Name: Chiara Brust, Krithika Karunakaran, Rehmat Babar, Kennex Lam

Date: 8/19/19

Goal:

1. Colony PCR on pcb302 in A. Tumefaciens from 8/16/19 transformation
    a. Papers A & B
2. Overnights on pcb302 in A. Tumefaciens from 8/16/19 transformation
    a. Papers A & B
3. Ethanol precipitation on Dino III RFP
4. Start overnight cultures for characterization of Test Device 1, Test Device 5, Positive Control, Negative Control colony 1 and 2 for each in LB, TB, YM, and YPD media.

Name: Krithika

Date: 8/19/19

Goal:

1. Overnights on pcb302 in A. Tumefaciens from 8/16/19 transformation
    a. Papers A & B

Protocol:

1. 7 mL of YM broth was added to a 15 mL Falcon tube along with 7 µL of kanamycin
2. A p10 tip was dipped into the selected colony and was dropped into its respective tube
3. Tubes were placed in the middle room shaking incubator at 220rpm at 30 °C at 6:00 pm and should be removed after ~48 hours.

Name: Chiara Brust

Date: 8/19/19

Goal:

1. Colony PCR on pcb302 in A. Tumefaciens from 8/16/19 transformation
    a. Papers A & B

Protocol:

PCR Protocol

20 µL Reaction

1. Prepared a PCR concentration cocktail with the following proportions: 7 µL of diH2O, 10 µL PCR Mastermix, 1 µL of the forward primer, and 1 µL of the reverse primer.
    - Primers ¾ & GFP Fwd/Rev
2. Added 19 µL of the concentration cocktail into a PCR tube along with 1 µL of the DNA.
2. Placed PCR tube in the thermocycler at the following generic settings:
    1. 94° C for 2:00 minutes
    2. 94° C for 1:00 minute
    3. 50° C for 0:45 minute
    4. 72° C for 0:45 minute
    5. 25X (Go to Step 2)
    6. 72° C for 1:30 minutes

    Lid Temperature: 105° C

Name: Chiara

Date: 8/19/19

Goal:

1. Run gel on pcb302 colony PCR

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

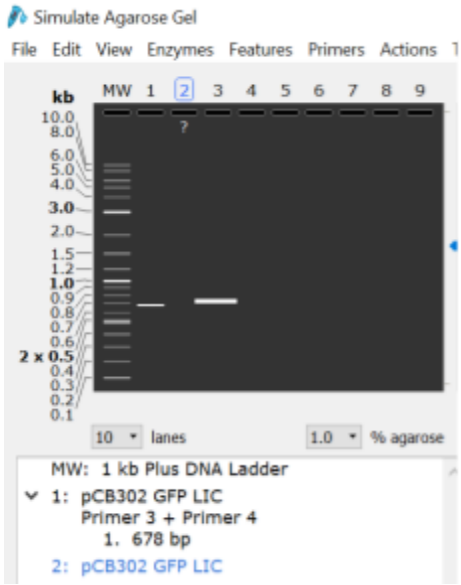
Loading

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 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 1.5 hours at 100 V

Expected Results:



Primers GFP Fwd & Rev

Our plasmid map on snapgene was an estimate. Because of this, the primers GFP Fwd/Rev provided by the writers of “Heterologous DNA Uptake by Cultured Symbiodinium spp. Aided by Agrobacterium Tumefaciens” are not being detected on our snapgene file. However, their paper states that these primers will amplify a region of about 700 base pairs.

Results:

Samples 1-10= Transformation 3
YM Kanamycin
300 µL
Colonies 1-10

Samples 11-20= Transformation 3
LB Kanamycin
200 µL
Colonies 11-20

Samples 21-23= Transformation 1
YM Kanamycin
300 µL
Colonies 21-23

Sample 24= 8/15/19 miniprep sample Plate B Colony 4

Gel 1 Key

Lane #	Sample
1	1 Kb Plus DNA Ladder
2	1
3	2
4	3
5	4
6	5
7	6
8	7
9	8
10	9
11	10
12	11
13	12
14	13

