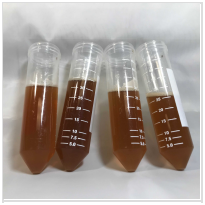

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Isolation of Symbiodiniaceae from scleractinian coral hosts suitable for cryopreservation and laser-warming

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This protocol is used to obtain a clean isolate of Symbiodiniaceae extracted from their coral hosts. The freshly isolated symbionts can then be cryopreserved or used for other experiments. This protocol is targeted towards Symbiodiniaceae that we are currently not able to maintain in culture conditions (e.g., most *Cladocopium* species), but can be used to extract culturable Symbiodiniaceae, as well.

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Troubleshooting

- If your Symbiodiniaceae cell suspension contains a lot of mucus or feels very sticky throughout the isolation, add additional cleaning cycles (i.e. centrifuging, resuspending, homogenising and measuring photosynthetic yield) between rounds of filtering
- If by the end of the Symbiodiniaceae protocol (between the 70-µm and the 20-µm filtering), the Symbiodiniaceae still feel sticky and are not easily resuspended with the pipette, add a full rinsing cycle using 1.5-mL Eppendorf tubes. After a centrifugation round, resuspend the pelleted symbiont in minimal FSW, homogenise in the tissue grinder, and distribute the homogenised sample in 1.5-mL tubes, as concentrated as possible. Spin the tubes for 3 min at RCF = 6100 x g. Gently remove the supernatant with a pipette, and using the 200 µl pipette, gently add 200 µl FSW, without disturbing the pellet. Then, still using the 200 µl pipette, gently blow FSW on the surface of the pellet to detach the white/yellow layer at the top of the pellet. Pipette out and discard the FSW with the white / yellow cells. Resuspend the cleaned pellet in FSW and resume the protocol.
- If you are finding skeleton shards in your sample even though you completed step 4, prior to step 6 centrifuging, gently spin the 50-mL tubes containing the blastate in a centrifuge for 3-5 min at RCF = 300 x g to force the skeleton shards to the bottom of the tubes without concentrating the algal symbionts into a pellet. Using a 1-mL pipette or a transfer pipette, gently transfer the blastate solution to clean 50-mL tubes without disturbing the bottom of the tubes and making sure not to pick up any skeleton shards in the process. Leave as much blastate as needed from the bottom as necessary to avoid stirring up the skeleton shards from the bottom. Adapt the centrifuge settings if needed, as different species might require a stronger or lighter spin.

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MATERIALS

Biological Material

- Coral fragments (~10 cm diameter, 3-5 genotypes)

Solutions for Symbiodiniaceae Isolation and Culture

- 0.22 µm filtered seawater (FSW), freshly collected and filtered each day
- Guillard's F2 Marine Water Enrichment Solution(G0154, Sigma-Aldrich)
- Polne-Fuller antibiotic cocktail (Polne-Fuller 1991, https://doi.org/10.1111/j.0022-3646.1991.00552.x)

Glassware and Consumables

- Clean bowl (15-20 cm diameter) or Ziploc bag
- 500-mL glass beaker
- 500-mL glass Erlenmeyer flask
- 50-mL conical Falcon tubes
- Wide 200-µl strainer (approx. 100-mm diameter; can be made by hand with fine mesh fabric and cut PVC pipe)
- 100-µl cell-strainer basket (Corning, NY, USA, #CLS431752)
- 70-µl cell-strainer basket (Corning, NY, USA, #CLS431751)
- 40-µl cell-strainer basket (Corning, NY, USA, #CLS431750)
- 20-µl cell strainer basket (Pluriselect, Leipzig, Germany, SKU 43-50020-03)
- Filter paper or circle of fine mesh fabric (< 200 µm) with diameter at least 2x the diameter of the Erlenmeyer mouth
- Rubber band
- Transfer pipettes
- 1-mL pipette tips
- Optional: 1.5-mL Eppendorf tubes (for sticky / mucus-heavy samples only)
- Optional: 200-µl pipette tips (for sticky / mucus-heavy samples only)

