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 CunningBulk gDNA extraction from coral samples. **protocols.io** dx.doi.org/10.17504/protocols.io.dyq7vv
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Protocol

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.

ANNOTATIONS

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

2. Add sample to 500 µL 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.

AMOUNT

500 µl

DURATION

01:00:00

PROTOCOL

1% SDS in DNA Buffer

CONTACT: Andrew Baker

SDS Incubation

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

REAGENTS

Sodium chloride
View

SDS Incubation

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

REAGENTS

Ethylenediaminetetraacetic acid

SDS Incubation

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

SDS Incubation

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

REAGENTS

MilliQ water

SDS Incubation

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

Proteinase K digest

3. Add 25 µL 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

REAGENTS

Proteinase K

DURATION

02:00:00

Organic extraction

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

ANNOTATIONS

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DNA can be extracted from any quantity of sample archive. The protocol given here is written for extractions of 100 µL 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

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

AMOUNT

200 µl

DURATION

00:30:00

PROTOCOL

CTAB mix

CONTACT: Andrew Baker

Organic extraction

5.1. Dissolve 0.75 g CTAB in 20 mL MilliQ water

AMOUNT

0.75 g

REAGENTS

Cetyltrimethylammonium bromide

Organic extraction

5.2. Add 12.5 mL of 4 M NaCl

REAGENTS

Sodium chloride

View

Organic extraction

5.3. Add 75 µL of E. coli tRNA at 20 mg/mL

ANNOTATIONS

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

Organic extraction

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

REAGENTS

- MilliQ water

Organic extraction

6. Allow samples to cool. In fume hood, add equal volume (300 µL) 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

REAGENTS

- Chloroform

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

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.

DURATION

00:25:00

First ethanol precipitation

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.

AMOUNT

500 µl

REAGENTS

Ethanol, pure
4455

DURATION

00:05:00

First ethanol precipitation

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.

DURATION

02:00:00

ANNOTATIONS

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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).

DURATION

00:10:00

Second ethanol precipitation

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

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

00:30:00

Second ethanol precipitation

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.

AMOUNT

100 µl

PROTOCOL

Sodium acetate solution

CONTACT: Andrew Baker

Second ethanol precipitation

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

AMOUNT

12.3 g

REAGENTS

- Sodium acetate anhydrous

Second ethanol precipitation

13.2. Adjust pH to 5.2 with glacial acetic acid

Second ethanol precipitation

13.3. Make up to 50 mL with MilliQ water

REAGENTS

- MilliQ water

Second ethanol precipitation

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

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

REAGENTS

- Ethanol, pure
  4455

ANNOTATIONS

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

Ethanol wash

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

Ethanol wash

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

PROTOCOL

70% Ethanol

CONTACT: Andrew Baker

Ethanol wash

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

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.

AMOUNT

100 µl

PROTOCOL

TE Buffer

CONTACT: Andrew Baker