

Protocol for Anemone Stress Challenge

Each table will receive one genet (clonal population) of at least 4 anemones.

1) Before transferring anemones, score each individual using the Coral Health Chart provided.

Take photos of your anemones with reference chart in background.

2) Label your Falcon tubes with genet ID (location), section number, table number, student name and treatment. E.g. ARB/U02/T3/Josh/Menthol

3) Each student will transfer 40ml of seawater into 50ml calibrated Falcon tube.

Note: For students working on Menthol, use 40ml of pre-made mentholated seawater instead.

4) Each student will transfer one anemone using a cut-off transfer pipette/q-tips to a

Falcon tube containing 40ml of seawater/mentholated seawater.

5) Loosely cover the top of each tube in plastic wrap. Place each tube in the appropriate incubator for seven days.

6) A student is assigned to incubate his/her anemone in one of the following treatments:

-32°C

-34°C

-25°C (0.19mM Menthol*)

-25°C (control)

Note: *Any student absent from the lab should be covered by the group members*

7) Return any remaining anemones to your TA (there should be at least one remaining anemone) to preserve the lineage. Never mind if you do not have any left.