Notebook Week 2 (June 5-9)

Project: iGEM 2017
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Dates: 2017-06-05 to 2017-06-09

MONDAY, 6/5

Cat & Locke- made Kool Aid Gel Info Poster for touch tomorrow
Made more kanamycin plates
Aylin & Edith- Finished lead pollution map
Mike & Haylea- Researched megaplate, found that lacobacillus is not motile so we need a new assay to test lactobacillus

- will be moving forward & attempting mega plate protocol using bacillus subtillis
  https://benchling.com/s/etr-sUEHij1NOw0AOEZTqWg0

Discussed NEGEM meetup -> will be attending on June 21st
Researched chromoproteins for testing in iGEM biobrick registry & studied their associated genes

pTOPOpbrRgfp and pBBpbrRgfp Plasmids.pdf

TUESDAY, 6/6

Cat- Meeting with Organizer of Engineering Ambassadors Program for possible outreach collaboration, will hear back Friday 6
Touch Tomorrow Volunteer Training
Finalized our 3 posters, and submitted them to printing to the ATC
Made MRS Broth for liquid culture
Plasmid research (AYLIN)
Prep for interlab study

pTOPOpbrRgfp and pBBpbrRgfp Plasmids (Bereza-Malcolm).pdf

Spot mating protocol.docx

lead biosensor and plasmid sequences.docx

WEDNESDAY, 6/7

Interlab Study Trial 1, has to be redone because of innacurate pipet

WPI_iGEM_InterLab_2017_Measurements (1).xlsx

Made more MRS broth to pour plates for lactobacillus growth
Discussed Possible Collaboations
Talked about Possible Oureach Opportunities
Researched Error Prone PCR
Grew lactobacillus in MRS in liquid cultures to better understand optimal growth conditions (LOCKE)
Mike & Haylea- wrote a protocol for lead assay after reading multiple papers

- Made 1M NaCl and L-glutathione solution for GSH solution for lead assay

Culture lactobacillus

- Could only find one source about culturing from a capsule:
  a. Open probiotic capsule and pour contents into 5 mL of MRS broth in a sterile culture tube. Shake the tube gently to dissolve, then place it into the incubator for a few hours to grow.

- http://www.openwetware.org/wiki/Lactobacillus_culture
- http://foodsafety.neogen.com/pdf/acumedia_pi/7543_pi.pdf
- http://www.sciencedirect.com/science/article/pii/S0022030203738211
    - MRS-vancomycine agar and anaerobic incubation at 43°C for 72 h were suitable to enumerate *L. rhamnosus*.
- https://en.wikipedia.org/wiki/Lactobacillus_rhamnosus
- Incubate in aerobic/semi-anaerobic conditions @ 37C for 24-96 hrs.

Protocol

1 capsule 25 mL mrs

Disslolve observe

Table1

	Temp (c)	Shake	Air (cap)	O.D (First day)	O.D (Second Day)
1	RT	No	T		
2	RT	no	L		
3	37	No	T		
4	37	No	L		
5	37	Yes	T		
6	37	Yes	L		

culture starting od @.1-.2 by adding 2 mL stock culture w/ 8 mL MRS broth

Lead/Cadmium AuNP Assay 96-well plate

	1	2	3	4	5	6	7	8	9
A	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)	Control	50 Cd (1uL/2mL)	100 C (1ul/1r
B	"	"	"	"	"	"	Control	"	"
C	"	"	"	"	"	"	Control	"	"
D	"	"	"	"	"	"	Control	"	"
E									
F									
G									
H									

THURSDAY, 6/8

Tried gel protocol for Kool Aid Touch Tomorrow Activity

Made 1M phosphate buffer for lead assay

Made Fluorescence Booth Plates for touch tomorrow

Completed data for Interlab Study Trial 1

Farny lectured on different plasmid and color interactions

- Picked pET-42a and pET-21a for plasmids

- Made plasmids on benchling with chromoproteins
- Ordered DNA

Flyer for Forum:

Lead Forum.docx

T7 promoter: >BBa_I719005 Part-only sequence (23 bp) taatacgactcactatagggaga (from http://parts.igem.org/cgi/partsdb/puttext.cgi)
Cambridge team used pSB1A2 for plasmid: http://2009.igem.org/Team:Cambridge/Project/Amplification

FRIDAY, 6/9

Final Touch Tomorrow Prep:

- Made 24 gels for the Kool Aid Gel Electrophoresis
    - Gel Protocol:
        - Contents:
            - 20ml of 10 millimolar salt water solution
            - .3 g of agarose
        - Microwave ~1min and pour gel. Let set ~15-20min. To run gel use 10 millimolar salt water for liquid to run gel in.
- Made 2 sets of colors of Kool Aid Mix
    - Kool Aid Protocol:
        - Contents:
            - 50µl of water
            - 50µl of 50% glycerol
            - Kool Aid powder (fill 1.5ml microcentrifuge tube to .25ml mark with powder)
        - Vortex
- Ran Strawberry DNA Extraction Demo
- Put together Fluorescence Booth
- Checked fluorsence booth plates

Edith & Aylin - Pamphlet for forum

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
2	"	"	"	"	"	"	Cor
3	"	"	"	"	"	"	Cor
4	"	"	"	"	"	"	Cor
5							
6							
7							
8							

23.