Name: Kennex Lam, Chiara Brust, Krithika Karunakaran, Rehmat, Asma
Date: 8/8/19

Goal:

1. Miniprep of DinoIII with RFP from 8/6/19 transformations.
2. Gel electrophoresis on PCR of DinoIII with RFP transformation.
    a. DinoIII GFP primer 1
    b. DinoIII GFP primer 2
3. Ethanol precipitation on codon optimized RFP digest from 8/7/19
4. Make more O. marina cultures
5. Glycerol stocks of DinoIII-RFP from 8/6/19 transformations
6. Send for sequencing
    a. DinoIII-GFP
    b. DinoIII-RFP
    c. Codon-optimized RFP

Name: Kennex Lam
Date: 8/8/19

Goal:

1. Gel electrophoresis on PCR of DinoIII with RFP transformation
2. Restriction digest on DnioIII with RFP minipreps from today

Protocol:

Preparing, Loading, and Running a 1% Agarose Gel for PCR on DinoIII with RFP

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. 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. Loaded 10 µL of the GeneRuler 1kb Plus ladder in the first well .
2. Loaded 6 uL of DinoIII with RFP into lanes 3-7.

Running

1. Ran for 2 hours at 90 volts.

Results:

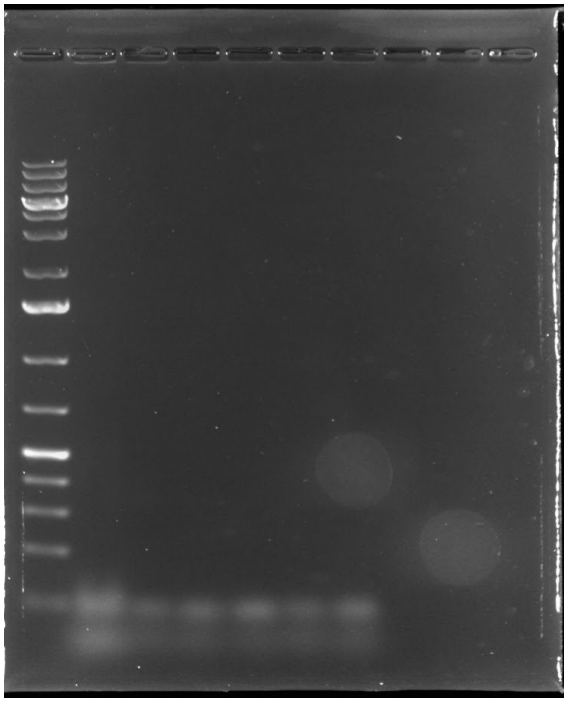

FIGURE 1. PCR products of DinoIII with RFP. No bands were seen except for primer dimers.

Conclusion:

No DNA was present in the gel. Meaning, the primers used for the DinoIII RFP did not work, and new primers must be found.

Name: Kennex Lam
Date: 8/8/19

Goal:

1. Restriction digest on DnioIII with RFP minipreps from today

Protocol:

Restriction Digest on DinoIII with RFP Minipreps from Today using HindIII

1. Each digest had a total volume of 10 uL; making the proportions for each digest 4 uL DNA, 2 uL of HindIII, 1 uL of FastDigest Green Buffer, and 3 uL of diH20.
2. A cocktail for 8 (there were 7 samples) was made using 16 uL of HindIII, 8uL of Fast Digest Green Buffer, and 24 uL of diH20.
3. Each of the 7 tubes, which contained 4 uL of DNA, had 6 uL of the digest cocktail mix added into them.
4. They were left to incubate in a water bath for 30 minutes at 37° C.
5. The digests were run on an E-gel for 30 minutes.

Results:

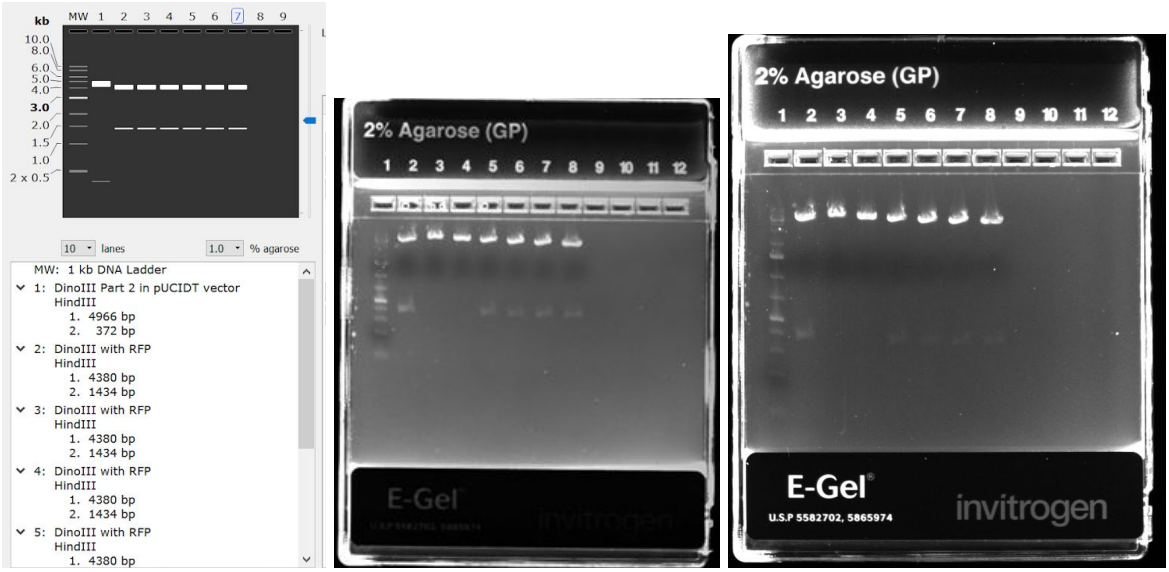

FIGURE **Restriction Digests of DinoII with RFP**
a.) The expected gel with DinoIII GFP in lane 2 and the DinoIII RFP Minipreps in lanes 3-8. Lanes 3-8 contained the DinoIII RFP minipreps in numerical order. b.) The gel ran for 20 minutes. c.) The gel was run for another 10 minutes for better separation of bands.

Conclusion:

DinoIII with GFP was used as a control to see if the DinoIII with RFP was successfully ligated and present.

Name: Asma and Kennex
Date: 8/8/19

Goal:

1. Miniprep on DinoIII with RFP Overnights

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. **Deviations**: We did not allow columns to sit for a minute.
l. Concentrations recorded for each sample.

Results:

Blanked with IDTE		
Samples	Conc. (ng/uL)	Purity Ratio
1	158	1,800

2	150	1.875
3	153	1.795
4	158	1.969
5	170	1.838
6	130	1.926

Blanked with EB		
Samples	Conc. (ng/uL)	Purity Ratio
1	150	1.875
2	148	1.844
3	143	1.839
4	153	1.794
5	170	1.789
6	130	1.857

Conclusion:
Although the samples did not sit for a complete minute once the EB was added, the concentrations still looked satisfactory. The overnights would have most likely given us higher concentrations had the columns sat at the end of the day.

Name: Krithika Karunakaran
Date: 8/8/19

Goal:

1. Ethanol precipitation on C.O. RFP Digest

Materials:
3M Sodium Acetate
100% ethanol
70% ethanol
Deionized Water

Protocol:

Ethanol Precipitation

1. 6μL of 3M sodium acetate, 150μL of 100% ethanol, and 60μL of the C.O. RFP digest were all added into an eppendorf tube
2. The tube was placed in the freezer for 20 minutes
3. It was then centrifuged at 17,000 rpm for 15 minutes at room temperature
4. The liquid was drawn out and 75μL of 70% EtOH was added to the tube with the pellet and then spun down for 10 minutes at 17,000 rpm
5. Tube placed in vacufuge for 20 minutes at 45°C and then air dried overnight
6. 20μL of diH2O was added to the tube and the pellet was resuspended
7. The tube was placed in a microcentrifuge for 10 minutes at 17,000 rpm and then the pellet was resuspended
    a. Can be found in pink “2019 iGEM Synbiodinim” box

Name: Chiara
Date: 8/8/19

Goal:

1. Glycerol stocks of DinoIII-RFP from 8/6/19 transformations

Protocol:

Glycerol Stocks

1. Take 1 mL of 50% glycerol and 1 mL of the overnight culture (after incubation) and add to a glycerol stock tube.
2. Label with your name, date, and the contents and store in the -80° C freezer in CLSO 442
    a. DinoIII-RFP glycerol 8/8/19 C.B.

Name: Chiara
Date: 8/8/19

Goal:

1. Send for sequencing
    a. DinoIII-GFP sample # 3 from 7/17/19 miniprep
    b. DinoIII-RFP sample #1, 2, & 3 from 8/8/19 miniprep
    c. Codon-optimized RFP sample # 7 from 7/24/19 miniprep

Protocol:

1. Diluted all the samples to reach a concentration of about 100 ng/µL with a final volume of 20-21 µL
    a. DinoIII-GFP: 11 µL DNA; 10 µL DI H_2O
    b. Codon-optimized RFP: 9 µL DNA; 12 µL DI H_2O
    c. DinoIII-RFP
        i. #3: 14 µL DNA; 7 µL DI H_2O