Gel 2 Key

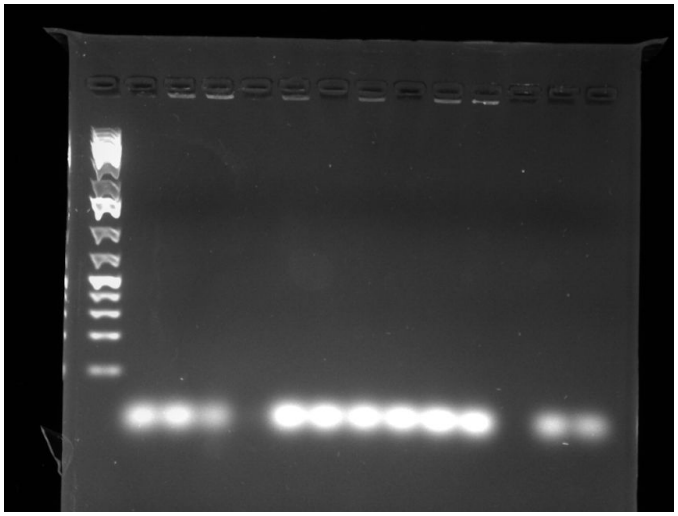
Lane #	Sample
1	1 Kb Plus DNA Ladder
2	14
3	15
4	16
5	17
6	18
7	19
8	20
9	21
10	22
11	23
12	24
13	G4
14	G5

Gel 3 Key

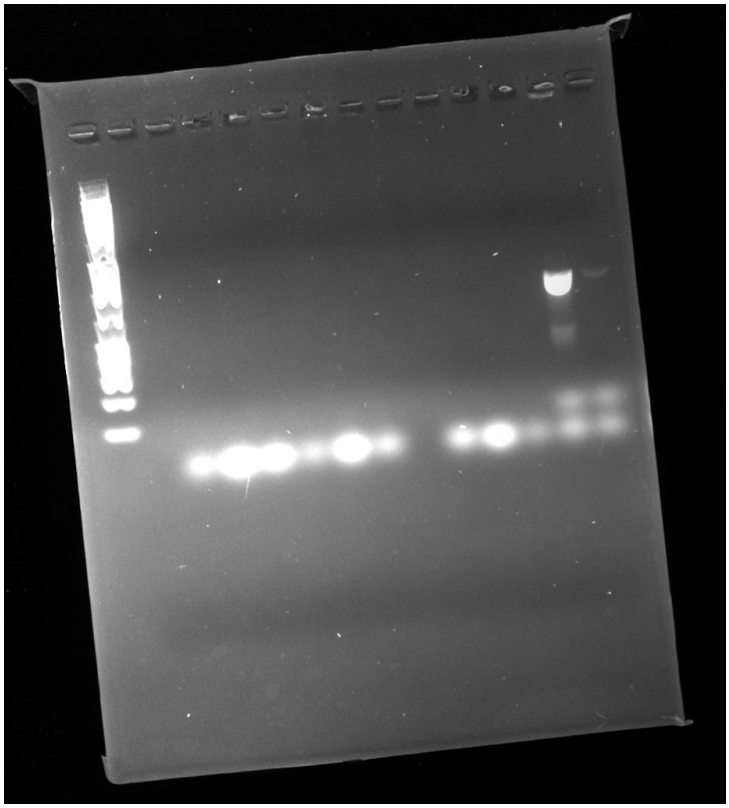
Lane #	Sample
1	1 Kb Plus DNA Ladder
2	G6
3	G7
4	G8
5	G9
6	G10
7	G11
8	G12
9	G21
10	G22
11	G24
12	Blank
13	Blank
14	Blank

