Name: Chiara, Kennex, Rehmat, Amirah, Shakera, Xuecheng Ye Yilin Lu Zexi Guo Jiayi Lan Yujie Huang
Date: 8/21/19

Goal:

1. Transform DinoIII-GFP into S. Microadriaticum and O. Marina
2. Finish DinoIII-RFP ethanol precipitation
3. New overnights of mcherry from glycerol stocks
4. Measure the ABS600 and fluorescence of the overnight culture Interlab

Name: Chiara
Date: 8/21/19

Goal:

1. Transform DinoIII-GFP into S. Microadriaticum and 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:
    - S. Microadriaticum: ASP 8a-SW media 75 mL labeled 8/21/19; [cell]= 2.52 x10^6 cells/mL
    - ii. O. Marina: f/2 , FSW media 75 mL labeled 6/28/19; [cell]= 3.8 x10^5 cells/mL

2. Harvested the cells by centrifugation at 800 g for 5 min at 4°C.

    a. S. Microadriaticum: Pelleted 5 mL= 12.6 x10^6 cells

    b. O. Marina: Pelleted 1 mL= 3.8 x10^5 cells

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

4. Centrifuged at 800 g for 2 min at 4°C.

5. Washed cells with 10% Glycerol 3 times, centrifuged at 800 g for 2 min in 4°C.

6. Re-measured the cell concentration of each sample

    a. [S. Microadriaticum]= 5.0 x10^6 cells/mL

    b. [O. Marina]= 0 cells":s\[PTRSGO|

i. O. Marina seem to have either died and bursted or were lost in a step. Therefore, we discontinued the protocol for this sample

7. Resuspended the pellet in 1 mL of 10% Glycerol1

8. Incubated ~100µl of cells with 17 µl (~1µg) of DinoIII-GFP DNA (60 ng/µL 8/16/19 ethanol precipitation sample) on ice for 5 min.

9. Put cells into a 0.2 cm cuvette, and electroporated using SHS (2.0 kV, 1 pulse), SC2 (1.5 kV, 1 pulse), or DIC (1.0 kV, 2 pulses, 1.0 msec) program with Bio-Rad MicroPulser 165-2100.

10. Added 1mL of ASP-8A SW medium to the 0.2 cm cuvette, mixed well, and transferred to a 15 mL tube

11. Left the cultures to grow under the programmed lights

12. Observed the cells under a normal microscope in 1-3 days and according to the need.

Name: Shakera
Date: 8/21/19

Goal:

1. New overnights of mcherry from glycerol stocks

Protocol:

1. Scraped ice from mcherry glycerol stocks and placed in a solution consisting of 7 µL LB and 7 µL ampicillin
2. Let incubate overnight at 37 degrees C

Name: Xuecheng Ye Yilin Lu Zexi Guo Jiayi Lan Yujie Huang

Goal:

1. Measure the ABS600 and fluorescence of the overnight culture

Results:

LB	positive control	control	device 1	device 5
colony1	0.149	0.165	0.157	0.170
	0.158	0.172	0.163	0.175
	0.158	0.174	0.170	0.173
	0.158	0.179	0.163	0.171
colony2	0.198	0.197	0.207	0.206
	0.210	0.210	0.213	0.209
	0.210	0.207	0.208	0.212
	0.214	0.207	0.206	0.209

Table 1: The ABS600 of the overnight culture in LB medium

	positive conyrol	negetive control	device1	device 5	YM medium

colony 1	0.111	0.109	0.149	0.141	0.041
	0.099	0.111	0.149	0.142	0.045
	0.108	0.114	0.142	0.145	0.043
	0.099	0.115	0.171	0.143	0.045
colony 2	0.128	0.145	0.138	0.143	0.045
	0.130	0.138	0.132	0.146	0.047
	0.134	0.143	0.131	0.141	0.046
	0.133	0.142	0.130	0.155	0.052

Table2: the Abs600 of overnight culture in YM medium

	positive	negetive	device 1	device 5	YPD medium
colony 1	0.206	0.192	0.155	0.200	0.053
	0.207	0.184	0.159	0.202	0.052
	0.200	0.193	0.147	0.198	0.052
	0.197	0.181	0.142	0.190	0.053
colony 2	0.206	0.214	0.202	0.189	0.051
	0.207	0.214	0.200	0.194	0.051
	0.200	0.203	0.198	0.186	0.081
	0.197	0.204	0.185	0.189	0.052

Table 3: the Abs600 of overnight culture in YPD medium

—	positive	negetive	device 1	device 5	TB medium

colony 1	0.274	0.294	0.238	0.302	0.059
	0.294	0.304	0.246	0.292	0.059
	0.302	0.305	0.248	0.309	0.058
	0.277	0.286	0.241	0.302	0.056
colony 2	0.246	0.252	0.225	0.242	0.058
	0.242	0.266	0.236	0.246	0.059
	0.241	0.257	0.232	0.258	0.058
	0.252	0.256	0.223	0.241	0.059

Table4 : the Abs600 of overnight culture in TB medium

__	positive	negetive	device1	device5
colony 1	23122	17274	18319	18383
	23330	17980	17996	19002
	23416	17618	18460	19288
	22707	17638	18186	19015
colony 2	27916	17312	19609	18248
	28484	18485	18452	18774
	29335	17821	18420	18806
	29018	18313	18564	18542

Table5 : the fluorescence of overnight culture in LB medium

__	positive	negetive	device 1	device 5	YM medium
colony	6881	6551	10220	6544	6380

1	6992	6459	10057	6478	6527
	6818	6930	9351	6678	6520
	6893	6528	10984	6499	6638
colony 2	6551	6433	9372	10138	6619
	6738	6194	9263	10907	6636
	6395	6367	8891	10744	6525
	6823	6638	8909	11096	6850

Table6 : the fluorescence of overnight culture in YM medium

—	positive	negetive	device 1	device 5	YPD medium
colony 1	19512	19361	19857	19447	19807
	19522	18431	20573	19712	19358
	19696	19178	19152	19675	18729
	19051	18257	19330	18473	18371
colony 2	19317	19041	20068	19763	19247
	19514	19347	20403	19822	19762
	19588	18751	20459	19214	38173
	18614	18552	19638	18759	19743

Table7 : the fluorescence of overnight culture in YPD medium

—	positive	negetive	device 1	device 5	TB medium
colony 1	27036	25805	39170	26497	24979

	27089	26100	39941	24860	24569
	27210	25849	39884	25485	23808
	25944	24820	39088	24538	23824
colony 2	26819	25522	40757	37385	25328
	26155	25852	40950	37091	24987
	26068	25564	40793	37177	24399
	26130	25114	39834	35650	24819

Table8 : the fluorescence of overnight culture in TB medium

Conclusion:

It seems device 5 colony 1 didn't grow well in any kind of medium. In comparison, the TB medium is better for the culture.