

Bulk gDNA extraction from coral samples

Andrew Baker, Ross Cunning

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.

Citation: Andrew Baker, Ross Cunning Bulk gDNA extraction from coral samples. protocols.io

dx.doi.org/10.17504/protocols.io.dyq7vv

Published: 04 Mar 2016

Protocol

Step 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.

NOTES

Andrew Baker 11 Oct 2015

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

Step 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 µl Additional info:

© DURATION

01:00:00

₹ PROTOCOL

. 1% SDS in DNA Buffer

CONTACT: Andrew Baker

Step 2.1.

Prepare stock solution of 4 M Sodium chloride in MilliQ water



Sodium chloride View by P212121

Step 2.2.

Prepare stock solution of 0.5 M EDTA in MilliQ water



Ethylenediaminetetraacetic acid by Contributed by users

Step 2.3.

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

Step 2.4.

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



MilliQ water by Contributed by users

Step 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.

REAGENTS

Sodium Dodecyl Sulfate View by P212121

Proteinase K digest

Step 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.

AMOUNT

25 µl Additional info:



Proteinase K by Contributed by users

O DURATION

02:00:00

Organic extraction

Step 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.

NOTES

Andrew Baker 11 Oct 2015

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).

Organic extraction

Step 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.

■ AMOUNT

200 µl Additional info:

© DURATION

00:30:00

PROTOCOL

. CTAB mix

CONTACT: Andrew Baker

Step 5.1.

Dissolve 0.75 g CTAB in 20 mL MilliQ water

■ AMOUNT

1 g Additional info:

REAGENTS

Cetyltrimethylammonium bromide by Contributed by users

Step 5.2.

Add 12.5 mL of 4 M NaCl

REAGENTS

Sodium chloride View by P212121

Step 5.3.

Add 75 µL of E. coli tRNA at 20 mg/mL

NOTES

Andrew Baker 11 Oct 2015

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

Step 5.4.

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



MilliQ water by Contributed by users

Organic extraction

Step 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.

AMOUNT

300 µl Additional info:

REAGENTS

Chloroform by Contributed by users

© DURATION

00:05:00

ANNOTATIONS

Stephan Bitterwolf 12 Jul 2017

This timer is incorrect. Protocol says 2-3 hrs not 5 mins.

First ethanol precipitation

Step 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 μ L into new tube. Dispose the rest of the contents into appropriate waste container.

© DURATION

00:25:00

First ethanol precipitation

Step 8

Add twice volume (500 μ L) 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.

AMOUNT

500 µl Additional info:



REAGENTS

Ethanol, pure 4455 by Omnipure Filter Company

© DURATION

00:05:00

First ethanol precipitation

Step 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.

O DURATION

02:00:00

NOTES

Andrew Baker 11 Oct 2015

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

Second ethanol precipitation

Step 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).

O DURATION

00:10:00

Second ethanol precipitation

Step 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.

Second ethanol precipitation

Step 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.

© DURATION

Second ethanol precipitation

Step 13.

Remove samples from vacufuge and add 100 μ L 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.

AMOUNT

100 μl Additional info:

₽ PROTOCOL

. Sodium acetate solution

CONTACT: Andrew Baker

Step 13.1.

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

■ AMOUNT

12 g Additional info:

REAGENTS

Sodium acetate anhydrous by Contributed by users

Step 13.2.

Adjust pH to 5.2 with glacial acetic acid

Step 13.3.

Make up to 50 mL with MilliQ water

REAGENTS

MilliQ water by Contributed by users

Step 13.4.

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

Second ethanol precipitation

Step 14.

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

AMOUNT

200 µl Additional info:

REAGENTS

Ethanol, pure 4455 by Omnipure Filter Company

NOTES

Andrew Baker 11 Oct 2015

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

Ethanol wash

Step 15.

Remove samples from freezer, and centrifuge for 10 minutes at 10,000g (RCF). Decant supernatant into appropriate waste container.

Ethanol wash

Step 16.

Add 100 µL 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.

■ AMOUNT

100 µl Additional info:

₽ PROTOCOL

. <u>70% Ethanol</u>

CONTACT: Andrew Baker

Ethanol wash

Step 17.

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

© DURATION

00:30:00

Resuspension of purified DNA

Step 18.

Take samples out of centrifuge and add 50-100 μ L 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.

■ AMOUNT

100 µl Additional info:

PROTOCOL

. TE Buffer

CONTACT: Andrew Baker