Name: Sijia Qin, Jiazi Tian, Chiara Brust, Amirah Hurst, Kennex Lam
Date: 7/22/19

Goals:

1. Miniprep pcb302 in A. Tume from papers 1 & 2 from transformations done on 7/17/19
2. Glycerol Stock of pcb302 in A. Tume from papers 1 & 2 overnight cultures from 7/19/19
3. Gel electrophoresis
    a. DinoIII P2 digested with BglIII & XbaI
    b. Codon optimized RFP digested with BglIII & XbaI
4. Overnight cultures
    a. Pcb302 in A. Tume from papers 1 & 2 from glycerol stocks made on unknown date
    b. K1357009 from glycerol stocks of unknown date
    c. Codon-optimized RFP from glycerol stocks of unknown date
5. Restriction Digest with new XbaI and BglII
    a. Codon-optimized-RFP
    b. DinoIIIP2

Name: Sijia Qin, Jiazi Tian, Amirah

Date: 7/22/19

Goal:

1. Glycerol stock transformations done on 7/17/19
2. Miniprep pcb302 from transformations done on 7/17/19

Protocol:

Glycerol Stock:

500ul of transformations done on 7/17/19 and 500 ul of 50% glycerol was added to make glycerol stock and was stored in the freezer, labeled as “pcb 302 from transformation 7/17/19”.

Mini Preps for Agrobacterium tumefaciens

1. Centrifuged 10 mL of overnight for 15 minutes at 3500 rpm and resuspended in 250 µl buffer P1 containing 0.1 mg/ml RNase A.
2. Added 250 µl lysis buffer P2 to the tube and inverted gently 6 times to mix.
3. Added 350 µl neutralization buffer N3 to the tube and inverted immediately but gently 6 times.
4. Centrifuged the lysate for 10 min at 13,000 rpm
5. Placed a QIAprep Spin Column in a 2 mL collection tube.
6. Transferred the cleared lysates from step 4 to the QIAprep Spin Column by decanting or pipetting.
7. Centrifuged for 60 seconds at 13,000 rpm and discarded flow-through.
8. Washed the QIAprep Spin Column by adding 500 µL of Buffer PB and centrifuged for 60 seconds at 13,000 rpm and discarded flow-through.
9. Washed the QIAprep Spin Column by adding 750 µL of Buffer PE and centrifuging at 60 seconds at 13,000 rpm and discarded the flow through.
10. Centrifuged for an additional 1 min to remove residual wash buffer at 13,000 rpm.
11. Placed the QIAprep Spin Column in a clean 1.5 mL microcentrifuge tube.
12. Added 50 µL of di H2O to the center of each QIAprep Spin Column, let stand for 1 min, and centrifuged for 1 min.

Results:

Sample	Concentration	Purity
400-2-4	Too low	1.25
400-2-2	Too low	1.40
400-2-3	Too low	1.00
300-2-1	Too low	1.50
300-2-2	Too low	1.00
300-2-3	Too low	1.00
300-2-4	Too low	0.667
200-2-1	Too low	1.200
200-2-2	Too low	1.00
200-2-3	Too low	1.00
200-2-4	Too low	1.00

Name: Chiara
Date: 7/22/19

Goal:

