Name: Chiara Brust, Kennex Lam, Laura Das Neves
Date: 6/26/19

Goal:

1. Run gel
    a. Restriction digest of pcb302 from miniprep colony 7 in E. Coli from papers A & B(6/20/19)
    b. PCR (6/25/19) of ligations (K592009 + J23102)
2. Check on all three algae
3. Filter some of the seawater
4. Check ligation transformations from 6/25/19 for growth
5. O. Marina
    a. Fed 5 mL of D. Tertiolecta

Date: 6/26/19

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1. Run gel
    a. Restriction digest of pcb302 from miniprep colony 7 in E. Coli (6/20/19)
    b. PCR (6/25/19) of ligations (K592009 + J23102)

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 GelGold 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 the 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

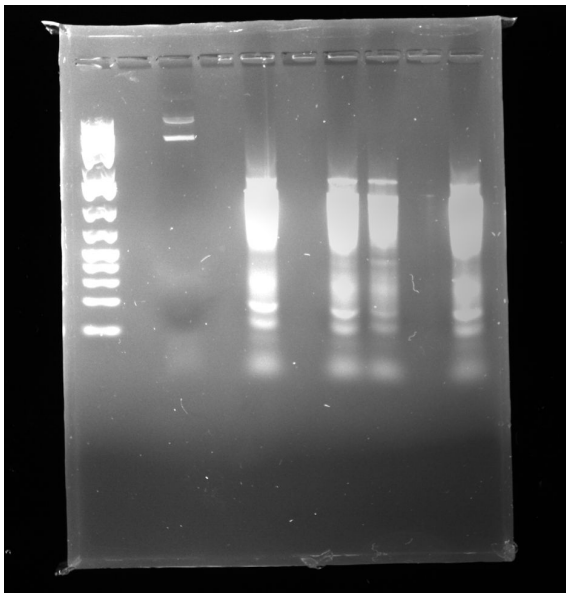
1. Once the gel had been loaded, slid on the cover making sure the negative electrode is closest to the DNA and the positive electrode is at the bottom of the gel
2. Ran at 119 V for about 45 minutes to an hour

Results:

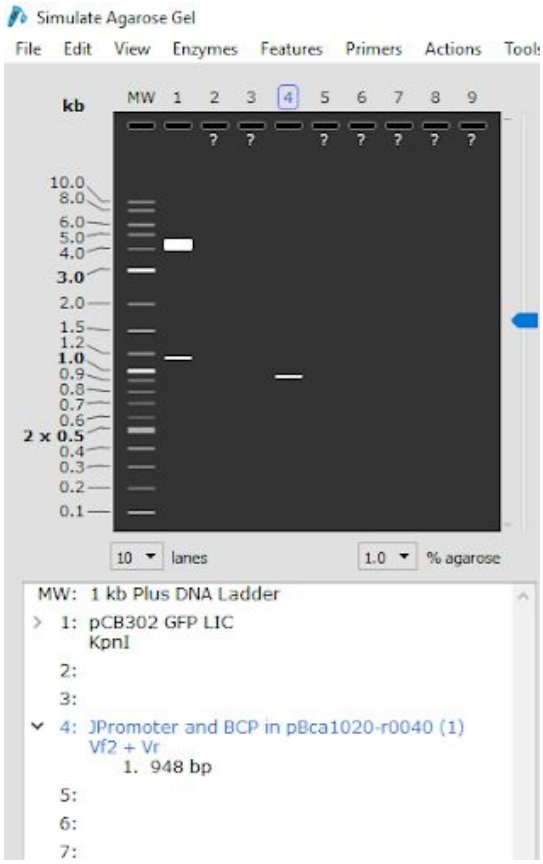
Gel Key

Lane #	**Sample**
1	MW 1 Kb Plus DNA ladder
2	Blank
3	Pcb302 Restriction digest with KpnI
4	Blank
5	Ligation 1, 100 µL, Colony 7
6	Blank
7	Ligation 1, 150 µL, Colony 11
8	Ligation 2, 100 µL, Colony 7
9	Blank
10	Ligation 2, 100 µL, Colony 12

Gel Pcb302 R.D. and Ligation of BP and promoter



Expected Results



Conclusion:

Pcb302 is much larger than expected according to this gel. We created that plasmid on snapgene based on assumptions from the article it was derived from (“Heterologous DNA Uptake in Cultured Symbiodinium spp. Aided by Agrobacterium Tumefaciens”). If the plasmid’s actual sequence is different from the one designed on snapgene, the restriction digest would have produced results unlike those simulated.

