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Date: 9/19/19

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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 100 mL labelled 8/12/19; [cell]= 1.0 x10^5 cells/mL

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

   a. S. Microadriaticum: Pelleted 10 mL 3 samples = 1.0 x10^6 cells/mL

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

   a. [S. Micro]= 2.58 x10^6 cells/mL
   b. [S. Micro]= 2.78 x10^6 cells/mL
   c. [S. Micro]= 2.00 x10^6 cells/mL

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

5. Washed cells with 500 µL of 10% Glycerol 3 times, centrifuged at 800 g for 2 min in 4°C.

6. Resuspended the pellet in 50 µL of 10% Glycerol

   a. [S. Micro]= 1.18 x10^6 cells/mL
   b. [S. Micro]= 5.2 x10^5 cells/mL
   c. [S. Micro]= 1.32 x10^6 cells/mL

8. Incubated ~40µl of cells with no DNA 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

   a. [S. Micro]= 8.0 x10^4 cells/mL
   b. [S. Micro]= 1.2 x10^5 cells/mL
   c. [S. Micro]= 4.0 x10^4 cells/mL

11. Left the cultures to grow under natural sunlight

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