Name: Kennex Lam, Laura Das Neves, Asma Khimani, Justin, Chiara
Date: 7/18/19

Goal:

1. Miniprep
    a. K1357009 overnight cultures
    b. Pcb302 from A.Tume from papers 1 & 2 glycerol stocks made on 7/15/19
2. Gel electrophoresis
    a. DinoIIIP2
    b. pGEX-HCGb

Name: Kennex Lam, Laura Das Neves
Date: 7/18/19

Goal:

1. Miniprep K1357009 overnight cultures

Protocol:

QIAprep Spin Miniprep Kit

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. Did two minipreps, both each 3 mL per tube.
b. Discarded the supernatant and resuspended pelleted bacterial cells in one tube with 250 µL Buffer P1 and transferred to the other and resuspended until one eppendorf tube contained the pelleted cells resuspended in 250 µL Buffer P1.
c. Added 250 µL of Buffer P2 and inverted 5 times.
d. Added 350 µL of Buffer N3 and immediately mixed 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 centrifuged for 60 seconds and discarded the excess liquid.
g. Added 500 µL of PB and centrifuged the spin columns for 60 seconds. Flow through discarded.
h. Added 750 µL of PE to the spin columns, centrifuged for 60 seconds, and discarded the flow through.
i. Centrifuged the spin columns again for 60 seconds to remove residual wash buffer and discarded the flow through.
j. Transferred the spin columns to a clean eppendorf tube and added 50 µL of EB to the center of the spin column to elute the DNA.
k. Spin column stood for one minute and then centrifuged for one minute.
l. Concentrations recorded for each sample.

Results:

Sample	Concentration	Purity Ratio
K1357009 100uL LB-C #1 A	20.0	2.667
K1357009 100uL LB-C #1 B	3.000	2.000
K1357009 150uL LB-C #1 A	15.0	-------
K1357009 150uL LB-C #1 B	10.0	-------

K1357009 100uL LB-C #2 A	17.5	2.333
K1357009 100uL LB-C #2 B	27.5	2.750
K1357009 150uL LB-C #2 A	45.0	2.000
K1357009 150uL LB-C #2 B	30.0	3.000
K1357009 100uL LB-C #3 A	15.0	2.000
K1357009 100uL LB-C #3 B	4.000	2.000
K1357009 150uL LB-C #3 A	17.5	2.333
K1357009 150uL LB-C #3 B	15.0	3.000
RFP Positive Control 100 uL LB-C #1 A	35.0	2.800
RFP Positive Control 100 uL LB-C #1 B	8.000	1.778
RFP Positive Control 100 uL LB-C #2 A	25.0	2.500
RFP Positive Control 100 uL LB-C #2 B	27.5	1.833
RFP Positive Control 100 uL LB-C #3 A	35.0	1.750
RFP Positive Control 100 uL LB-C #3 B	55.0	2.200

Conclusion:

Concentrations were low. May run a restriction digest tomorrow to see if DNA bands are present and correct. The positive controls were also pretty low.

Name: Chiara Brust

Date: 7/18/19

Goal:

1. Miniprep pcb302 in A. Tume from papers 1 & 2 from glycerol stocks made on 7/15/19

Protocol:

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 Buffer EB to the center of each QIAprep Spin Column, let stand for 1 min, and centrifuged for 1 min.

Results:

The concentrations were too low to measure.

Name: Asma Khimani

Date: 7/18/19

Goal:

1. Gel electrophoresis (Neither digested nor PCRed)
    a. DinoIIIP2
    b. pGEX-HCG

Protocol:

Preparing, Loading, and Running a 1% Agarose Gel

Preparing

1. Add 1 g of Agarose in 100 mL of 1X TBE in an Erlenmeyer flask
2. Heat in the microwave until fully dissolved (usually about 45 seconds to 1 minute)
    a. Solution should be completely clear
3. Allow the solution to cool until comfortable to touch
4. Add 10 µL GelRed Nucleic Acid Gel Stain and mix
5. Insert casting tray, make sure the rubber on the sides is not overlapping
6. Carefully pour the agarose into the tray and place the comb to create the wells
7. Allow the gel to solidify
8. Once solidified, change the orientation of casting tray where the rubber sides are not in contact with the sides of the system.
9. Pour in 1X TBE into the gel electrophoresis system to the fill line, be sure to submerge the gel, and remove the comb

Loading

1. Load ~5 µL of the ladder in the first well
2. Prepare your samples to load by adding in 1 µL of 6X Loading dye for every 5 µL of DNA and load

