Name: Chiara Brust, Krithika Karunakaran, Laura Das Neves
Date: 8/28/19

Goal:

1. Transform DinoIII-GFP into O. Marina
2. Transform original mcherry
3. Pellet & Resuspend pcb302 after fresh media

Name: Chiara Brust
Date: 8/28/19

Goal:

1. Transform DinoGFP into O. Marina

Protocol:

1. Cultured *Symbiodinium Microadriaticum* cells in ASP-8A with filtered seawater medium under natural sunlight for about 2 months and *O. Marina* cells in f/2 media for about 2 months with no antibiotics

- Cultures:
  - ii. O. Marina: f/2 , FSW media 75 mL labeled 6/28/19; [cell]= 1.8 x10^5 cells/mL

2. Harvested the cells by centrifugation at 400 g for 5 min at 4°C.

   b. O. Marina: Pelleted 2 mL= 3.6 x10^5 cells

3. Used 500 µL of 0.1M EDTA to resuspend the cell pellet

4. Centrifuged at 400 g for 2 min at 4°C.

5. Washed cells with 10% Glycerol 3 times, centrifuged at 400 g for 2 min in 4°C.

6. Re-measured the cell concentration of each sample

Results:

After step 5, we no longer saw any O. Marina cells under the microscope

Conclusion:

Because a pellet was seen after the first centrifugation, it is not likely that the centrifugation speed is causing the cells to burst. Instead, the resuspensions may have disrupted the cells enough to burst them. Next time, we should resuspend by simply swirling around the liquid rather than pipetting up and down.

Name: Laura Das Neves
Date: 08/13/19

Goal:

1. Transform original mCherry Part into One Shot Top10 chemically competent cells

Protocol:

Heat Shock

1. Thawed One Shot TOP10 chemically competent cells on ice.
2. Added 2 µL of DNA sample into competent cells
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 200 rpm.
7. After shaking for 1 hour, streaked 150 µL of the solution onto an agar plate with the respective antibiotics **(ampicillin)**.
8. Incubated plates at 37°C for at least 24 hours.

Conclusion:

No growth after ~16 hours of incubation because mcherry was plated on ampicillin plates. Redo transformation with electrically competent cells and chlor plates.

Name: Krithika Karunakaran

Date: 8/28/19

Goal:

1. Pellet & Resuspend pcb302 after fresh media

Protocol:

1. 3 pCB302 tubes' ODs were checked:
    a. 0.872, 0.813, 0.815
2. The pcb302 tubes were spun down in the centrifuge at 5000xg for 5 minutes
    a. 4 tubes were thrown out due to clumping
3. The supernatant (media) was poured out and 7mL of fresh YM media and (7µl) Kanamycin was added
4. The pellet was resuspended using a 2mL pipette
5. The resuspended pCB302 was placed in the back shaker at 30 °C at 220rpm at 6:30pm
    a. The OD should be checked after ~24 hours