Equipment

- Dental Water Flosser (Water Pik, Fort Collins, CO, USA)
- Centrifuge for 50-mL Falcon tubes
- 15-mL Dounce tissue grinder (Wheaton, DWK Life Sciences, Millville, NJ, USA) – use the loose pestle
- Lightweight 1-mL pipette for resuspending Symbiodiniaceae pellets (the lightweight model eases hand manipulation stress during repetitive movements)
- Junior Pulse Amplitude Modulated fluorometer (Junior PAM, Walz, Effeltrich, Germany)
- Optional: Centrifuge for 1.5-mL Eppendorf tubes (for sticky / mucus-heavy samples only)
- Optional: 200-µl pipette (for sticky / mucus-heavy samples only)

Tips

- While running the Symbiodiniaceae isolation protocol, if a break (> 10 min) is needed, ensure to leave the algal symbiont solution sufficiently diluted. We recommend aiming for a volume of 30-40 mL in a falcon tube, angled at ~30-45°
- Use clean 50-mL falcon tubes at every step
- Thoroughly clean and dry the tissue grinder between each cycle
- Pre-wet all filters with FSW before use

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Isolation of Symbiodiniaceae Day 1

1 Select 3-5 genotypes for each target species to ensure a sample representative of the Symbiodiniaceae commonly found in that coral species.

2 Take a photosynthetic yield measurement of each genotype using a PAM fluorometer to use as reference control value throughout the Symbiodiniaceae isolation protocol.

3 Blast coral tissue containing algal endosymbionts from the skeleton of each coral by using a dental flosser filled with FSW and collect the blastate (coral tissue and symbionts suspended in FSW) in a bowl or Ziploc bag. If isolating symbionts from a mushroom coral (e.g., *Lobactis scutaria*) stay away from the mouth of the polyp to minimise mucus in the blastate and to allow for recovery of the fungiid. Aim for 250-900 mL of total blastate.

4 Extra step for corals with brittle skeletons, such as some *Pocillopora* and *Acropora* species: filter the blastate through a 200-µm filter to exclude any skeleton shards. In step 8, pay attention to any remaining skeleton shards. See the Guidelines, *Troubleshooting* section for further steps to remove skeleton shards. Step 9 can be skipped.

5 Pour the blastate, including the mucus, in 50-mL falcon tubes. Balance the tubes so that they have similar volumes of liquid and mucus using a transfer pipette.

6 Spin the samples in their 50-mL tubes, in a centrifuge for 10 min at RCF = 2300 x g.

7 For each 50-mL tube: transfer the floating raft (above the supernatant) into a 15-mL glass tissue grinder, discard the supernatant, and resuspend the pellet in 1-2 mL FSW using a 1-mL pipette. Add the resuspended pellet to the floating raft in the glass tissue grinder.

8 Add some FSW to the sample and homogenise the contents of the glass tissue grinder using the loose pestle until the solution appears homogeneous. This may take some time for coral species that naturally harbour a lot of mucus such as *Lobactis* and *Porites* species, and may be faster for other species such as *Acropora* and *Pocillopora* species (pers. obs.)

9 Transfer the homogenised solution through a 200-µm filter and into a glass beaker.

10 Repeat steps 7-9 for each 50-mL tube.

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11 Transfer the filtered solution into clean 50-mL falcon tubes (aim for maximum 6 tubes).

12 Take a photosynthetic yield measurement in one of the falcon tubes using a PAM fluorometer, and record the value to ensure the symbionts are still healthy and yielding photosynthetic values near control levels.

13 Spin the 50-mL tubes in a centrifuge for 10 min at RCF = 2300 x g.

14 For each 50-mL tube: discard the supernatant and resuspend the pellet in 1-2 mL FSW using a 1-mL pipette. Transfer all resuspended pellets to a clean glass tissue grinder.

