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Bulk gDNA extraction from coral samples

 [Evolutionary Applications](#)

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Abstract

This is a modified version of the DNA extraction methods published in:

Rowan, R., & Powers, D.A. (1991) Molecular genetic identification of symbiotic dinoflagellates (zooxanthellae). *Marine Ecology Progress Series*, 71(1), 65–73.

Baker, A.C., Rowan, R., & Knowlton, N. (1997) Symbiosis ecology of two Caribbean acroporid corals. *Proceedings of the 8th International Coral Reef Symposium, Panama 2*: 1295–1300

This protocol is used to extract and purify genomic DNA from coral samples (tissue and skeleton) that is suitable for PCR, qPCR, sequencing, and other downstream applications. DNA is recovered from the coral host, *Symbiodinium*, and other members of the holobiont.

Materials

STEP MATERIALS

⊗ Proteinase K

⊗ Chloroform

⊗ Ethanol, pure **Omnipure Filter Company Catalog #4455**

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⊗ Proteinase K

⊗ Chloroform

⊗ Ethanol, pure **Omnipure Filter Company Catalog #4455**

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Protocol materials

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1 Acquire sample material.

Small amounts of coral tissue, with or without skeleton, may be obtained by clipping off branch tips, scraping with a razor blade, etc.


Note

Greater success in downstream PCR amplification is generally achieved when the amount of starting material is small. Tissue scrapings or biopsies are ideal for the in situ digests described here using SDS and Proteinase K. Larger core samples or colony fragments are not recommended - in this case blast the tissue off the skeleton first using an airbrush or Water Pik and then centrifuge the blastate and lyse cells in 1% SDS and digest with Proteinase K.

SDS Incubation

- 2 Add sample to 500 μ L 1% SDS in DNAB in a microcentrifuge tube. Be sure that sample is fully immersed in the buffer. Incubate sample for 60-90 minutes at 65°C. Sample is now stabilized for storage at room temperature, and can be treated as an "archive" for future use. These archives can be used for multiple attempts at DNA extraction.

 500 μ L

 01:00:00

Protocol

NAME

1% SDS in DNA Buffer

CREATED BY

Andrew Baker

PREVIEW

- 2.1 Prepare stock solution of 4 M Sodium chloride in MilliQ water

 Sodium chloride **P212121**

- 2.2 Prepare stock solution of 0.5 M EDTA in MilliQ water

 Ethylenediaminetetraacetic acid

- 2.3 Mix 50 mL 4 M NaCl and 50 mL 0.5 M EDTA



- 2.4 Make up to a final volume of 500 mL with MilliQ water

MilliQ water

- 2.5 Dissolve SDS in DNA Buffer to a final concentration of 1% (w/v).
e.g., 5 g SDS in 500 mL of DNA Buffer.

Sodium Dodecyl Sulfate **P212121**

Proteinase K digest

- 3 Add 25 μ L Proteinase K (10 mg/mL) to sample archive and vortex well. Incubate overnight at 37°C, for 6-7 hours at 45°C, or for 2-3 hours at 55°C.

25 μ L

02:00:00

Proteinase K

Organic extraction

- 4 Prepare a new set of 1.5 mL tubes for the samples you intend to process, and add 100 μ L of each sample archive (in 1% SDS in DNAB) to the new set of tubes. Return the remainder of the sample archive to storage.

Note

DNA can be extracted from any quantity of sample archive. The protocol given here is written for extractions of 100 μ L of the sample archive, but volumes can be proportionally adjusted up to the second ethanol precipitation if desired (e.g., to increase absolute DNA yields).

- 5 Defrost CTAB mix (stored at -20°C) and add twice volume (200 μ L) to each sample. Vortex and incubate at 65°C for 30-60 minutes.

