Name: Kennex, Laura, Asma, Amirah, Sijia, Jaizi, Krithika, Chiara
Date: 7/19/19

Goals:

1. Overnight cultures
    a. Pcb302 in A. Tume from transformations done on 7/17/19
2. Restriction Digest
    a. DinoIII-P2 digested with BglII and XbaI
3. Gel electrophoresis
    a. DinoIII P2 digested with BglIII & XbaI
    b. Codon optimized RFP digested with BglIII & XbaI

Name: Chiara
Date: 7/19/19

Goal:

1. Overnight cultures
    a. Pcb302 in A. Tumefaciens from transformations done on 7/17/19

Protocol:

Overnight Cultures

1. Added about 7 mL of LB with 7 µL kanamycin to a 15 mL Falcon tube
2. Dipped a p10 tip into the selected colonies and drop into the tube
3. Incubated in the water bath at 28° C at 200 rpm over the weekend

Results:

N/A

Conclusion:

N/A

Name: Asma
Date: 07/19/2019

Goals:

1. Restriction Digest
    a. DinoIII-P2 samples digested with BglII and XbaI

Protocol:

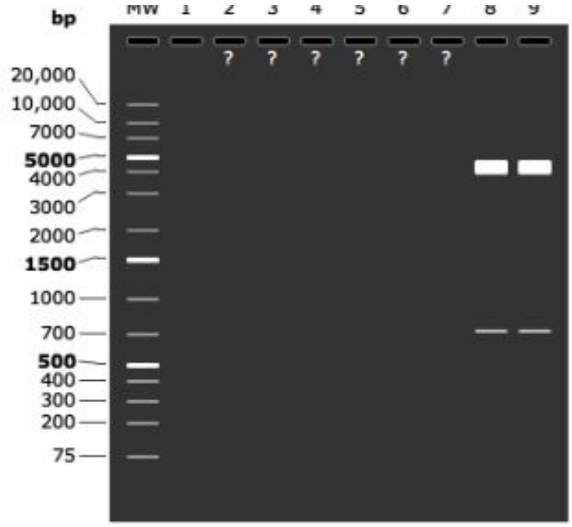
30 µL Fast Digest Restriction Digest

1. Prepared a Fast Digest concentration cocktail with the following proportions: 1 µL XbaI, 1 µL BglII, 3 µL of 10X Fast Digest Buffer, and 15 µL of diH2O.
2. Added 20 µL of this cocktail to a clean 1.5 Eppendorf tube and then added 10 µL of DNA
3. Incubated at 37° C for 30 minutes.

Results:

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Generuler 1kb plus MW ladder												D4	D2

Expected Gel-



1.0% agarose

MW: GeneRuler™ 1 kb Plus DNA Ladder

2:

3:

4:

5:

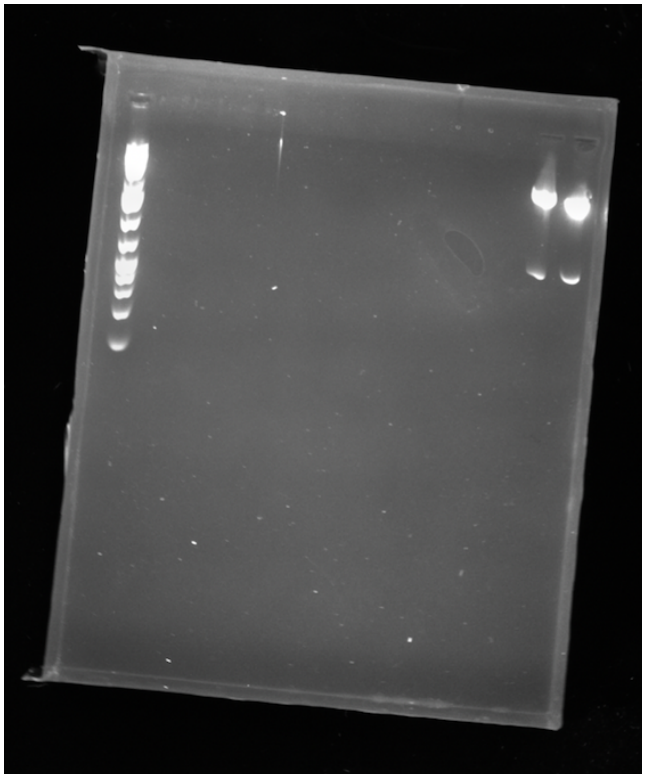
6:

7:

8: DinoIII Part 2 in pUCIDT vector
BglII + XbaI
1. 4612 bp = BglII (2240) – XbaI (1514)
2. 726 bp = XbaI (1514) – BglII (2240)

9: DinoIII Part 2 in pUCIDT vector
XbaI + BglII
1. 4612 bp = BglII (2240) – XbaI (1514)
2. 726 bp = XbaI (1514) – BglII (2240)

Observed gel-



Conclusion:

The digest was successful; the observed bands are similar to the expected bands. The next step is gel extraction.

Name: Amirah
Date: 7/19/19

Goal:

1. Ran RFP codon optimized pre-done restriction digest on gel

Protocol:

Preparing, Loading, and Running a 1% Agarose Gel

Preparing

1. Added 1 g of Agarose in 100 mL of 1X TBE in an Erlenmeyer flask
2. Heated in the microwave until fully dissolved
3. Allowed the solution to cool until comfortable to touch
4. Added 10 µL GelRed Nucleic Acid Gel Stain and mixed
5. Inserted casting tray, made sure the rubber on the sides was not overlapping
6. Carefully poured the agarose into the tray and placed the comb to create the wells
7. Allowed the gel to solidify
8. Once solidified, changed the orientation of casting tray where the rubber sides are not in contact with the sides of the system.
9. Poured in 1X TBE into the gel electrophoresis system to the fill line, being sure to submerge the gel, and removed the comb

Loading

1. Loaded ~5 µL of the ladder in the first well
2. Prepared your samples to load by adding in 1 µL of 6X Loading dye for every 5 µL of DNA and loaded
    a. 10 µL of DNA was loaded

Running

1. Once the gel had been loaded, slid on the cover making sure the negative electrode is closest to the DNA and the positive electrode is at the bottom of the gel
2. Ran for about an hour at 150 V

Results:

Gel did not look like simulation

Conclusion:

Did not do extraction. Left gel in fridge