

## **CULTURE AND FREEZING SYMBIODINIUM**

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Cultures were kindly provided by Dr. Scott Santos (Auburn University).

Table 1. List of the cultures that are currently been kept at IMAges LAB. *Symbiodinium* clades A-E (confirmed by ITS2-DGGE and ITS2-HRM).

<b>Culture ID</b>	<b>Invert Host</b>	<b>Geographic Location</b>	<b>Isolated by</b>	<b>18S-rDNA RFLP</b>
FLAp1 10AB	Aiptasia pallida	Long Key	S.R. Santos	A
2a	Plexaura kuna	Three Sisters	M.A. Coffroth	A
708	Plexaura kuna	San Blas	M.A. Coffroth	A
719	Pseudoplexaura porosa	San Blas	M.A. Coffroth	A
Mf 12.5f	Montastrea faveolata	Florida Keys	M.A. Coffroth	A
Ap (HIAp)	Aiptasia pulchella	Kaneohe Bay	R.A. Kinzie	B
PurPflex	Plexaura flexuosa	TN Reef	S.R. Santos	B
PeSC166	Pseudotergorgia elisabeth	San Salvador	S.R. Santos	B
13 (Pk13)	Plexaura kuna	Three Sisters	M.A. Coffroth	B
703 (Pk702)	Plexaura kuna	San Blas	M.A. Coffroth	B
Gv 5.6c	Gorgonia ventlina	Florida Keys	M.A. Coffroth	B
Mf 1.5b	Montastrea faveolata	Florida Keys	M.A. Coffroth	B
FLAp2 10AB	Aiptasia pallida	Long Key	S.R. Santos	B+
PtBr	Briareum sp.	Long Key	S.R. Santos	C
Pd 45a	Porites asterodies	Florida Keys	M.A. Coffroth	C
Mf 8.3Tb	Montastrea faveolata	Florida Keys	M.A. Coffroth	C
Pd 44b	Porites divaricata	Florida Keys	M.A. Coffroth	C
A001	Acropora sp-0	Okinawa	M. Hidaka	D
A013	Porites annae	Okinawa	M. Hidaka	D
A014	Porites australiensis	Okinawa	M. Hidaka	D
Mf 10.8a	Montastrea faveolata	Florida Keys	M.A. Coffroth	D
Ap2 (Ua#2)	Unknown anemone	Sesoko Jima	S.R. Santos	D+
Ap37	Unknown anemone	Sesoko Jima	S.R. Santos	D+
CCMP421	Acquired from CCMP	Wellington Harbor	Bigelow	E

## **NEW CULTURES OF *SYMBIODINIUM* ON A MONTHLY BASIS (from the month before)**

We have been doing two sets of cultures.

### Reagents

- F/2 media (original stock 50X)
- Filtered seawater

### Materials

- 25mL flasks

### Procedure

1. Prepare F/2 media 1X with filtered seawater and keep at room temperature or 25°C.
2. Label 25mL flasks for the new cultures.  
Note: Our labels contain the name of the culture, the date (month, day), and the room or place where the culture is been kept.
3. Add 15mL of F/2 1X media (Sigma, 50X) to new flask.
4. Close old culture tightly.
5. Mix the old culture, in between gentle and hard in order to resuspend the *Symbiodinium*.
6. Transfer 10 $\mu$ L of old culture to the corresponding new flask.
7. Fill old culture to 15mL with F/2 1X.
8. Leave caps of both cultures loose.
9. In incubator, place them in their sides, with the cap facing up.
10. *Symbiodinium* is kept on a photoperiod 12 hours:12 hours light:dark, at  $\sim 80$  photons  $\text{m}^{-2} \text{s}^{-1}$ , 25°C.
11. Left F/2 media can be kept at -20°C for the next month.

## **FREEZING CULTURES OF *SYMBIODINIUM* ON A MONTHLY BASIS (from the oldest cultures)**

### Reagents

- 100% Ethanol

### Materials

- 15mL falcon tubes
- 1.5mL tubes
- Transfer pipettes
- Centrifuge with the rotor for the 15mL falcon tubes
- Extra 15mL falcon tubes to balance the centrifuge
- Container to discard F/2 after centrifugation

### Procedure

1. Label a set of falcon tubes, one per culture, with the “code” (e.g. S213) and the name of the culture (e.g. PeSc166).
2. Label a set of 1.5mL tubes with the code (e.g. S213) on the cap and on the side.
3. Bring the oldest cultures.
4. Close tightly and vigorously shake until resuspending completely the culture. If needed, use a transfer pipette.
5. Transfer all the culture to the 15mL falcon tube.
6. Repeat with all cultures.
7. Centrifuge at 1,500 rpm for 10min.
8. Immediately, discard liquid of all tubes (to avoid resuspending the pellet).
9. Take ~1mL of 100% Ethanol with a transfer pipette.
10. Resuspend the pellet and transfer to corresponding 1.5mL tube.
11. Repeat with all the samples.
12. Place 1.5mL tubes in a Ziploc bag labeled with the range of the codes (e.g. S200-S222).
13. Store at -20°C.