*G= samples with primers GFP Fwd/Rev

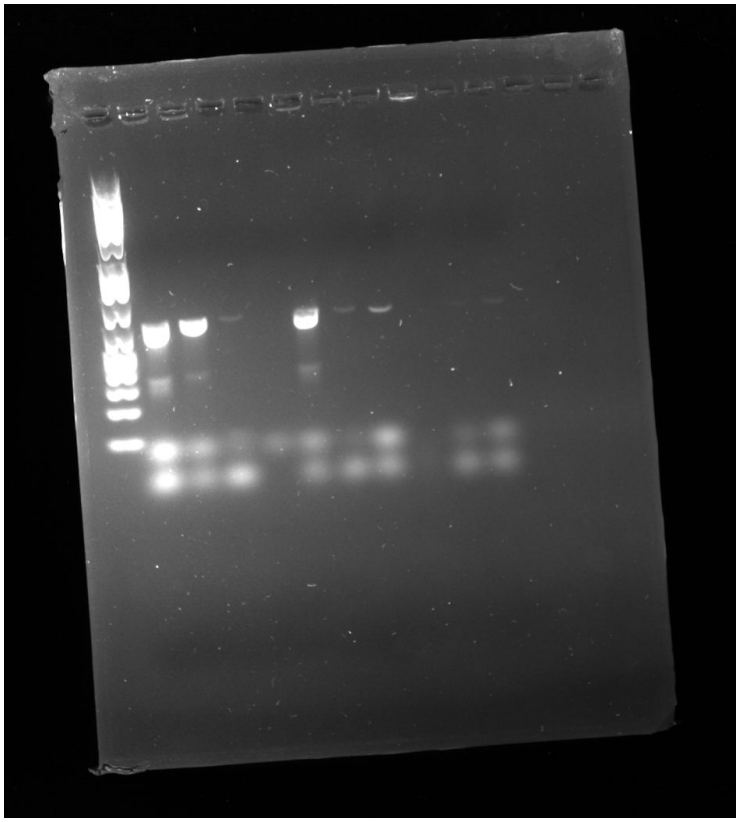
Gel 1



Gel 2



Gel 3



Conclusion:

The reactions with primers 3 & 4 did not result in the expected bands although they have worked successfully in the past. On the other hand, the GFP primers produced bands of the correct size. Because of this, we can assume that we have pcb302 in A. Tumefacines.

Name: Rehmat

Date: 8/16/19

Goal: Ethanol precipitate on gel extraction Dino III RFP samples

Protocol:

Ethanol Precipitate Dino III RFP

1. Added 23 µL of Sodium Acetate
2. Added 500 µL ethanol
3. Stored in the freezer over the weekend
4. Defrosted and centrifuged at 13,000 rpm for 30 minutes, removed as much ethanol as possible
5. Added 200 µL of 70% ethanol from freezer
6. Spinned that down for 15 minutes, removed as much ethanol as possible, allowed to air dry and resuspended in 75 µL EB

Results

Name: Rehmat

Date: 8/19/19

Goal:

Linearize the two Dino III plasmids using EcoRI

Protocol:

1. Combined 100 µL of diH2O, 25 µL of EcoRI, 25 of Fast Digest Buffer, and 100 µL of Dino III GFP mini prep DNA at 260 ng/µL in one eppendorf tube.
2. Combined 100 µL of diH2O, 25 µL of EcoRI, 25 of Fast Digest Buffer, and 100 µL of Dino III RFP mini prep DNA at 180 ng/µL in another eppendorf tube.
3. Incubated at 37°C for 2-3 hours.

Name: Rehmat

Date: 8/19/19

Goal: Run a gel for gel extractions on the linearized Dino III plasmids

Protocol:

Preparing, Loading, and Running a 0.8% Agarose Gel

Preparing

1. Added about 0.8 g of Agarose in 100 mL of 1X TBE in an Erlenmeyer flask.
2. Heated until fully dissolved.
3. Added 10 µL SYBR Gold 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. Gel solidified
6. Changed the orientation of casting tray so the rubber sides were not in contact with the sides of the system.
7. Poured in 1X TBE into the gel electrophoresis system to the fill line, making sure to submerge the gel.

Loading

1. Prepared samples to load by adding in 50 µL of 6X Loading dye to the 250 µL digest reactions for each plasmid.
2. Loaded 40 µL of the sample into the six wells.

Running

1. Ran for overnight at 34 volts.

Name: Yujie Huang, Jiayi Lan

Date: 8/19/19

Goal:

1. Start overnight cultures for characterization of Test Device 1, Test Device 5, Positive Control, Negative Control colony 1 and 2 for each in LB, TB, YM, and YPD media

Protocol:

Overnight Cultures

1. Added 7 mL of LB to a 50 mL Falcon tube along with 7 µL of Chloramphenicol
2. Scraped some of the ice with the p10 tip and drop into the tube for:
    a. Test Device 1 colony 1 and 2, Test Device 5 colony 1 and 2, Positive Control colony 1 and 2, Negative Control colony 1 and 2
3. Incubated at 37° C at 220 rpm for 16 hours
4. Repeat the procedure with other three media: TB, YM, YPD