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Coral tissue and skeleton DNA extraction

In 1 collection

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Protocol status: Working
We use this protocol and it's working

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Abstract

This extraction protocol is based on the Qiagen DNeasy PowerBiofilm kit with modifications from Sunagawa et al. 2010.

Sunagawa, S., Woodley, C. M. & Medina, M. Threatened Corals Provide Underexplored Microbial Habitats. PLoS ONE 5, e9554 (2010).

Materials

MATERIALS

- Proteinase K (20 mg/ml)
- nuclease free water
- Lysozyme
- 1000uL sterile filter tips
- DNeasy PowerBiofilm Kit **Qiagen Catalog** #24000-50
- 20ul sterile filter tips
- 200ul sterile filter tips

Equipment

NAME	BRAND	SKU
Block heater	--	--

Equipment

NAME	BRAND	SKU
24 Vortex Adapter for 2mL tubes	-	-

Equipment

Centrifuge	NAME
Benchtop Centrifuge	TYPE
Eppendorf	BRAND
5405000441	SKU
https://online-shop.eppendorf.us/US-en/Centrifugation-44533/Centrifuges-44534/Centrifuge-5425-PF-243560.html	LINK
Any benchtop centrifuge will suffice	SPECIFICATIONS

Preparation

1 If needed, prepare lysozyme by dissolving 12.5 mg of lysozyme powder (≥ 40,000 units/mg) per 1 mL of nuclease free water. Multiple aliquots can be prepared and stored at -20 °C.

2 Clean surfaces and pipettes with 10% bleach and 70% ethanol.

3 Thaw samples and lysozyme (if frozen).

4 Warm solution solution MBL at 65 °C for 00:10:00.

5 Pre-label tubes.

Extraction

6 Add between 200 µL to 400 µL of airbrushed coral tissue or 200 cm3 - 500 cm3 of pulverized skeleton to each bead tube.

The optimal amount of starting material may vary for each sample, depending on how much PBS was used to airbrush sample, the amount of material of the skeleton's endolithic community, and the coral species.

Too much starting material can result in low yields, particularly for coral species with thick tissue.

7 Add 350 mL warm MBL solution to each bead tube.

8 Add 100 µL solution FB to each bead tube. Vortex briefly.

9 Add 10 µL lysozyme (12.5mg/ml) (see Step 1) to the bead sample mixture.

Incuate at Room temperature for 00:10:00 .

10 Add 20 µL proteinase-K (20mg/ml) to each sample and incubate at 65 °C for 01:00:00 .

11 Bead beat the sample with a Vortex Adapter for 00:15:00 .

12 Centrifuge the tubes at 13000 x g, Room temperature, 00:01:00 .

13 Transfer the supernatant to a clean 2ml collection tube.

14 Add 200 µL solution IRS and vortex briefly to mix. Incubate at 4 °C for 00:05:00

15 Centrifuge the tubes at 13000 x g, Room temperature, 00:01:00 .

16 Avoiding the pellet, transfer all of the supernatant to a 2ml collection tube.

17 Add 900 µL solution MR and vortex briefly.
(Note: if solution MR has precipitated, warm at 55°C for 5-10 minutes)

18 Load 650 µL supernatant onto an MB spin column and centrifuge at 13000 x g, Room temperature, 00:01:00 . Discard the flow-through and repeat until all the supernatant has been processed.

19 Place the MB Spin Column into a clean 2ml Collection Tube.

20 Shake Solution PW. Add 650 µL solution PW and centrifuge at 13000 x g, Room temperature, 00:01:00 .

21 Discard the flow-through and add 650 µL ethanol and centrifuge at 13000 x g, Room temperature, 00:01:00 .

22 Discard the flow-through and centrifuge again at 13000 x g, Room temperature, 00:02:00 to ensure all ethanol is removed from the filter.

23 Place the MB Spin Column basket into a clean 2ml collection tube.

24 Add 60 µL to 100 µL of nuclease free water to the center of the white filter membrane, depending on expected yield and desired concentration.

Make sure entire membrane is wet. Incubate at room temperature for 00:05:00 .

25 Centrifuge at 13000 x g, Room temperature, 00:01:00 and discard the MB Spin Column.

26 Proceed to DNA quantification and dilute (if needed) with nuclease-free water.

Store at -20 °C for short term storage (e.g. to be used within the same week) or -80 °C for long term storage.