200 μ L

00:30:00



Protocol

NAME

CTAB mix

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PREVIEW

- 5.1 Dissolve 0.75 g CTAB in ~ 20 mL MilliQ water



1 g



Cetyltrimethylammonium bromide

- 5.2 Add 12.5 mL of 4 M NaCl

Sodium chloride **P212121**

- 5.3 Add 75 μ L of E. coli tRNA at 20 mg/mL

Note

E. coli tRNA is used as a carrier nucleic acid to boost total nucleic acid concentrations and increase the extraction yield

- 5.4 Make up to a final volume of 50 mL with MilliQ water



MilliQ water

- 6 Allow samples to cool. In fume hood, add equal volume (300 μ L) of chloroform. Be sure to 'charge' (i.e., fill and empty pipette tip with chloroform 2 to 3 times) the pipette tip before first use, or your tip will leak chloroform. Vortex sample and invert several times, but be careful that caps are tight – leaking chloroform will erase your sample labels! Put in rack on rotating platform for 2-3 hours.

300 μ L

00:05:00




Chloroform

First ethanol precipitation


- 7 Centrifuge at 10,000g (RCF) for 10 minutes. Align tubes in centrifuge so that hinges are on the outside. While spinning, prepare a new set of labeled 1.5 mL tubes. Remove

samples from centrifuge and very carefully pipette off top ~250 μL into new tube. Dispose the rest of the contents into appropriate waste container.

 00:25:00


- 8 Add twice volume (500 μL) of 100% (200-proof) ethanol (EtOH). Ensure caps are shut tightly and invert samples in their rack several times, together with a few brief shakes to make sure samples are well mixed.

 500 μL

 00:05:00

 Ethanol, pure **Omnipure Filter Company Catalog #4455**

- 9 Put samples in freezer for at least 2 hours to promote DNA precipitation. If the EtOH is pre-chilled, you can leave it in the -20°C freezer for only a 1/2 hour.


 02:00:00

Note

Potential stopping point. Keep samples in freezer to allow DNA to precipitate.


Second ethanol precipitation

- 10 Put samples in centrifuge (ensuring that the hinges of the tubes are on the outside) and spin for 10 minutes at 10,000g (RCF).

 00:10:00

- 11 Remove samples from centrifuge and carefully decant off ethanol from all the tubes into a waste container. The DNA pellet should remain stuck to the inside of the tube.

- 12 Put tubes, with their caps open, in the Vacufuge/Speedvac. Be careful when putting the tubes in and don't touch the inside of the caps. Speedvac at 45°C for 30-60 minutes.

 00:30:00

- 13 Remove samples from vacufuge and add 100 μL of 0.3 M NaOAc (do not use the stock 3 M solution!). Vortex sample well to dissolve pellet. When the pellet is dissolved the sample will appear "syrupy" and will not bounce around as droplets inside the tube.

 100 μL



Protocol

NAME

Sodium acetate solution

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Andrew Baker

PREVIEW

- 13.1 Dissolve 12.3 g of Sodium acetate (anhydrous) in ~ 40 mL MilliQ water.

 12 g Sodium acetate anhydrous

- 13.2 Adjust pH to 5.2 with glacial acetic acid

- 13.3 Make up to 50 mL with MilliQ water

 MilliQ water

- 13.4 Dilute an aliquot of the 3 M stock solution to 0.3 M for use in the DNA extraction protocol.

- 14 Once the pellet is dissolved, add 200uL of 100% Ethanol, vortex and invert several times and put in freezer for at least 2hrs.

 200 μ L

Note

Potential stopping point. Keep sample in freezer to allow DNA to precipitate

 Ethanol, pure **Omnipure Filter Company Catalog #4455****Ethanol wash**

- 15 Remove samples from freezer, and centrifuge for 10 minutes at 10,000g (RCF). Decant supernatant into appropriate waste container.
- 16 Add 100 μ L of 70% Ethanol, and vortex thoroughly (this is the "Ethanol Wash" step). Centrifuge for 10 minutes at 10,000g (RCF), and again decant supernatant into appropriate waste container.

 100 µL**Protocol**

NAME


70% Ethanol

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Andrew Baker

PREVIEW

- 17 Put samples in Vacufuge with the caps open, and speedvac at 45°C for 30-60 minutes to thoroughly dry the pellet.

 00:30:00**Resuspension of purified DNA**

- 18 Take samples out of centrifuge and add 50-100 µL TE buffer. Vortex briefly to mix and store at -20°C in freezer. Sample is now ready for PCR. Store DNA samples at -20°C.

 100 µL**Protocol**

NAME

TE Buffer

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Andrew Baker

PREVIEW