Running

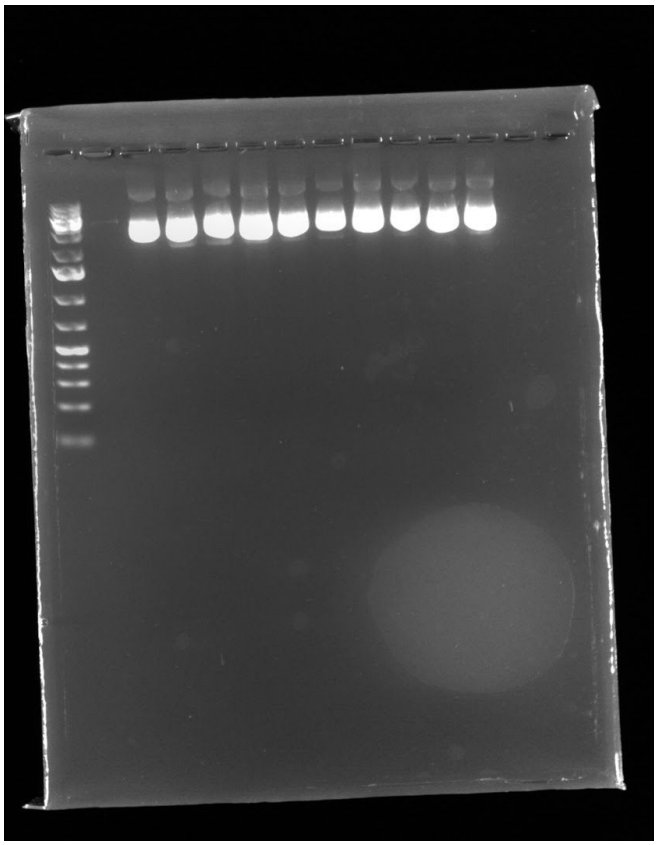
1. Once the gel has been loaded, slide on the cover making sure the negative electrode is closest to the DNA and the positive electrode is at the bottom of the gel
2. Run for about 45 minutes to an hour

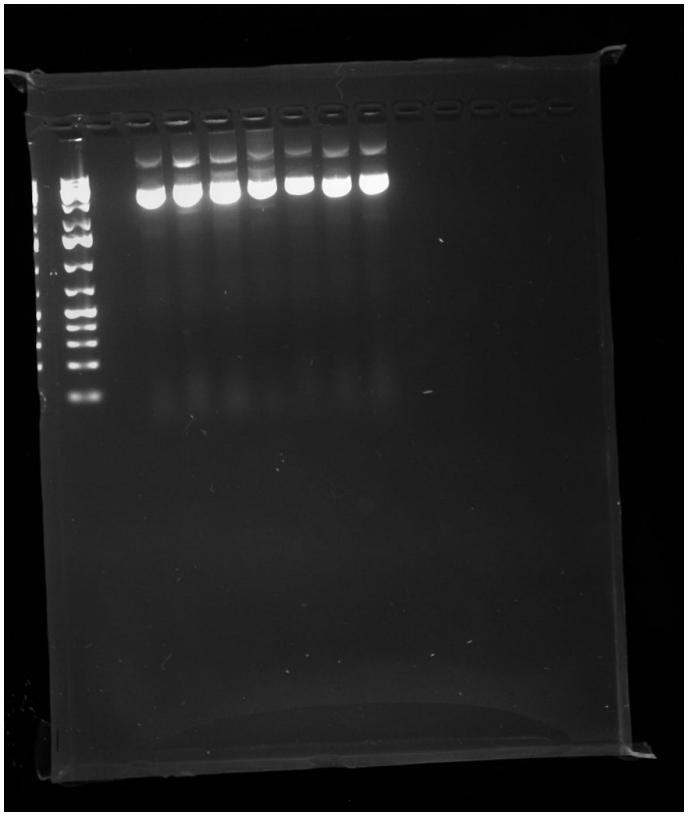
Lane 1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 KB Plus MW	blank	P1	P2	P3	P4	P5	P6	P7	----	----	----	-----	-----

Ladder													

1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 KB PLUS MW LADDER	BLANK	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10		

Results:





Conclusion:

A gel electrophoresis was performed to verify the DNA in the samples. Although the concentrations of the miniprep samples were too low to read (07/17/2019), there is still a possibility of DNA in the samples. The samples were not cut with restriction enzymes because we want to see if there are any bands to indicate DNA is present in the sample. The gels reveal bands which indicate DNA; the next step is to perform a restriction digest to verify the identity of the plasmid.