1. Gel electrophoresis
    a. Codon optimized RFP
    b. DinoIII P2

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

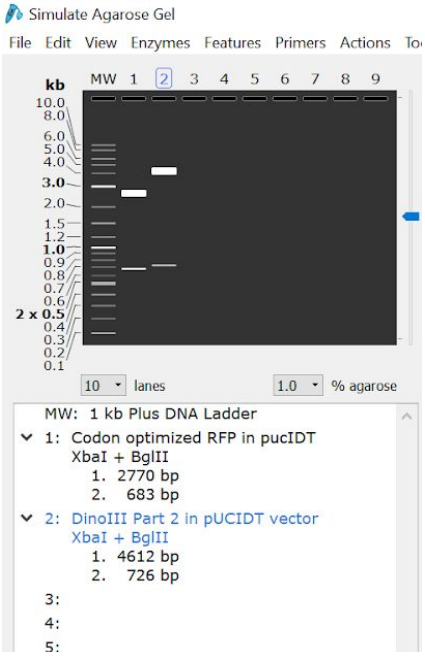
Loading

1. Loaded ~5 µL of the ladder in the first well
2. Prepared samples to load by adding in 1 µL of 6X Loading dye for every 5 µL of DNA and load
    a. 5 µL of codon-optim.-RFP digest was loaded
    b. 10 µL of DinoIIIP2 digest was loaded

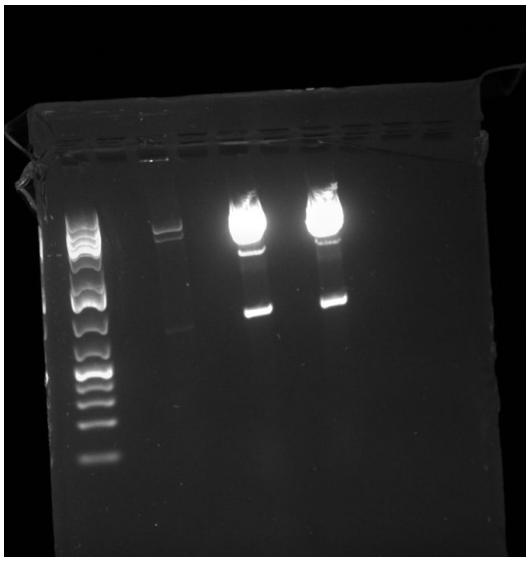
Running

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 1.5 hours at 93 V

Expected Results:



Results:



Gel Key

Lane #	**Sample**
1	MW 1 Kb Plus DNA Ladder
2	Blank
3	Codon-optimized-RFP colony 10
4	Blank
5	DinoIIIP2 Colony 2
6	Blank
7	DinoIIIP2 Colony 4

Conclusion:

XbaI restriction enzyme has expired and has, therefore, lost efficiency. We obtained a new one and will digest these same samples again tomorrow.

Name: Sijia Qin, Jiazi Tian, Amirah

Date: 7/22/19

Goal:

1. Measure concentrations of mini preps from pcb302 on 7/

Protocol:

1. Used nanophotometer and blanked with DI water

Results:

Sample	**Concentration**	**Purity**
1	Too low	Too low
2	Too low	1.5
3	Too low	1.5

Conclusion:

Will do new overnight cultures then do midi preps to get more dna

Name: Chiara
Date: 7/22/19

Goal:

1. Restriction Digest with XbaI and BglII
    a. Codon-optimized-RFP
    b. DinoIIIP2

Protocol:

Restriction Digest Protocol

30 µL Fast Digest Restriction Digest

1. Prepared a Fast Digest concentration cocktail with the following proportions: 1 µL Restriction Enzyme XbaI, 1 µL Restriction Enzyme 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:

N/A

Conclusion:

We will run this gel tomorrow

Name: Amirah Hurst
Date: 7/22/19

Goal:

1. Overnight cultures
    a. Pcb302 in A. Tume from glycerol stocks
    b. K1357009 from glycerol stocks
    c. Codon-optimized RFP from glycerol stocks

Protocol:

Overnight Cultures

1. Added about 5-7 mL of LB to a 25 mL Falcon tube along with 5-7 µL of antibiotics
    a. Kanamycin for pcb302
    b. Chloramphenicol for K1357009
    c. Ampicillin for codon-optimized-RFP
2. Scraped some of the glycerol stock ice with the p20 tip and dropped into the tube
3. Incubated K1357009 and C.O. RFP in the water bath at 37° C at 220 rpm for 16-18 hours
    a. Pcb302 was in 28 degrees C for 48-56 hours

Results:
N/A

Conclusion:
N/A