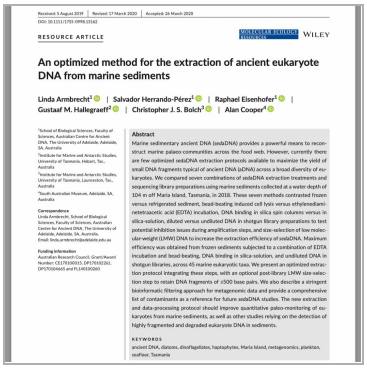

RESOURCE ARTICLE

An optimized method for the extraction of ancient eukaryote DNA from marine sediments

Linda Armbrecht | Salvador Herrando-Pérez | Raphael Eisenhofer | Gustaaf M. Hallegraeff | Christopher J. S. Bolch | Alan Cooper

Abstract

Marine sedimentary ancient DNA (sedaDNA) provides a powerful means to reconstruct marine palaeo communities across the food web. However, currently there are few optimized sedaDNA extraction protocols available to maximize the yield of small DNA fragments typical of ancient DNA (aDNA) across a broad diversity of eukaryotes. We compared seven combinations of sedaDNA extraction treatments and sequencing library preparations using marine sediments collected at a water depth of 104 m off Maria Island, Tasmania, in 2018. These seven methods contrasted frozen versus refrigerated sediment, bead-beating induced cell lysis versus ethylenediaminetetraacetic acid (EDTA) incubation, DNA binding in silica spin columns versus in silica-solution, diluted versus undiluted DNA in shotgun library preparations to test potential inhibition issues during amplification steps, and size-selection of low molecular-weight (LMW) DNA to increase the extraction efficiency of sedaDNA. Maximum efficiency was obtained from frozen sediments subjected to a combination of EDTA incubation and bead-beating, DNA binding in silica-solution, and undiluted DNA in shotgun libraries, across 45 marine eukaryotic taxa. We present an optimized extraction protocol integrating these steps, with an optional post-library LMW size-selection step to retain DNA fragments of ≤500 base pairs. We also describe a stringent bioinformatic filtering approach for metagenomic data and provide a comprehensive list of contaminants as a reference for future sedaDNA studies. The new extraction and data-processing protocol should improve quantitative paleo-monitoring of eukaryotes from marine sediments, as well as other studies relying on the detection of highly fragmented and degraded eukaryote DNA in sediments.

KEYWORDS

ancient DNA, diatoms, dinoflagellates, haptophytes, Maria Island, metagenomics, plankton, seafloor, Tasmania

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LINK

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Ambrecht et al. 2020: An optimized method for the extraction of ancient eukaryote DNA from marine sediments

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Mark Louie Lopez

ABSTRACT

Four combinations of sedaDNA extraction treatments using marine sediments collected at a water depth of 104 m off Maria Island in Tasmania are compared. These methods contrast frozen versus refrigerated sediment, bead-beating induced cell lysis versus ethylenediaminetetraacetic acid (EDTA) incubation, DNA binding in silica spin columns versus in silica-solution.

All four methods worked to varying degrees; see paper for recommended shotgun library preparation

ATTACHMENTS

Armbrecht et al. 2020 - MolEcolRes sedaDNA extractions.pdf

GUIDELINES

DNA extractions:

- **Bead-beating + spin column (DNeasy PowerLyzer PowerSoil Kit, Qiagen; “Kit”)**
- **Bead-beating + liquid silica in QG Buffer (“Si4” and “Si20”)**
- **EDTA + MinElute (“EDTA”)**
- **EDTA + bead-beating + liquid silica in QG Buffer (“Combined”, or “Com”)**

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

Created: Feb 13, 2023

Last Modified: Apr 17, 2023

PROTOCOL integer ID: 76894

Keywords: ancient DNA, diatoms, dinoflagellates, haptophytes, Maria Island, metagenomics, plankton, seafloor, Tasmania

BEFORE START INSTRUCTIONS

Sediment processing and pretreatment:
Core section processing, sedaDNA extractions and sequencing library preparations took place at ACAD's ultraclean forensic facilities following aDNA decontamination standards (Willerslev & Cooper, 2005). We placed the three sediment core sections into zip-lock bags sterilised with UV light and manually homogenized them for ~5 min. From each section, two 1 cm3 subsamples were transferred into two separate 15 ml centrifuge tubes using a sterile disposable spatula. One subsample was kept at 4°C and the other at −20°C for one month. The samples were prepared for the different extraction methods in a glove box decontaminated (3% bleach) between consecutive subsamples.

