Name: Asma, Chiara, Justin, Laura, Kennex, Amirah

Date: 7/17/19

Goals:

1. Minipreps
    a. DinoIII-GFP part 2
    b. Pgex-HCG
2. Glycerol stocks
    a. DinoIII-GFP Part 2
3. Transform
    a. Pcb302 in A.Tumefaciens from papers 1 & 2
4. Restriction Digest
    a. Codon-Optimized-RFP
5. Overnights
    a. K1357009

Name: Asma, Justin

Date:7/17/19

Goal:

1. Glycerol stocks
    a. DinoIII Part 2

Protocol:

1 ml of glycerol and 1 ml of the sample (D1-D10) in each tube.

Results:

N/A

Conclusion:

The glycerol stocks are in the -83 degrees freezer labeled “ glycerol stocks of DinoIII-P2”

Name: Asma, Justin, Laura

Date: 7/17/19

Goal:

1. Minipreps (17 samples)
    a. DinoIII Part 2
    b. pGEX-HCG

Protocol:

QIAprep Spin Miniprep Kit Protocol

a. Centrifuged 3 mL of bacterial overnight culture in two separate Eppendorf tubes (1.5 mL in each) at 8,000 rpm for 3 minutes at room temperature.
b. Discarded the supernatant and resuspend pelleted bacterial cells in one tube with 250 µL Buffer P1 and transfer to the other and resuspend until one eppendorf tube contains the pelleted cells resuspended in 250 µL Buffer P1.
c. Added 250 µL of Buffer P2 and invert 5 times.
d. Added 350 µL of Buffer N3 and immediately mix by inverting 5 times.
e. Centrifuged for 10 minutes at 13,000 rpm.
f. Micropipetted 800 µL of the clear supernatant into a spin column and centrifuge for 60 seconds and discard the excess liquid.
g. Added 500 µL of PB and centrifuge the spin columns for 60 seconds. Discard the flow through.
h. Added 750 µL of PE to the spin columns, centrifuge for 60 seconds, and discard the flow through.
i. Centrifuged the spin columns again for 60 seconds to remove residual wash buffer and discard the flow through.
j. Transfered the spin columns to a clean eppendorf tube and add 50 µL of EB to the center of the spin column to elute the DNA.
k. Allowed the spin column to stand for one minute and then centrifuge for one minute.
l. Recorded the concentrations for each sample.

Results:

Sample	Concentration (ng/ µl)	Purity Ratio (A260/A280)
P1	178	1.778
P2	135	1.800
P3	113	1.731
P4	62.5	1.667
P5	65	1.733
P6	55	1.692
P7	50	1.818
D1	163	1.757
D2	280	1.778
D3	198	1.795
D4	285	1.781
D5	265	1.738
D6	260	1.763
D7	215	1.792
D8	250	1.786
D9	248	1.768
D10	285	1.781

Conclusion:

Both pGEX-HCG and DinoIII-GFP P2 have good enough concentrations to do a gel extraction and subsequent ligation.

Name: Chiara, Kennex, Justin, and Amirah

Date:7/17/19

Goal:

1. Transform pcb302 in A. Tume

Materials:

Agrobacterium tumefaciens LBA4404

Protocol:

Electroporation of Agrobacterium tumefaciens

1. Thawed Agrobacterium tumefaciens cells on wet ice
2. Combined 1 µL of pCB302-gfp-MBD plasmid DNA and 20 µL of cells in an Eppendorf tube
3. Pipetted the cells into a cuvette and electroporated at 2 kV
4. Added 1 mL of YM media and transferred to a 15 mL falcon tube
5. The tubes were incubated at 30°C at 200 rpm for 3 hours
6. 400 µL of each culture was streaked onto a YM kanamycin plate.
7. 300 µL of each culture was streaked onto a YM kanamycin plate.
8. 200 µL of each culture was also streaked onto a YM kanamycin plate.
9. The plates were incubated at 30°C for 48 hours

Results:

N/A

Conclusion:

N/A

Name: Kennex Lam
Date:7/17/19

Goal:

Restriction Digest on codon-optimized RFP

Materials:
BgIII, XbaI, RFP optimized codon

Protocol:

30 µL Fast Digest Restriction Digest

1. A Fast Digest concentration cocktail was prepared with the following proportions: 1 µL XbaI, 1 µL BgIII, 3 µL of 10X Fast Digest Green Buffer, and 15 µL of diH2O.
2. 20 µL of this cocktail was added to a clean 1.5 Eppendorf tube with 10 µL of DNA (RFP opt. codon).
3. Incubated at 37° C for 30 minutes.

