Notebook Week 11 (August 7-11)

Project: iGEM 2017
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MONDAY, 8/7/17

- Gradient PCR of pbrR promoter
  - 12 different tubes each containing 25mL of reaction
  - Gradient PCR uses a different annealing temp. for each tube

Table6

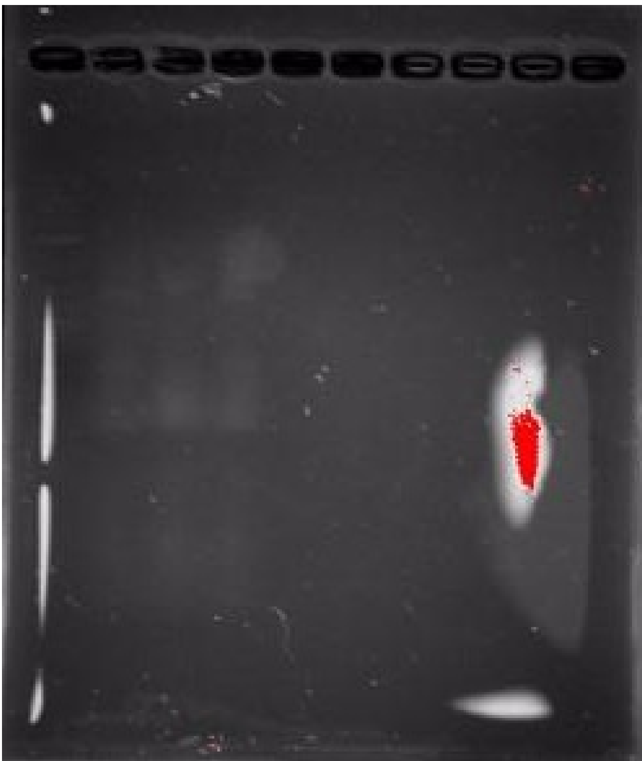
	Tube Number	Annealing Temp (C)
1	1	52
2	2	52.3
3	3	52.9
4	4	53.7
5	5	54.8
6	6	56.3
7	7	58
8	8	59.4
9	9	60.5
10	10	61.3
11	11	61.8
12	12	62.0

- Run 5 uL of product of each on a gel
  - One with the brightest top band run the rest (20uL) on another gel
- Ran 12 columns with the ranging temperatures below
  - Gel 1 - Lane 1 - ladder, lane 2- 52 degrees ... cont

GradientPCRGel1.jpg

- Gel 2- Lane 1- ladder, lane 2 - 61.3 degrees ... cont

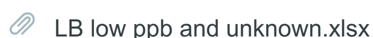
thumbnail_GradientPCRGel2.jpg



- Gels did not come out as expected but can see a cleaner PCR cleanup around 58 degrees(lane 7) , with less background noise
- Set up a new 50uL PCR reaction with an annealing temperature at 58 and ran a PCR, held overnight and tomorrow will run on a gel

- Overnight cultures of B.subtilis and Lactobacillus for plating tomorrow
- Overnight culture of RFP vector to mini prep tomorrow

- Lead Assay with Low Concentrations of lead in LB
    - The plan for the lead assay was to make a standard curve using low concentrations of lead in LB. We decided to do this because the results from the last assay seemed to be promising but only up to 250ppb. We used 25, 50, 75, and 100ppb to try to generate this new standard, and we used 30ppb as the known unknown that we tried to fit in the curve. This did not work. The absorbance for the 25ppb was below the control; the absorbance for 50ppb was above the 100ppb, and the absorbance for 30ppb (the known unknown) was similar to the 100ppb.


LB low ppb and unknown.xlsx

    - To proceed Aylin and Cat will meet with Prof. Farny Tuesday to talk about other possible routes to go. The water standard curve and testing can be tried because water showed the most promising results.
- Gibson assembly was performed for chromoprotein PCRs. Ran two assemblies at different thermocycler times (a 15 minute trial and a 60 minute trial). Assemblies were plated overnight.

Table7

	A	B	C	D
1	Name	Insert	Vector	Total
2	aeBlue	1	5	6
3	amilCp	1.25	5	6.25
4	fwYellow	1	2.9	3.9
5	amilGFP	1.35	2.9	4.25
6	efoRed	1.36	2.9	4.26
7	tsPurple	1	2.9	3.9
8				

- Run gel with the 50uL reaction with 58 degrees as the annealing temperature
    - cut top band and gel purify for gibson assembly
- Control for Lead Plates
    - Serial dilutions of liquid cultures of both
    - Take OD before plating
    - Spread with glass beads
    - Couldn't not do this today, overnight cultures did not grow as expected.. lactobacillus did not grow at all, while B.subtilis grew a small amount
- Restreak b.subtilis and lactobacillus plates for better growth for growth curves Thursday and control plates
- Mini prep more RFP vector to get higher yield
    - yields: 84.3 ng/uL and 132.1 ng/uL
- Gibson Assembly of pbrR promoter and RFP plasmid
- Go back to literature- Research growth curves and try to determine what went wrong in the last trial
- Lead Assay: Trial with water standard curve and known unknown?
- Type Interlab Data for Wiki
- Checked Gibson assembly growth: No growth on any plates including negative control

plategroup1.jpg

plategroup2.jpg

- Overnight cultures for both growth curves and control plates (2 cultures of lactobacillus and 2 cultures of b.subtilis)

- Growth curves of Lactobacillus and B.subtilis
    - Re-do from last Tuesday- See math from last Tuesday for lead amounts
        - B.subtilis
            - control, 100ppm, 400 ppm, 800 ppm
            - Parent culture starter OD -
        - Lactobacillus
            - control, 5ppm, 25 ppm, 50ppm
            - Parent culture starter OD-
    - Math dilutions to get to a starter OD of 0.01

- Control for Lead Plates
    - Serial dilutions of liquid cultures of both
    - Take OD before plating
    - Spread with glass beads