15 Add some FSW to the sample if needed and homogenise the contents of the glass tissue grinder using the loose pestle until the solution appears homogenous.

16 Transfer the homogenised solution through a 100-µm filter and into a clean 50-mL tube (aim for 1-2 tubes).

17 Take a photosynthetic yield measurement using a Junior PAM fluorometer, and record the value.

18 Spin the 50-mL tube(s) in a centrifuge for 10 min at RCF = 2300 x g.

19 For each 50-mL tube: discard the supernatant and resuspend the pellet in 1-2 mL FSW using a 1-mL pipette. Transfer all resuspended pellets to a clean glass tissue grinder.

20 Add some FSW to the sample if needed and homogenise the contents of the glass tissue grinder using the loose pestle until the solution appears homogenous.

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21 Transfer the homogenised solution through a 70-µm filter and into a clean 50-mL tube (aim for 1 tube).

22 Take a photosynthetic yield measurement using a Junior PAM fluorometer, and record the value.

23 Spin the 50-mL tube in a centrifuge for 10 min at RCF = 2300 x g.

24 For each 50-mL tube: discard the supernatant and resuspend the pellet in 1-2 mL FSW using a 1-mL pipette. Transfer all resuspended pellets to a clean glass tissue grinder.

25 Add some FSW to the sample if needed and homogenise the contents of the glass tissue grinder using the loose pestle until the solution appears homogenous.

26 Transfer the homogenised solution through a 40-µm filter and into a clean 50-mL tube (aim for 1 tube).

27 Take a photosynthetic yield measurement using a Junior PAM fluorometer, and record the value.

28 Spin the 50-mL tube in a centrifuge for 10 min at RCF = 2300 x g.

29 For each 50-mL tube: discard the supernatant and resuspend the pellet in 1-2 mL FSW using a 1-mL pipette. Transfer all resuspended pellets to a clean glass tissue grinder.

30 Add some FSW to the sample if needed and homogenise the contents of the glass tissue grinder using the loose pestle until the solution appears homogenous.

31 Transfer the homogenised solution through a 20-µm filter and into a clean 50-mL tube (aim for

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a final volume of 50 mL / 1 tube).

32 Take a photosynthetic yield measurement using a Junior PAM fluorometer, and record the value.

33 Prepare an Erlenmeyer flask with 50 mL of double-strength Guillard's F2 Marine Enrichment Solution containing double-strength Polne-Fuller antibiotic cocktail.

34 Gently add the Symbiodiniaceae solution to the Erlenmeyer while swirling the Erlenmeyer. The total volume should add up to 100 mL.

35 Close the opening of the Erlenmeyer flask with a filter paper or a circle of fine mesh fabric and tie it down with a rubber band.

36 Place overnight in an incubator at 24-26°C with a day/night light cycle.

Isolation of Symbiodiniaceae Day 2

37 The next morning, resuspend the Symbiodiniaceae solution using a 1-mL or a transfer pipette, and pour into 2-3 50-mL falcon tubes.

38 Spin the 50-mL tubes in a centrifuge for 10 min at RCF = 2300 x g.

39 For each 50-mL tube: discard the supernatant and resuspend the pellet in 1-2 mL FSW using a 1-mL pipette. Transfer all resuspended pellets to a clean glass tissue grinder.

40 Add some FSW to the sample if needed and homogenise the contents of the glass tissue grinder using the loose pestle until the solution appears homogenous.

41 Transfer the homogenised solution through a 20-µm filter and into a clean 50-mL tube (aim for max 50 mL / 1 tube).

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42 Take a photosynthetic yield measurement using a Junior PAM fluorometer, and record the value.

43 Your Symbiodiniaceae solution is now ready. Use immediately for experiments, dilute to 50 mL in FSW if to be used later in the day, or dilute to 100 mL in an Erlenmeyer flask in Guillard's F2 Marine Enrichment Solution if to be used on a later day.

44 Depending on the Symbiodiniaceae community composition, the solution will remain stable for at least 2-3 days.

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