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 until fully dissolved.
3. Added 10 µL GelRed Nucleic Acid Gel Stain when it cooled enough to touch.
4. Inserted casting tray.
5. Poured the agarose into the tray and placed the comb to create the wells
6. Gel solidified
7. Changed the orientation of casting tray so the rubber sides were not in contact with the sides of the system.
8. Poured in 1X TBE into the gel electrophoresis system to the fill line, making sure to submerge the gel.

Loading

1. Loaded 6 µL of the GeneRuler 1kb Plus ladder in the first well
2. Loaded 8 µL of the digested RFP optimized codon into second well

Running

1. Ran for about 55 minutes at 100 volts.

Results:

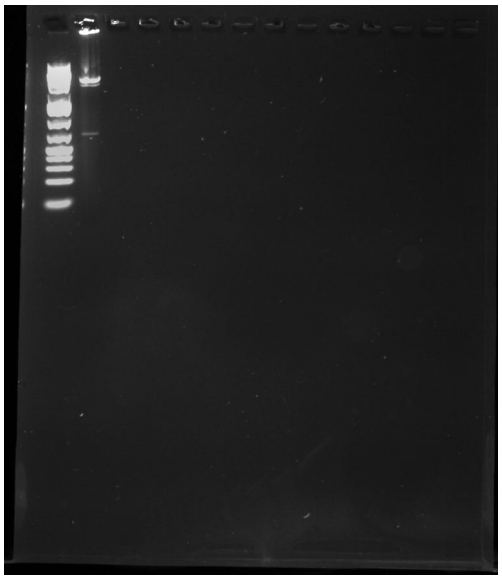


Figure 1:

Initial image of agarose gel.

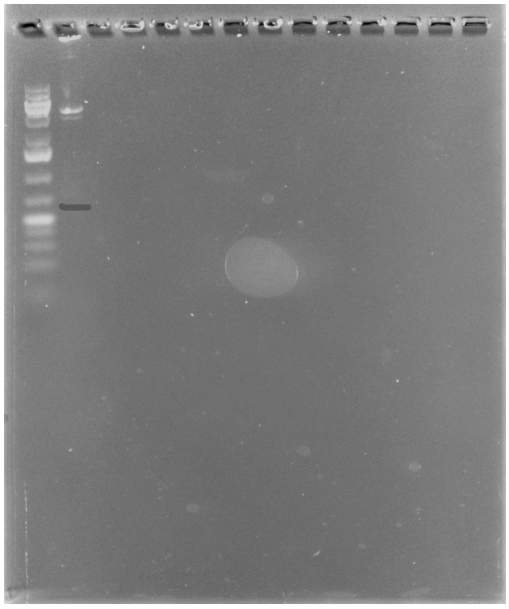
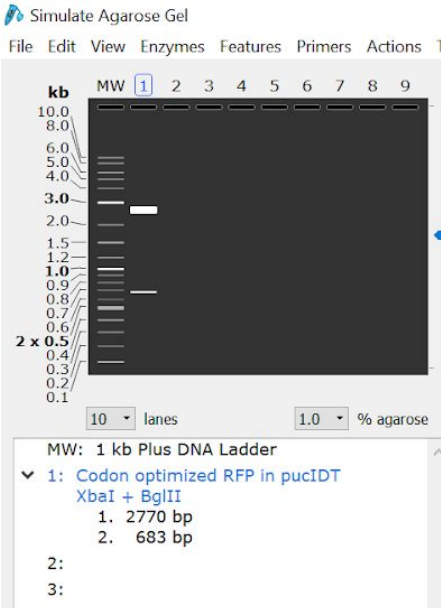


Figure 2:

Gel after being run for an additional 30 minutes at 100V. Due to low visualization of the second band in the image a marker was added to indicate its placement.

Expected Results:



Conclusion:

The restriction digest was successful

Name: Amirah

Date:7/17/19

Goal:

1. Overnight cultures of transformed K1357009 colonies plated on 7/15/19

Protocol:

*DONE UNDER STERILE CONDITIONS

1. Chose 3 colonies from 3 different plates:
    a. K1357009 100 ul
    b. K1357009 150 ul
    c. RFP control 100 ul
2. Poured 7 ml of LB + 7 ul of Chloramphenicol to 6 15ml tubes
3. Used 10 ul pipette tip to dip into the selected colonies on the plate
4. Dropped tip with bacteria into allocated 15 ml tube
5. Placed into shaking incubator overnight
    a. 37.0 degrees C
    b. 200 rpm

Results:

N/A

Conclusion:

N/A