Method 1: Bead-beating + spin column (DNeasy PowerLyze... 3m 25s

1 This technique was applied to 0.25 g of sediment subsamples stored at 4 °C, following the **manufacturer's protocol** with the some modifications:

1.1 **TRANSFER** sediment into individual bead-tubes using a disposable, sterile spatula

1.2 **APPLY** bead-beating in three runs of 00:00:20 with 00:00:05 breaks using a Precellys 24 homogenizer 55s

CENTRIFUGE at 10319 undetermined for 00:00:30

1.3 **RETAIN** all optional 00:05:00 incubation steps at 4 °C per the kit's protocol 5m

1.4 **ELUTE** DNA in 80 µL of Buffer EB instead of the customary C6 solution and store at -20 °C

Method 2: Bead-beating + liquid silica in QG Buffer ("Si4" a...

1h 32m

2 This lysis process was applied to 0.25 g of the subsamples stored at both 4 °C and -20 °C

2.1 **FOLLOW** the same protocol as described in Section 1 down to step 10 of the manufacturer's instructions (addition of Solution C3 and subsequent centrifugation)

AFTER this step...

2.2 **TRANSFER** the supernatant to 15 mL centrifuge tubes containing a DNA-binding buffer

Note

DNA Binding Buffer

- 100 µL silica-solution (Sigma Aldrich)
- 3 mL modified Buffer QG (2.7 mL Buffer QG, 46 µL H_2O, 39.08 µL Triton X-100, 24.66 Molarity NaCl, and 164.5 Molarity NaOAc (Brotherton et al., 2013)

2.3 **STIR** on a rotary mixer for 00:00:00 at Room temperature

5m

CENTRIFUGE at 4500 rpm for 00:05:00

DISCARD supernatant

RESUSPEND pellet in 900 µL of DNA-binding buffer

2.4 **RE-CENTRIFUGE** at 14000 rpm for 00:01:00

16m

DISCARD the supernatant

WASH the pellet twice in 80 % ethanol

DRY pellet for 00:15:00 at 37 °C

RESUSPEND pellet in 80 µL Buffer EB

2.5 **FOLLOWING** incubation for 00:10:00 at 50 °C, centrifuge at 14.000 rpm for 00:01:00 11m

STORE the supernatant (free of silica) in a sterile Lo-bind tube (Eppendorf) at -20 °C

Method 3: EDTA + MinElute ("EDTA") 3m

3 This technique was applied to 0.25 g sediment subsamples stored at 4 °C following **Slon et al. (2017)** with minor modifications

3.1 **ADD** 1 mL of ethylenediaminetetraacetic acid (EDTA) to the sediment in a 2 mL screw-cap tube

PLACE samples on a rotary mixer and mix at 25 rpm, Overnight at Room temperature

3.2 **CENTRIFUGE** at 13.000 rpm for 00:03:00 3m

PURIFY the DNA using the MinElute Kit (Qiagen) as **per the manufacturer's instructions**

3.3 **BIND** DNA using the kit's spin column

ELUTE the DNA in 80 µL of Buffer EB

Method 4: EDTA + bead-beating + liquid silica in QG Buffer .. 3m

4 **INCUBATE** 0.25 g of three frozen sediment subsamples in EDTA overnight as in **step 3.1 of the above section "EDTA + MinElute ("EDTA")"**, EXCEPT use only 0.75 mL to keep volumes consistent with a subsequent step (below)

4.1 **CENTRIFUGE** at 13.000 rpm for 00:03:00 3m

KEEP supernatant at 4 °C

PROCESS pellet separately using bead-beating and DNA purification, as in **Method 2** (above)

4.2 **RECOMBINE** the resulting 0.75 mL DNA-solution purified from the pellet (step 10 of DNeasy Kit protocol) with 0.75 mL EDTA supernatant to make 1.5 mL total

ADD 6 mL modified QG buffer with 100 µL liquid silica

PROCEED as described in **Method 2** (above)

4.3 **ELUTE** the DNA in 100 µL Buffer EB