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Coral tissue and skeleton DNA extraction

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1 Works for me dx.doi.org/10.17504/protocols.io.bi9bkh2n

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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).

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MATERIALS TEXT

MATERIALS

Proteinase K (20 mg/ml) **Contributed by users**

nuclease free water **Contributed by users**

Lysozyme **Contributed by users**

1000uL sterile filter tips **Contributed by users**

DNeasy PowerBiofilm Kit **Qiagen Catalog #24000-50**

20ul sterile filter tips **Contributed by users**

200ul sterile filter tips **Contributed by users**

Block heater

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24 Vortex Adapter for 2mL tubes

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Centrifuge

Benchtop Centrifuge

Eppendorf **5405000441**

Any benchtop centrifuge will suffice

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Preparation

1 If needed, prepare lysozyme by dissolving **12.5 mg** of lysozyme powder (≥ 40,000 units/mg) per **1 mL** of

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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** .

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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.

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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.

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