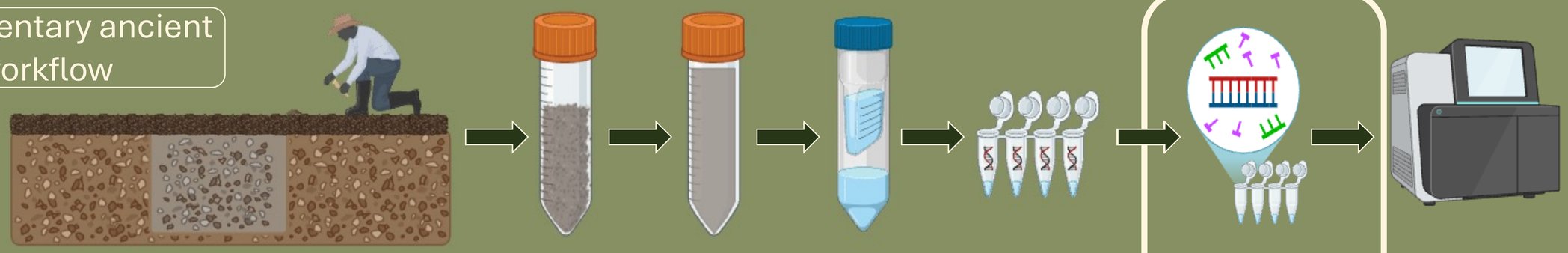
Recovering sedimentary ancient DNA in Northwestern Australia

Authors: Dawn A Lewis, Vilma Pérez Godoy, Bastien Llamas

The authors thank the Australian Archaeological Association and the Australian Research Council for supporting this work

Sedimentary ancient DNA workflow



Background

Ancient environmental DNA preserved in sediments (sedimentary ancient DNA; sedaDNA) is a powerful tool for reconstructing detailed records of past biodiversity. In this study, we investigated the potential for recovering sedaDNA from an archaeological site in northwest Western Australia to reconstruct biodiversity changes over time.

Bulk sediment samples were taken from the wall of an excavation at an archaeological site located close to the coast (location undisclosed for cultural reasons). DNA was successfully recovered from the sediments using an established in-house protocol for sedaDNA. See “Sedimentary ancient DNA workflow” above for an overview.

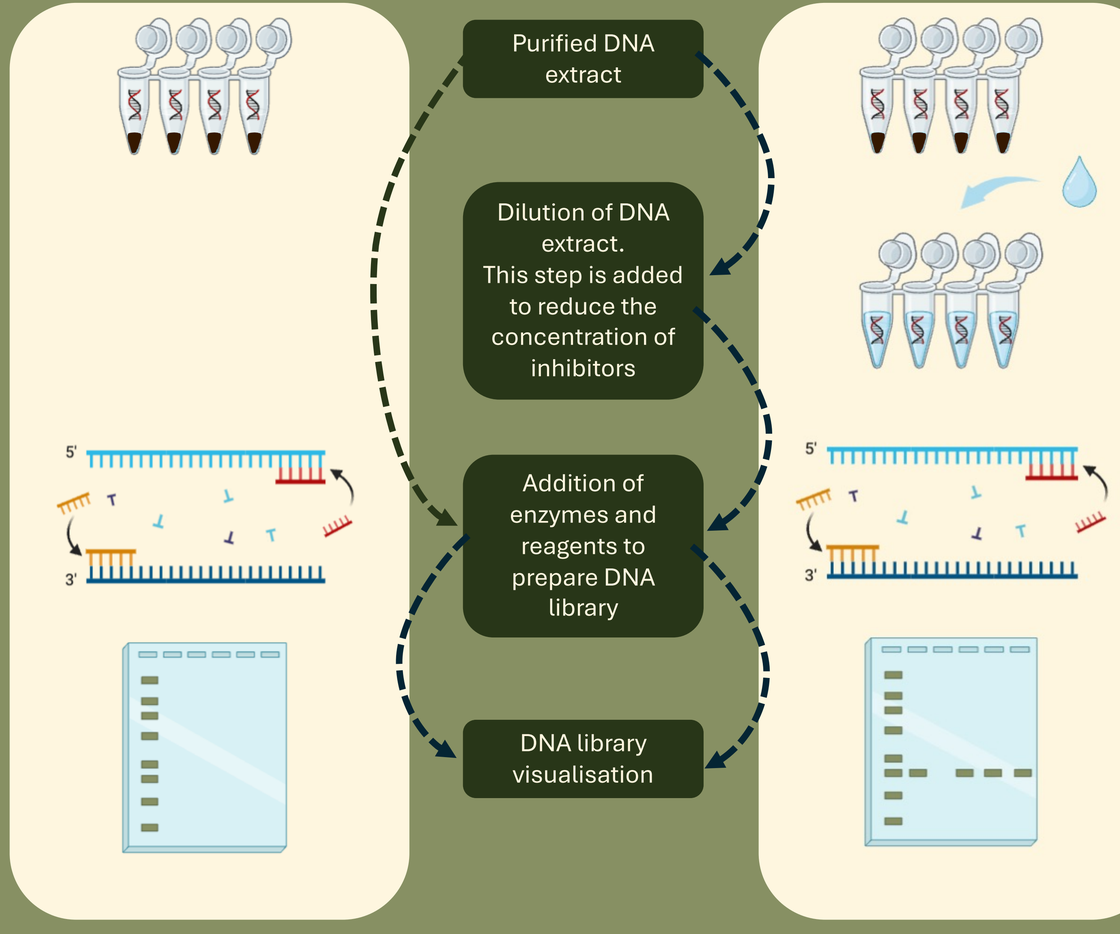
The DNA recovered however, could not be successfully converted into a sequencing-compatible format, known as sequencing “libraries”, preventing the molecular information from being accurately read by DNA sequencing instruments.

