Name: Kennex, Rehmat
Date: 8/5/19

Goal:

1. Linearize Dino III with GFP miniprep
2. Culture a Larger Volume of Symbiodinium and Oxyrrhis Marina
3. Filter and Autoclave Seawater

Name: Rehmat Babar
Date: 8/5/19

Goal:

1. Restriction Digest on
    a. Dino III mini preps from 7/17/19 samples #1-#10 to linearize the plasmid

Protocol:

Restriction Digest Protocol

30 µL Fast Digest Restriction Digest

1. Prepared a 11X Fast Digest concentration cocktail: 22 µL EcoRI, 88 µL of 10X Fast Digest Buffer, and 55 µL of diH2O.
2. Add 15 µL of this cocktail to a clean 1.5 Eppendorf tube and then add 15 µL of DNA.
3. Incubate at 37° C for 30 minutes.

Name: Kennex Lam
Date: 8/5/19

Goal:

1. Create larger cultures of Symbiodinium Microadriaticum and Oxyrrhis Marina
2. Filter and autoclave seawater

Protocol:

S. Microadriaticum in 400 mL ASP-8A with DI H2O

1. 15 mL of stock S. Microadriaticum was added into 400 mL of ASP-8A medium and placed next to a window.

O. Marina in 500 mL Autoclaved, Filtered Seawater

1. 15 mL of stock O. Marina was added into filtered seawater and placed next to a window.

Filter and Autoclave Seawater

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

Results:

FIGURE 1. S. Microadriaticum in 400 mL of ASP-8A and O. Marina in 500 mL of autoclaved, filtered seawater.

Conclusion:
Larger volumes of cultures are needed to be prepared due to transformation protocol calling for a high cell density due to low successful gene expression probability (reference Nuclear gene…). S. Microadriaticum was cultured in a lower volume simply because that was all that was remaining of unused ASP-8A medium. The Symbiodinium seem to reproduce the most in the ASP-8A medium, so that also played a factor. One F2 medium with O. Marina cells in 500 mL will be made within this week. Another culture of 500 mL ASP-8A can be made for Symbiodinium. About 3000 mL of seawater was autoclaved and filtered today for later use.