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Isolation of axenic Symbiodinium cultures

In 1 collection

Aiptasia Symbiodiniaceae Model System

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MATERIALS

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50g Streptomycin Sulphate USP Grade **G-Biosciences Catalog #RC-197**

Agar-Agar **Contributed by users**

Ampicillin, sodium salt USP **Bio Basic Inc. Catalog #AB0028.SIZE.25g**

Kanamycin sulfate **Bio Basic Inc. Catalog #KB0286.SIZE.100g**

Homogenizer **Fisher Scientific Catalog #PowerGen 125**

DAPI **Thermo Fisher Scientific Catalog #D1306**

4% Paraformaldehyde (PFA) Solution in PBS **Boster Bio Catalog #AR1068**

CORAL PRO SALT **Contributed by users Catalog #R_99344**

DAIGOS IMK MEDIUM FOR MARINE MICROALGAE **Contributed by users Catalog #398-01333**

BEFORE START INSTRUCTIONS

Before starting, make 10X sterile Daigo solution in artificial seawater (ASW):
 Dissolve 2.56 g Daigo powder in 1L ASW and filter sterilize through a 0.22 µm filter.

Purify algae from anemone host

- Several anemones were disrupted using a glass homogenizer so as to leave the algal cells largely intact.

- 2 The algal cells were pelleted by centrifugation at 1,000g and then washed by resuspension and repelleting the cells several times in filter-sterilized ASW containing 50µg mL⁻¹ kanamycin, 100µg mL⁻¹ ampicillin, and 50µg mL⁻¹ streptomycin antibiotics KAS (Kan, Amp and Strep)

Plating algal cells on solid medium

- 3 Prepare solid IMK medium: add 1g Agar into 90 mL ASW, and autoclave at 122°C for 30 minutes.
- 4 After autoclave, let it cool down to around 65 °C, add sterile 10X Daigo's IMK Medium stock solution as well as KAS antibiotic cocktail: 50µg mL⁻¹ kanamycin, 100µg mL⁻¹ ampicillin, and 50µg mL⁻¹ streptomycin.
- 5 The algae in liquid solution were then spread onto solid IMK + KAS medium.

Note

1. To be able to have single colonies for later pick up or purification, plating different amount of cells are recommended.
2. Wrap plates in parafilm to prevent evaporation/drying of the medium.
3. Expected amount of time needed for visible colony growth is around 30 days, although some stains may take more or less time.

- 6 Prepare a flame-sterilized inoculation loop, allowed to cool to room temperature for use;
- 7 Then individual colonies were picked with the inoculation loop under microscope and streaked onto solid IMK + KAS medium.
- 8 Repeat step 8 procedure for several times until a clean single colony was formed. Those are potentially axenic colnal colonies.

Verify clonal and axenic Symbiodinium culture

9 Identify if the colonies are bacteria-free by transferring them to rich solid medium Marine Broth (MB). If there is bacterial or other contaminant, they will grow on MB plate.

10 If there is no contamination on MB plate, further verify by PCR using specific bacterial 16S primers. Extract genomic DNA from the colony/cells grown on the plate.

PCR reactions contain 0.3 M of each primer in 2X GoTaq Master Mix (Promega, Madison, WI, USA) and were performed by incubating at 94°C for 5 min followed by 30 cycles of 94°C for 30 s, 50°C for 1 min, and 72°C for 1.5 min, with a final incubation at 72°C for 5 min.

Note

16S bacterial primers:

first set:

63F: 5'-CAGGCCTAACACATGCAAGTC-3'

1542R: 5'-AAGGAGGTGATCCAGCCGCA-3'

second set:

27F: 5'-AGAGTTTGATCCTGGCTCAG-3'

1492R: 5'-GGTTACCTTGTTACGACTT-3'

11 Further verify the axenic culture by imaging the cells under the microscope.

- (1) Fix the cells by using 4% PFA for more than one hour in 4°C;
- (2) Wash the cells using PBS buffer for 6 times;
- (3) Stain the cells with DAPI;
- (4) Examine the cells by bright-field and fluorescence microscopy. If the culture is axenic, bacteria would not be observed in the culture medium or associated with the Symbiodinium cells.

Note

Here is an example image of Symbiodinium cell from an axenic culture stained with DAPI.

Scale bar: 3 μ m

- 12** To identify the Symbidonium, PCR using 18S and Chloroplast Cp23S sequences to assign the clades.

Detailed protocol can be found in the following link.

Note

Primer sequences:

18S-F: 5'-GGTTGATCCTGCCAGTAGTCATATGCTTG-3'

18S-R: 5'-GATCCTTCCGCAGGTTACCTACGGAAACC-3'

Cp23S-F: 5'-GACGGCTGTAACCTATAACGG-3'

Cp23S-R: 5'-CCATCGTATTGAACCCAGC-3'

<https://www.protocols.io/view/symbiodinium-cp23s-rflp-and-sequence-analysis-qyidxun>