

Culturing Symbiodiniaceae

Last edited: Feb 2018 – HKU Baker Lab

- Preparing Artificial Seawater + growth media (ASW+)
 1. Weigh Instant Ocean sea salt (~35g/L)
 2. Add Instant Ocean to DI water
 3. Mix well on stir-plate with magnetic stir bar
 4. Check salinity with refractometer: 35 ppt
 5. Add 1ml/L NaNO₃, 1ml/L NaH₂PO₄ 1ml/L f/2 Trace metal, and 0.5ml/L f/2 Vitamin into the seawater (f/2 medium)
 6. Filter with autoclaved filtration set and 0.22um membrane filter paper (dull size up)
 7. Pour filtrate in a clean autoclaved bottle (check autoclave tape)
 8. DONE!

- Culturing
 1. Collect cultures from the plant incubator at 7/F
 2. Clean fumehood with alcohol
 3. Turn on the UV light for 5-10 minutes
 4. Label new Erlenmeyer flasks
 5. Pour 100mL ASW+ into flasks
 6. Choose a patch and scrap the culture from bottom while withdrawing with a pipette (~100uL)
 7. Transfer population into the new flask
 8. Place new cultures in incubator
 9. DONE!

- Cleaning and preparation
 - 250mL Erlenmeyer flask
 1. Clean old culture flasks with detergent and hot water
 2. Rinse and soak in 1% HCL acid bath overnight
 3. Rinse with DI water and dry
 4. Furnace at 500 degree for 4-5 hours
 - Filtration set and bottle
 1. Rinse the equipment with water
 2. Soak in 1% HCL acid bath overnight
 3. Rinse with DI water and dry
 4. Autoclaved