Furthermore, several bands were produced by the ligation PCRs, which should be impossible since the reaction should only amplify one region of the plasmid. It is possible that the annealing temperature is too low, which would explain why the primers seem to be binding to more than just the regions of interest.

Date: 6/26/19

Goal:

1. Filter some of the seawater

Protocol:

Filtered seawater

1. Two 500 mL volumes of seawater were vacuumed filtered using a 0.22um Millipore filter paper.
2. Both flasks were autoclaved.

Date: 6/26/19

Goal:

1. Verify algal life

Protocol:

Algae Verify life

1. Use a plastic pipette to add one drop of algae to the microscope slide
2. Check for movement in all three algae cultures using magnifications 4 and 10

Results:

Algae

- All three cultures moved on the slides
- When D. tertiolecta was mixed with O. Marina on a slide,
    - O. Marina sped up and exhibited a larger range of motion
    - We were able to visualize green circles similar in shape and size to D. Tertiolecta within O. Marina

Conclusion:

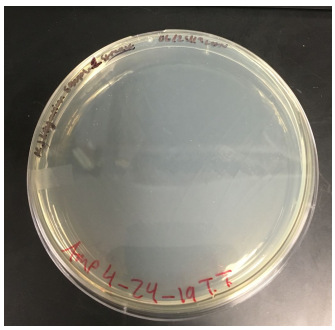
- All three algae cultures seem to be alive based on their movement.
- O. Marina may be consuming D. Tertiolecta as anticipated

Date: 6/26/19

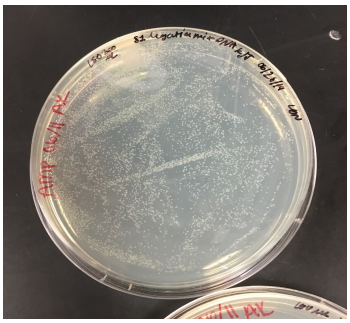
Goal:

1. Observe the plates of the transformations done on the ligation of K592009 & J23102

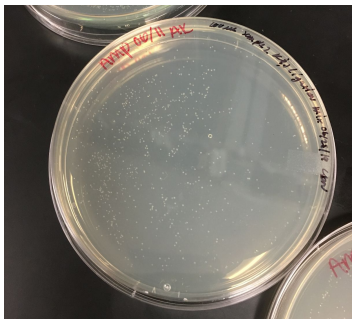
Results:



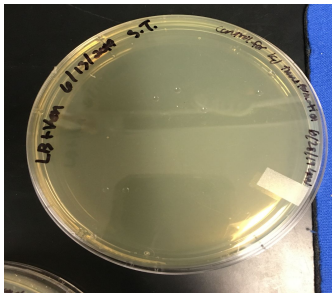
Sample KJ1-streak



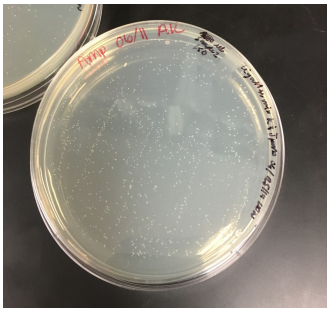
Sample KJ1 150 microliters



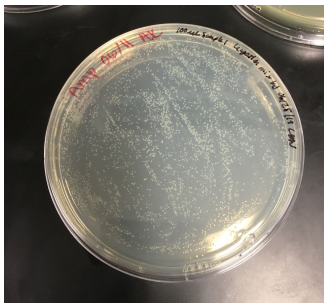
Sample KJ2 100 microliters



Sample KJ1 on Kanamycin plate (control)



Sample KJ2 150 microliters



Sample KJ1 100 microliters

Conclusion

Since there is no visible growth on the control plate and lots of growth on all of the others, the transformation was most likely successful using the KJ Ligation DNA parts. The plates were placed in the fridge, and colonies will be picked for overnight cultures and colony PCR.