Dinoflagellate isolation from cnidarians host tissue procedure

**Preparation of cultures**

1. Homogenize cnidarians host tissue in 5-10 ml of Sigma-Guillard's (F/2) medium (Sigma-Aldrich).
2. Filter the homogenate using a 100 μm nylon mesh to a final volume of 10-15 ml.
3. Add EDTA to a final concentration of 0.005M.
4. Recover alga from tissue homogenate by centrifugation for 5 min at 5000 rpm.
5. Wash algal pellets in 5 to 10 ml of fresh f/2.
6. Place in a 35 mm Petri dish for picking.
7. From this stage on all procedures will be treated as sterile and performed under a laminar flow hood.

**Picking and first stage growth technique**

2. Transfer individual cells into single wells of a 96 well cartridge (0.3 ml PCR plates are recommended) pre-filled with f/2 solution (add Antibiotic Antimycotic Solution (100x) (Sigma-Aldrich)).
3. Place the plate in culture room or incubator in the temperature range that best fits the host environment under a 12:12 h light dark cycle for 3-7 days.
4. Check growth and viability of the culture using a microscope (and a microscope PAM).

**Large Subunit rRNA-Encoding DNA (rDNA) Sequencing**

1. Isolate a single dinoflagellate cell from the selected well (as described above) and extract to 1 μl of TE buffer and freeze until analysing.
2. Add Proteinase K (1 II 200 μg/ml), maintain tubes at 55 °C for 50 minutes with a UNOII Thermoblock (Biometra). Incubate at 95 °C for 10 minutes to inactivate the proteinase K and facilitate DNA denaturation. Cool to 4°C in preparation for PCR amplification. Standard PCR amplification of nuclear ribosomal DNA was performed by using two sets of primers: (i) S-DINO (cgctcctaccgattgtag) and L-DIN-1.

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**Equipment**: P-2000 micropipette puller (Sutter Instrument), laminar flow hood, water bath, gloves, safety goggles and lab coat, centrifuge (any type with a ss-34 compatible frame), dissecting microscope Carl-Zeiss Ltd.

**Solutions**: Sigma-Guillard's (F/2) (Sigma-Aldrich)

**Plasticware**: 96 wells plastic case with cover
(aacgattgcaagctacgctacgc), which are Symbiodinium-specific and cover the ITS-1/5.8S/ITS-2/partial large subunit (LSU) rDNA, and (ii) D1R (acccgctgaatttaagcatat) and D2C (ccttggctctgtt), which are dinoflagellate-specific and target a 5’ fragment of the LSU rDNA. PCR products were purified by using Shrimp Alkaline Phosphatase and Exonuclease (modified from Ki et al. 2005).

**Mass culture**

1. Transfer the culture from the plate to a 25 ml Erlenmeyer flask prefilled with Sigma-Guillard’s (F/2) medium (Sigma-Aldrich) after one week. Incubate under the selected temperature and light conditions.

2. Transfer again the culture in log phase (check using a flow cytometer or measuring chl a) to a 100 ml Erlenmeyer flask with Sigma-Guillard’s (F/2) (Sigma-Aldrich). Incubate under the selected temperature and light conditions.

3. Repeat with increasing volume until reaching the desired mass.