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

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Virus and Prokaryote Enrichment in Coral DNA Metagenomes

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ABSTRACT

Tissue preparation and DNA extraction protocol for enrichment of viruses and prokaryotes in DNA metagenomes of corals.

IMAGE ATTRIBUTION

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Keywords: coral, bacteria, virus, bacteriophage, metagenome

MATERIALS

10X DNase Buffer:

- [M] 100 millimolar (mM) Tris-HCl (pH 7.5)
- [M] 25 millimolar (mM) $MgCl_2$
- [M] 5 millimolar (mM) $CaCl_2$

- Fresh or thawed ~1cm^3 coral sample
- Sterile mortar and pestle
- Sterile forceps
- 1.5 mL centrifuge epi tubes
- 0.02 µm filtered and autoclaved artificial seawater (ASW)
- 425-600 µm acid-washed glass beads (Sigma CAT#G9268)
- 15 mL conical centrifuge tubes
- Vortex
- Centrifuge
- Pipettes
- DNase [M] 10000 U/mL
- 100X EDTA [M] 0.5 Molarity (m)
- Sterile 10 mL Luer-Lok™ syringes
- 8 µm pore size, 25mm flat filters (Whatman, Nucleopore Track-Etched Polycarbonate (CAT#110614))
- Sterile 25mm filter holders with Luer-Lok™ connection (Swinnex CAT#SX0002500)
- Amicon™ Ultra-15 Centrifugal Filter Units (MilleporeSigma™ CAT#UFC910008)
- NucleoSpin® Tissue Kit (MACHEREY-NAGEL Inc. REF:740952.10/.50/.250)

BEFORE START INSTRUCTIONS

- Glass beads must be washed with 0.1 N HCl
- Incubator set to 56 °C & 70 °C
- Check if Buffer B5 and Proteinase K were prepared according to section 3 of NucleoSpin® Tissue Kit Manual.

Sample Preparation

3h

1 Collect a coral sample of approximately 1cm^3 containing coral tissue, mucus, and skeleton. Use

a fresh sample if possible. If not, flash-freeze the sample and keep it at -80 °C until processing. Thaw the sample on ice before proceeding.

2 Weigh and record coral fragment weight.

3 Crush the coral sample using a sterile mortar and pestle until the skeleton and tissue fragments are the size of coarse sand. Do not overdo this step. Transfer the fragments, along with the mucus (coral homogenate), to a 2 mL microcentrifuge tube.

4 Suspend sample in 100 µL of Artificial Seawater (ASW) and 0.2 g of sterile glass beads.

5 Vortex at speed 3 for 00:05:00 min to dislodge tissue and mucus. 5m
* To avoid lysis of bacterial cells, do not vortex the sample at higher speeds or longer durations than described.

6 Pulse-centrifuge the sample (3-5 seconds at 3.200 x g).

7 Collect supernatant and transfer to a clean 15 mL microcentrifuge tube.

8 Bring sample up to 1 mL with sterile ASW.

9 Add 100 µL of 10X DNase Buffer and 2 µL of DNase 10,000 U/mL for a final concentration of 20 U/mL. Mix by gently inverting the tube.

10 Incubate at room temperature for 02:00:00 hours . 2h

11 Stop DNase activity by adding 10 µL of 100X EDTA.

12 Bring sample volume up to 5 mL with sterile ASW.

13 Transfer thesample to a 10 mL syringe and push through an 8.0 µm, 25 mm flat filter, collecting the flowthrough in a clean 15 mL centrifuge tube.
* If the sample clogs the filter, replacing the 8.0 µm filter may be necessary. In this case, pull back on the syringe plunger to recollect the sample in the syringe before transferring it to a new filter.
* In the case of samples with excessive mucus, you may further dilute the sample with sterile ASW prior to filtering.

14 Transfer flowthrough to an Amicon™ Ultra-15 Centrifugal Filter Unit.

15 Centrifuge at room temperature for 00:30:00 min at ≥ 2600 x g . 30m

DNA Extraction

1h 12m

16 1. Add 200 µL Buffer T1 and 20 µL of Proteinase K from the NucleoSpin® Tissue Kit to the Amicon™ filter unit.

17 Rinse one side of the filter with the lysis buffer 3-5 times using a pipette.

18 Securely close the Amicon™ filter unit and incubate for 01:00:00 hour at 56 °C, being sure to place the tube in a position where the rinsed side of the filter remains covered in the Buffer T1 & Proteinase K mixture. 1h
* Some Amicon™ filter unit lids may not seal properly. Check for leaks before placing in the incubator. Seal with parafilm if necessary.

19 Repeat steps 17 and 18 on the other side of the filter.

20 Add 210 µL Buffer B3 to the Amicon™ filter unit and vortex to mix.

21 Incubate for 00:10:00 min at 70 °C . 10m

22 Add 210 µL ethanol (100%) to the Amicon™ filter unit and vortex to mix.

23 Proceed with steps 5-7 of the NucleoSpin® Tissue Kit Standard protocol for human or animal tissue and cultured cells.

24 On step 8 of the NucleoSpin® Tissue Kit, perform the following: 2m
a. Place the NucleoSpin® Tissue Column into a 1.5 mL microcentrifuge tube (not provided in kit)
b. Add 50 µL PCR-grade water
c. Incubate at room temperature for 00:01:00

d. Centrifuge for 00:01:00 min at 11000 x g

e. Repeat steps b, c, & d once, for a final volume of 100 uL