Name: Chiara Brust, Kennex Lam, Sijia Qin, Jiazi Tian, Laura Das Neves
Date: 7/11/19

Goals:

1. Minipreps and glycerol stocks of overnight cultures of codon optimized RFP from 7/10/19
2. Restriction digest of codon optimized RFP mini preps
3. Gel electrophoresis of restriction digest codon optimized RFP
4. Culture algae in ASP-8A with seawater.
5. Make plates for Symbiodinium culture.

Name: Chiara
Date: 7/11/19

Goal:

1. Glycerol stocks of codon optimized RFP

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

Name: Laura Das Neves, Chiara Brust
Date: 7/11/19

Goal:

1. Mini preps of codon optimized RFP from transformations done on 7/9/19

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 resuspended until one eppendorf tube contains the pelleted cells resuspended in 250 µL Buffer P1.
c. Added 250 µL of Buffer P2 and inverted 5 times.
d. Add 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 centrifuged for 60 seconds and discarded the excess liquid.
g. Added 500 µL of PB and centrifuged the spin columns for 60 seconds. Discarded the flow through.
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. Allowed the spin column to stand for one minute and then centrifuged for one minute.
l. Recorded the concentrations for each sample.

Results:

Codon optimized RFP

Sample (Colony #)	**Concentration (ng/μL)**	**260/280**
1	29.0	1.933
2	19.5	1.857
3	36.0	1.895
4	20.0	1.905
5	21.5	1.870
6	13.5	2.077
7	48.5	1.830
8	22.5	1.957
9	22.0	1.833
10	59.5	1.831

Conclusion:

Colonies 7 and 10 are very promising. A restriction digest will be performed to verify if this is the correct plasmid.

Additional note for next time: warm up EB buffer before the late elution step for a possible higher yield/ concentration.

Name: Jiazi Tian, Sijia Qin
Date:7/11/2019

Goal:

1. Culture algae in ASP-8A with seawater.
2. Make plates for Symbiodinium culture.

Protocol:

Culture algae in ASP-8A with seawater

10ml ASP-8A medium with seawater and 1 ml algae samples.
25ml ASP-8A medium with seawater and 2 ml algae samples.
75ml ASP-8A medium with seawater and 3 ml algae samples.

In total, 9 samples were made and covered with foil.

Make plates for Symbiodinium culture

1. 200ml filter-seawater and 2g bacto-agar was autoclaved.
2. After it cooled, 200ul f/2 medium was added
3. Poured 20ml of this mixture to make 2 control plates
4. 20ul Chloramphenicol, 20ul Ampicillin, 20 ul kanamycin were added separately to make other 6 plates.
5. After 24 hours, spread symbiodinium to these plates.

Results:

Conclusions:

Kennex and Laura will plate them with Symbiodinium

Name: Chiara Brust
Date: 7/11/19

Goal:

1. Restriction digest of codon optimized RFP from today's mini preps

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:

N/A

Name: Chiara Brust
Date: 7/11/19

Goal:

1. Run gel electrophoresis of restriction digest on codon optimized RFP from minipreps done today

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

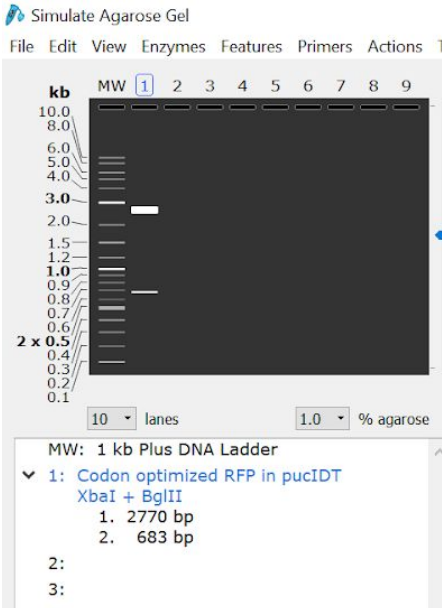
Loading

1. Loaded ~5 µL of the ladder in the first well
2. Prepared your 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 45 minutes at 120 V

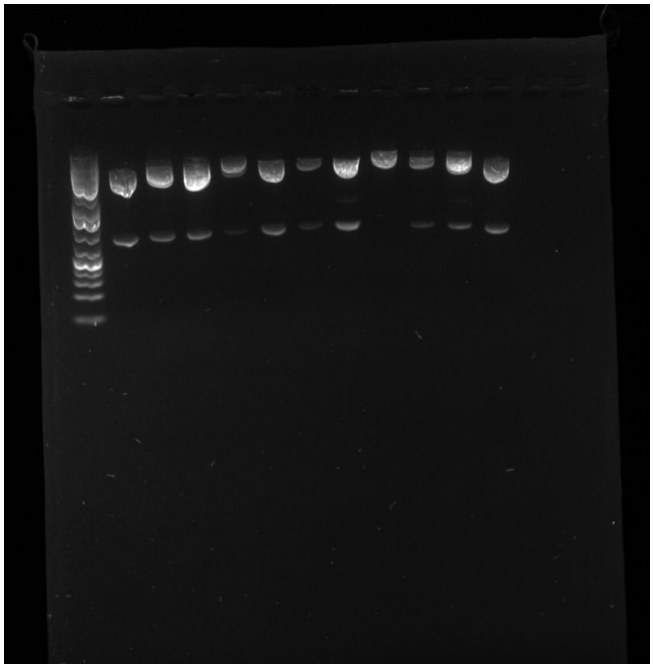
Expected Results:



Results:

Gel Key

Lane #	**Sample**
1	1 Kb Plus MW DNA Ladder
2	Colony 1
3	Colony 2
4	Colony 3
5	Colony 4
6	Colony 5
7	Colony 6
8	Colony 7
9	Colony 8
10	Colony 9
11	Colony 10
12	Colony 1 redo (Pierced lane 2 well)



Conclusion:

Although the gel is not totally clear, the digest appears to have worked. We can now proceed to ligate RFP and psb1c3 together.