    - Lead assay was performed again in the same manner, this time with a greater number of lead concentrations: 1000 ppb, 750 ppb, 500 ppb, 250 ppb, 100 ppb, 50 ppb, 30 ppb, 15 ppb, 10 ppb, 5 ppb, 0 ppb, 0 ppb (H20 instead of GSH) by column from left to right

lead all.xlsx

- Restreaked B.subtilis onto 4 new plates to get isolated colonies
    - in fridge

For Outreach Outline completed today see: Outreach 6/15

## FRIDAY, 6/16

Plan:

- Finish Lead Assay
    - Second lead assay from 6/15 was repeated with the same concentrations: 1000 ppb, 750 ppb, 500 ppb, 250 ppb, 100 ppb, 50 ppb, 30 ppb, 15 ppb, 10 ppb, 5 ppb, 0 ppb, 0 ppb (H20 instead of GSH) by column from left to right

    - Due to lack of descrepancy between low lead concentrations, next time with will try adding 10.5 uL of extra lead-spiked water instead of distilled water to complement the 1.5 uL of GSH

lead all 61617.xlsx

- Prep for Worcester Tech (set up 24 tubes)
- Prep for NEiGEM
- Fill tip boxes
- Look @ Error Prone PCR Protocol
- Research MatLab SimBio for Modeling
    - get software licesene approved

All of these things were completed.

# DIY Lead Assay

## Introduction

## Materials

- Gold Nanoparticles
- Lead Nitrate
- GSH
- NaCl (1 M)
- Phosphate Buffer (50 mM, pH=7)
- D.I Water
- 15 mL conical tubes
- 1.5 centrifuge tube

## Procedure

### Make Glutathione Liquid

1. Make 100mM solution of L-Glutathione

   30.72 g / 100mM
   Add 0.5 g of the Glutathione powder to 16.3 mL of D.I water
   Make 1 mL alaquotes and store in freezer

### Make Phosphate Buffer

2. Make a 1 M stock of Phosphate Buffer
3. Add to a graduated cylinder 65.82 g/L of Sodium Monobasic and 93.1 g/L of sodium dibasic in 100 mL of water
4. Test pH with pH meter and adjust using 10 normal NaOH
5. Top graduated cylinder to 1 L

### Make NaCl solution

6. Make 1 M stock of NaCl
7. Add 11.86 g into 200 mL of water into a bottle

### Make GSH solution

8. Make dilute of the phosphate buffer to 50 mmol, add 1 mL of stock to 19 mL of D.I water
9. In a 15 mL conical tube add 110 µL of NaCl, 620 µL of Phosphate buffer, 480 µL of L-Glu liquid and 790 µL of D.I water.
10. Vortex thoroughly for 5 seconds

## Prepare  Lead Spiked Water Stock

11. Measure out 100 mg of Lead nitrate to get 1000 ppb
12. Dilute as necessary to achieve concentrations of  5 ppb, 10 ppb, 15 ppb, 30 ppb, 50ppb, and 100 ppb.

    5 ppb - 1 µL Pb in 20 mL of D.I
    10 ppb - 1 µL Pb in 10 mL of D.I
    15 ppb - 1 µL Pb in 6.67 mL of D.I
    30 pbb - 1 µLL Pb in 3.3 mL  of D.I
    50 ppb- 1 µL Pb in 2 mL of D.I
    100 ppb - 1 µL Pb in 1 mL  of D.I

## Prepare Cadmium Spiked Water Stock

13. Measure out 100 mg of Cadmium nitrate tetrahydrate to get 1000 ppb
14. Dilute as necessary to achieve concentrations of  5 ppb, 10 ppb, 15 ppb, 30 ppb, 50ppb, and 100 ppb.

    50 ppb- 1 µL Cd in 2 mL of D.I
    100 ppb - 1 µL Cd in 1 mL  of D.I

## Well Preparation and Plate Reading

15. In wells A1-A7, place samples 5 ppb-control
16. In each well add 12 µL of GSH and 34.6 µL of AuNP along with 41.5 µL of the lead spiked water
17. Mix contents with pipette by pipetting up and down
18. Place replicates in B1-B7, C1-C7, and D1-7
19. In wells A, B, C, D8-9 add 12 µL of GSH and 34.6 µL of AuNP along with 41.5 µL of the cadmium spiked water (50-100 ppb)
20. Place in plate reader at Absorbance $A_6$10
21. Incubate for 10 min and read over period of time
22. Perform second well test and record color change every 1 mins f

Lead/Cadmium AuNP Assay 96-well plate

| | A | B | C | D | E | F | |
|---|---|---|---|---|---|---|---|
| 1 | 5 ppb Pb (1 uL Pb-H2O/20 mL DI H2O) | 10 ppb Pb (1uL/10mL) | 15 Pb (1uL/6.67mL) | 30 Pb (1uL/3.33mL) | 50 Pb (1uL/2mL) | 100 Pb (1uL/1mL) | Cor |
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