Name: Rehmat Babar
Date: 10/19/19

Goal:

1. Ethanol precipitate DinoIII-RFP

Protocol:

1. Added 1:10 ratio of Sodium Acetate: Gel extraction volume
2. Added chilled ethanol in a ratio of 2 times the volume of the gel extraction
3. Centrifuged at 13,000 rpm for 30 minutes
4. Removed supernatant, being careful not to disturb the clear pellet
5. Resuspended in 200 µL of 70% chilled ethanol
6. Centrifuged for 15 minutes at 13,000 rpm
7. Removed supernatant
8. Air dried under hood overnight
9. Resuspended in 100 µL of EB
10. Measured the concentration

Results:

Sample	**[DNA]**
DinoIII-GFP	ng/µL
DinoIII-RFP	ng/µL

Conclusion:

We now have µg of DinoIII-RFP ready to transform.

Name: Kennex Lam
Date: 10/19/19

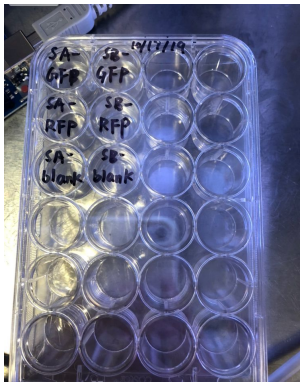
Goal:

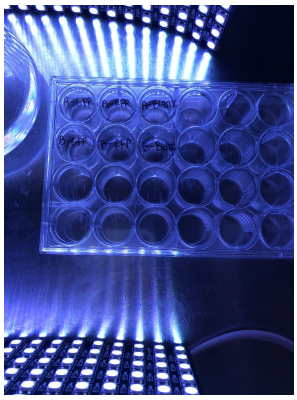
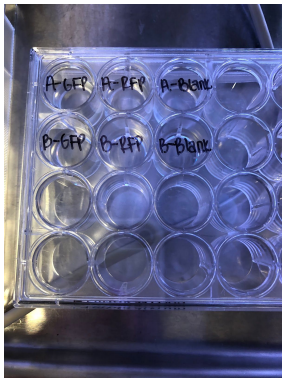
1. Observation of Transformed Algae

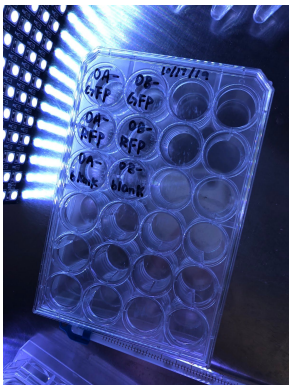
Protocol:

1. Observe under a microscope.

Results:





* The blanked out ones were not viewed.

SA GFP	-
SA RFP	3 Symbiodinium were swimming in circles. https://www.youtube.com/watch?v=vHc2ZdOgslM https://www.youtube.com/watch?v=6Ha1-5feamo
SA Blank	Two were non-motile while two were motile.
SB GFP	20 uL were loaded and 6 in total were found swimming (2 swimming in loops). https://www.youtube.com/watch?v=cQdbDgyLKt8
SB RFP	-
SB Blank	-
O. marina A GFP	Nothing was seen except for organelles.
O. marina A RFP	-
O. marina A Blank	Nothing was seen except for organelles.

O. marina B GFP	-
O. marina B RFP	Nothing was seen.
O. marina B Blank	-
S. Microadriaticum A GFP	-
S. Microadriaticum A RFP	-
S. Microadriaticum A Blank	Some organelles were floating around but 3 were seen swimming in circles.
S. Microadriaticum B GFP	-
S. Microadriaticum B RFP	-
S. Microadriaticum B Blank	-
OA GFP	Nothing was seen.
OA RFP	-
OA Blank	-
OB GFP	-
OB RFP	-
OB Blank	Nothing was seen.
Lambert Bead Beating	20 uL were loaded, and 4 could be seen alive while 4 were immobile. https://www.youtube.com/watch?v=vMIEH03G4KQ https://www.youtube.com/watch?v=vMIEH03G4KQ
Commercial Bead Beating	Only organelles with agrobacterium caught in them were seen. https://www.youtube.com/watch?v=KdY2gFSQlk8

Conclusions:

In regards to the bead beating, the reason the Symbiodinium may not have survived the transformation with the commercial bead beater is due to the commercial one being faulty and shaking too aggressively. The machinery appeared to have been broken, but the transformation was still done. With the loaned Lambert High School bead beater, the algae may have been

transformed successfully as some algae were still swimming, but we would have to test if the transformation was actually successful by looking at the Symbiodinium's fluorescence under a fluorescent-detecting microscope. The Lonza transformations on O. marina did not appear to work as even the blanks lacked viable cells. This is most likely due to being too aggressive in one of the steps. However, the S. microadriaticum that were viewed did showcase some viable cells, so there is a chance that those cells may also have successfully transformed.