Name: Kennex Lam, Chiara Brust, Jaizi, Sijia, Rehmat
Date: 7/3/19

Goals:

1. Create new media for algae
2. Glycerol Stocks
3. PCR analysis of pcb302 in E. Coli from papers 1 & 2
4. Transform K592015 into E. coli
5. Miniprep pcb302 in E. Coli from papers 1 & 2
6. Feed O. *marina*

Name: Kennex Lam
Date: 7/3/19

Goal:

1. Filter and autoclave seawater

Protocol:

Filter and Autoclave Saltwater

1. 2 flasks of 1000 mL were filtered using vacuum filtration and 0.22 um Millipore filtration paper.
2. Filtered saltwater was put into autoclave.

Conclusion:

The filtered saltwater overboiled in the autoclave. One flask lost 200 mL, and the other lost 100 mL. However, both solutions will still be used for mediums that will be made tomorrow. The ASP-8A and F2 medias will be mixed with the L1 media to see if this will help culture S. Microadriaticum.

Name: Kennex Lam
Date: 7/3/19

Goal:

1. Prepare L1 media

Protocol:

L1 Media

1. 1 mL of L1 stock media was put into 1000 mL of filter, autoclaved saltwater.

Name: Kennex Lam
Date: 7/3/19

Goal:

1. Miniprep on pCB302 in A. Tume transformations

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 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. 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:

The concentrations were too low to even be read on the spectrophotometer.

Conclusion:

A restriction digest will be done tomorrow to see DNA bands.

Name: Rehmat Babar
Date: 7/3/19

Goal:

1. Miniprep pcb302 in E. coli cultures

Materials:

QIAprep Spin Miniprep Kit Lot 160021667

Protocol:

QIAprep Spin Miniprep

a. Centrifuged the overnight culture of bacterial overnight culture at 8,000 rpm for 3 minutes at room temperature.
b. Discarded the supernatant and resuspended pelleted bacterial cells in one tube with 250 µL Buffer P1 and transferred it 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.
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k. Allowed the spin column to stand for one minute and then centrifuged for one minute.
l. Recorded the concentrations for each sample.

Results:

Sample	Concentration
pCB302-gfp-MBD plasmid Colony 1 75 µL plate	10 ng/µL
pCB302-gfp-MBD plasmid Colony 2 75 µL plate	10 ng/µL
pCB302-gfp-MBD plasmid Colony 3 75 µL plate	10 ng/µL
pCB302-gfp-MBD plasmid Colony 4 75 µL plate	15 ng/µL
pCB302-gfp-MBD plasmid Colony 5 75 µL plate	10 ng/µL
pCB302-gfp-MBD plasmid Colony 6 75 µL plate	10 ng/µL
pCB302-gfp-MBD plasmid Colony 7 75 µL plate	17.5 ng/µL
pCB302-gfp-MBD plasmid Colony 1 150 µL plate	17.5 ng/µL
pCB302-gfp-MBD plasmid Colony 2 150 µL plate	7.5 ng/µL
pCB302-gfp-MBD plasmid Colony 3 150 µL plate	12.5 ng/µL
pCB302-gfp-MBD plasmid Colony 4 150 µL plate	12.5 ng/µL
pCB302-gfp-MBD plasmid Colony 5 150 µL plate	7.5 ng/µL
pCB302-gfp-MBD plasmid Colony 6 150 µL plate	12.5 ng/µL
pCB302-gfp-MBD plasmid Colony 7 150 µL plate	5 ng/µL

Conclusion

We will do a restriction digest on these tomorrow to see if we get the bands placed correctly.

Name: Chiara
Date: 7/3/19

Goals:

1. Gel electrophoresis of colony PCR on pcb302 in E. Coli

Protocols:

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 remove the comb

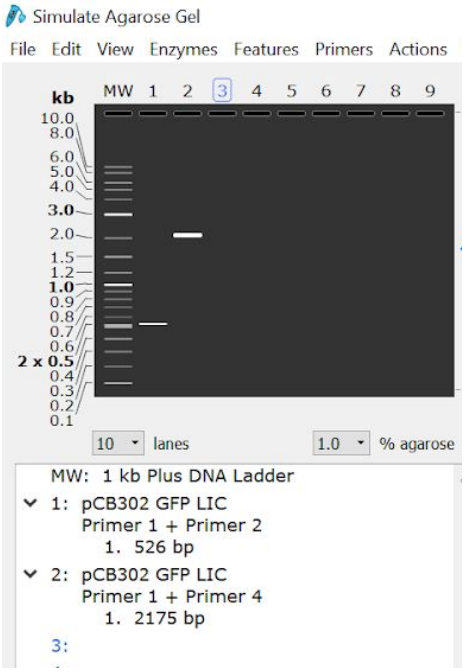
Loading

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

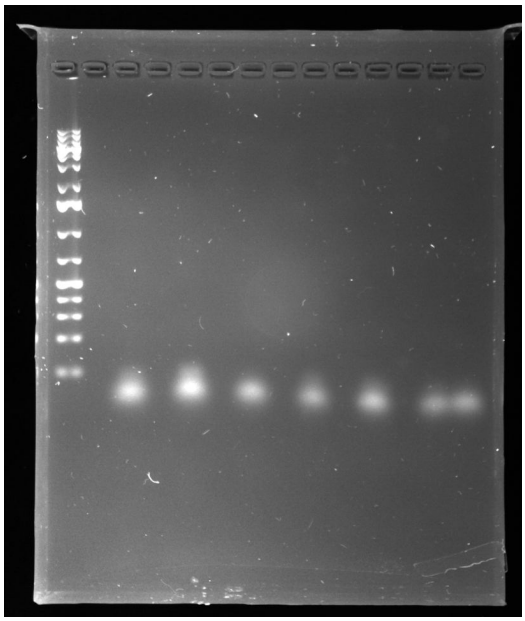
1. Once the gel had been loaded, slid on the cover making sure the negative electrode is closest to the DNA and the positive electrode is at the bottom of the gel
2. Ran at 95 V for 1.5 hours

Expected Results:

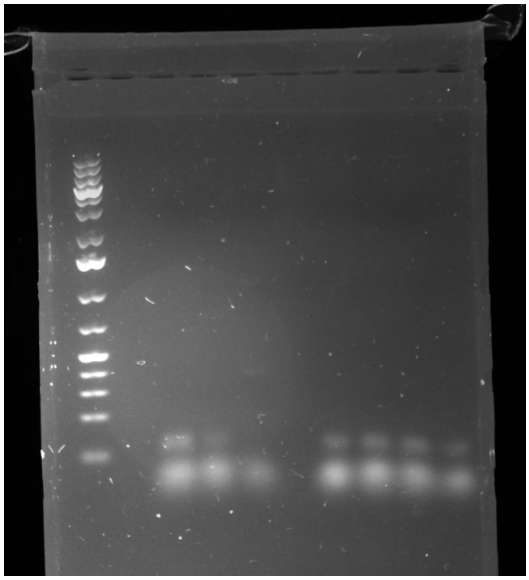


Results:

Pcb302 Primers 1 & 2



Pcb302 Primers 1 & 4



Conclusion:

The primers formed dimers. It is possible that the primers' sequences are wrong because they were based on a hypothetical plasmid. We could try to use known restriction sites to design the primers instead. However, another PCR reaction may be warranted since the previous PCR reactions were using incorrect annealing temperatures.

Name: Chiara Brust
Date: 7/3/19

Goal:

1. Transform K592015 into E. Coli

Materials:

- One Shot Top 10 chemically competent cells

Protocol:

Transformation Heat Shock

1. Thawed One Shot TOP10 chemically competent cells on ice.
2. Added 2 µL of DNA sample into competent cells
    a. K592015
    b. Puc19 negative control
    c. RFP construct in psb1C3 positive control
3. Incubated the cells on ice for 35 minutes.
4. After the ice incubation, placed the samples into a 42° C water bath for 30 seconds.
5. **Quickly** took them out and **immediately** added 250µL of SOC medium
6. Placed the samples into a 37° C shaking water incubator for 1 hour at 300 rpm.
7. After shaking for 1 hour, spread 150 µL of the solution onto an agar plate with chloramphenicol antibiotics.
8. Incubated plates at 37°C for at least 24 hours.

Results:
N/A

Conclusion:

N/A

Name: Justin Benton
Date: 3 Jul 19
Protocol:

Feeding of O. *marina*

1. 3mL of D. *tertiolecta* were added to the 75mL culture of O. *marina*.
2. 1mL of D. *tertiolecta* was added to the 25mL and to the 75mL O. *marina.*

Name: Amirah Hurst
Date: 7/3/19

Goal:

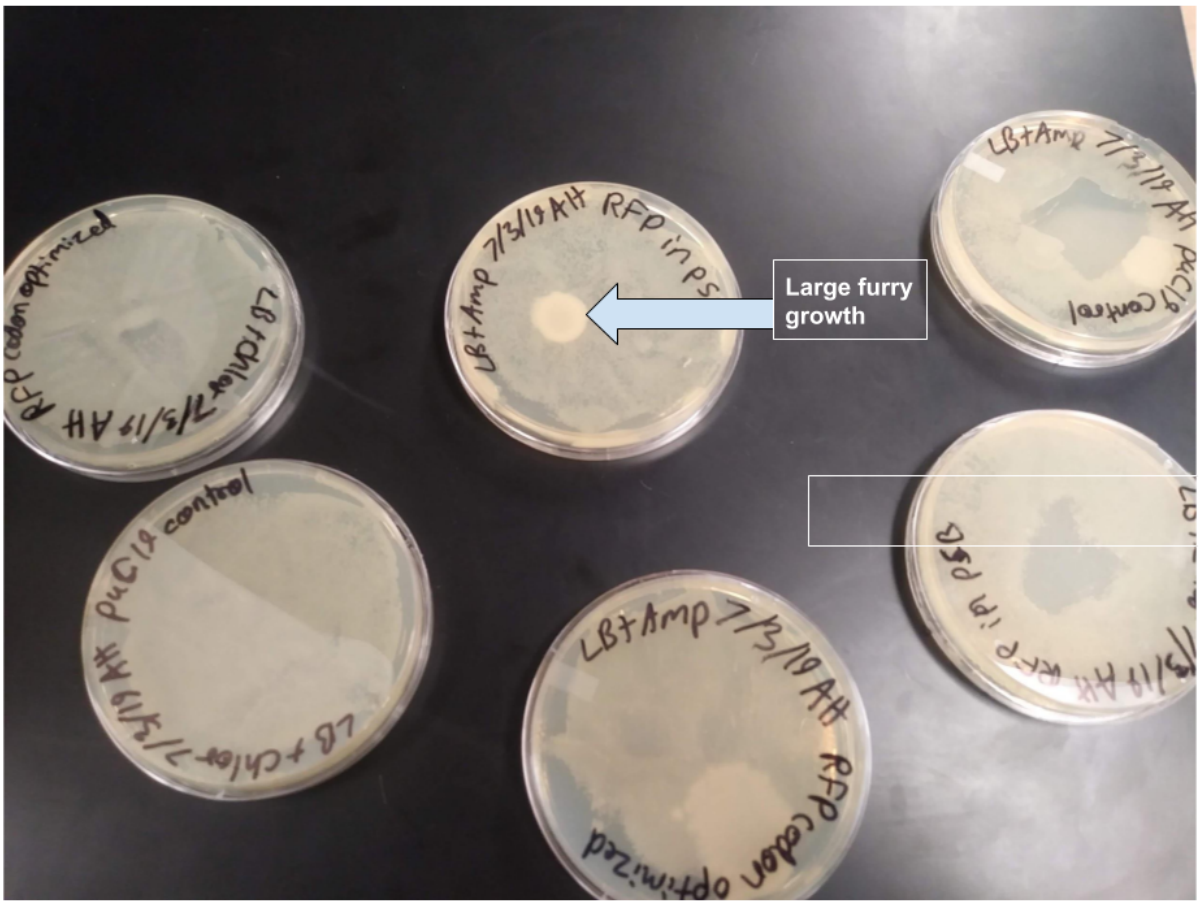
1. Transform electrocompetent E. coli cells with codon optimized RFP and K592015

Protocol:

- Home made electrocompetent cells were thawed on ice
- 40 ul of those electrocompetent cells were mixed with 1 ul of DNA in a 1.5 ml eppendorf tube and placed on ice for about 5 mins
    - 4 transformations were done:
        - RFP codon optimized
        - pUC19 (positive control)
        - RFP construct in psb1C3
        - K592015 (blue chromoprotein)
- The transformations were transferred into an electroporation cuvette
- Electroporated by placing the cuvette into the Bio-Rad MicroPulser and delivering the electric shock.
- Immediately added 950 ul of SOC medium to the cells in the cuvette
- Used sterile pipet to mix solution in cuvette and transfer back into eppendorf tubes
- Incubated transformation in 37°C water bath shaking at 300 rpm for ~ 3 hrs
- Made new LB plates:
    - 40 g of LB powder
    - 1L ddh2o
    - Dissolved with stir bar and stir plate
    - Autoclaved
        - Liquid cycle
        - 15 mins
        - Allowed to cool
    - Microwave autoclaved a 500ml flask
        - Ddh2o 6 mins in microwave
    - Poured 500 ml of autoclaved LB into 500 ml flask
    - Added 500 ul of chloramphenicol to 2 L flask
    - Added 500 ul of ampicillin to 500 ml flask
    - Swirled
    - Poured into plates
    - Allowed to congeal
- Plated 150 ul of each transformation onto both ampicillin and chloramphenicol plates
- To serve as negative control for one another
- Placed in incubator at 37°C.

Results: **Observed on 7/5/19**

All plates had growth. Ampicillin plates had a large furry growth in the middle.



Conclusion:

I cannot tell if the transformations worked because there was overgrowth on all of the plates. The RFP codon optimized and pUC19 (positive control) should have grown on amp plates but not chloramphenicol plates and the RFP construct in psb1C3 and K592015 (blue chromoprotein) should have grown on chloro plates but not amp plates. The (over) growth on all plates suggests that the growth seen may have been unintentional and that the plates were contaminated. Some of the colonies on the plates may have been the desired transformed cells, but it cannot be confirmed based off these results.