Hypothesis & experimental design

Inhibitors contained in the sediment samples are the likely cause of failure. Indeed, naturally occurring heavy metals and humic acids can inhibit key steps in the library preparation process.

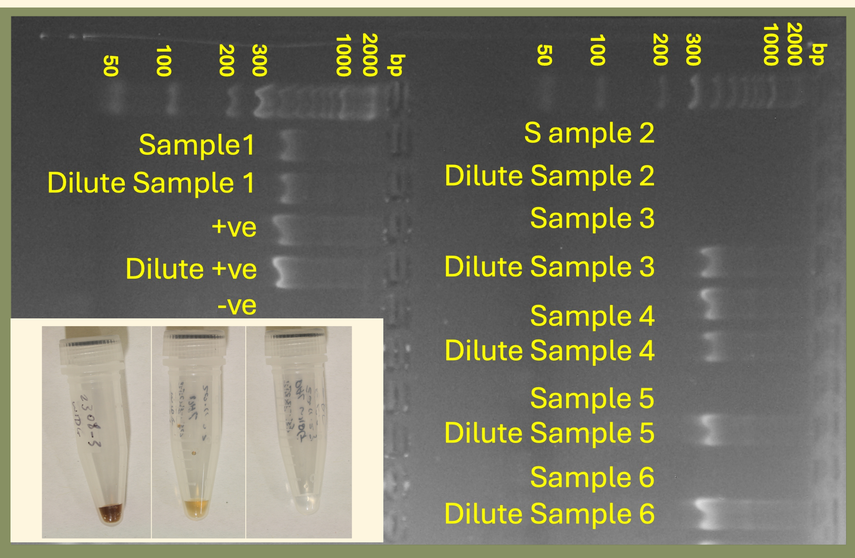
To address this challenge, we diluted the recovered DNA to minimise the impact of inhibitors that disrupt library preparation. See “Preparing a sedaDNA library in the presence of inhibitors” for a comparison of diluted and undiluted library preparation. We then used a simple, cost-effective method—polymerase chain reaction (PCR)—to amplify (make copies of) a microbial 16S rRNA gene expected to be abundant in sediments.

Preparing a sedaDNA library in the presence of inhibitors



Results

Figure 1. Visualisation of DNA from the16S rRNA gene amplicon using an agarose gel. Samples are shown alongside their diluted counterpart. Positive (+ve) and negative (-ve) controls were included for validation.
Insert image: representative samples indicating the variability in extract colour.



Discussion

The 16S rRNA gene amplicon results demonstrated that dilution of recovered DNA improved downstream processes (Figure1). Based on these results, library building was repeated with extracts diluted either 1/10 or 1/100 dependent on their 16S amplicon results.

New libraries have been successfully built and have been sent for sequencing by a service provider. After processing the sequencing data, we will be able to authenticate the recovery of ancient DNA.

Future Work

We plan to investigate the geochemical composition of archaeological sediments to determine which potential inhibitors are present in the samples. This would allow the optimisation of sedaDNA sequencing preparation.

Images created in BioRender.com

ACAD Australian Centre for Ancient DNA

THE UNIVERSITY of ADELAIDE

AUSTRALIAN RESEARCH COUNCIL
Centre of Excellence for Australian Biodiversity and Heritage

Dawn A. Lewis
Australian Centre for Ancient DNA
The University of Adelaide
Kaurna Country, Australia
@dawnlewis.bsky.social
dawn.lewis@Adelaide.edu.au