Journal of Archaeological Science 181 (2025) 106317

Contents lists available at ScienceDirect

Journal of Archaeological Science

journal homepage: www.elsevier.com/locate/jas

The necessity for authentication of ancient DNA from archaeological artefacts

Roberta Davidson [a,1,*], Shyamsundar Ravishankar [a,1], Yassine Souilmi [a,b,c,d], Xavier Roca-Rada [a,e,f], Colin Sobek [a], Leonard Taufik [a,g,h], Siobhan Evans [a], Gludhug A. Purnomo [a,h], Adam B. Rohrlach [i,j], Thomas Harvey [a], Christian Haarkötter [k], Karina Pfeiler [a], Dawn Lewis [a], Bastien Llamas [a,c,d,g], Jamie R. Wood [a,b,l,2], Vilma Pérez [a,g,2]

[a] Australian Centre for Ancient DNA, School of Biological Sciences, The University of Adelaide, Adelaide, SA, 5005, Australia
[b] The Environment Institute, The University of Adelaide, Adelaide, SA, 5005, Australia
[c] National Centre for Indigenous Genomics, John Curtin School of Medical Research, Australian National University, Canberra, ACT, Australia
[d] Indigenous Genomics, Telethon Kids Institute, Adelaide, SA, 5000, Australia
[e] Department of Ecology, Evolution, and Organismal Biology, Brown University, Providence, RI, USA
[f] Center for Computational Molecular Biology, Brown University, Providence, RI, USA
[g] Centre of Excellence for Australian Biodiversity and Heritage, University of Adelaide, Adelaide, South Australia, Australia
[h] Mochtar Riady Institute for Nanotechnology, Tangerang, Indonesia
[i] Department of Archaeogenetics, Max-Planck Institute for Evolutionary Anthropology (MPI-EVA), Leipzig, Germany
[j] School of Biological Sciences, The University of Adelaide, Adelaide, SA, 5005, Australia
[k] Laboratory of Genetic Identification & Human Rights (LABIGEN-UGR), Department of Legal Medicine, Faculty of Medicine, University of Granada, PTS Granada, Av. Investigación 11, 18016 Granada, Spain
[l] Environmental Evolutionary Genomics, School of Biological Sciences, The University of Adelaide, Adelaide, SA, 5005, Australia

ARTICLE INFO

Keywords:
Ancient DNA
Methodology
Research integrity
Artefacts

ABSTRACT

The study of ancient DNA (aDNA) has revolutionised the fields of archaeology, human evolution and paleoecology, offering new insights into the past. In particular, aDNA can be used to study the origin of unique archaeological artefacts and adds an exciting line of evidence to the multifaceted research of these artefacts. However, aDNA methodology requires thorough consideration due to the inherent risks of modern DNA contamination, misinterpretation of results, and the irreversible destruction of culturally significant materials. This article revisits the importance of adhering to standardised aDNA protocols and established criteria for aDNA authentication. Through the discussion of several peer-reviewed studies from recent literature, we illustrate the benefits and challenges of integrating aDNA into archaeological research. Ultimately, we advocate for consultation and collaboration between archaeologists and aDNA specialists and present a study design schematic that integrates the roles of archaeologists, paleogeneticists and other stakeholders to ensure appropriate protocol selection, and accurate interpretation of aDNA data for the study of archaeological artefacts.

* Corresponding author.
E-mail addresses: roberta.davidson@adelaide.edu.au (R. Davidson), shyamsundar.ravishankar@adelaide.edu.au (S. Ravishankar), yassine.souilmi@adelaide.edu.au (Y. Souilmi), xavier.rocarada@adelaide.edu.au (X. Roca-Rada), colin.sobek@adelaide.edu.au (C. Sobek), leonard@adelaide.edu.au (L. Taufik), siobhan.evans@adelaide.edu.au (S. Evans), gludhug.purnomo@adelaide.edu.au (G.A. Purnomo), adam_ben_rohrlach@eva.mpg.de (A.B. Rohrlach), thomas.harvey@adelaide.edu.au (T. Harvey), chaarkotter@ugr.es (C. Haarkötter), karina.pfeiler@adelaide.edu.au (K. Pfeiler), dawn.lewis@adelaide.edu.au (D. Lewis), bastien.llamas@adelaide.edu.au (B. Llamas), jamie.wood@adelaide.edu.au (J.R. Wood), vilma.perez@adelaide.edu.au (V. Pérez).
[1] Co-first authors.
[2] Co-senior authors.

https://doi.org/10.1016/j.jas.2025.106317
Received 2 August 2024; Received in revised form 1 July 2025; Accepted 17 July 2025
Available online 29 July 2025

Nomenclature

DNA -	Deoxyribonucleic acid
aDNA -	ancient DNA
PCR -	Polymerase chain reaction
SPAAM	Standards and Precautions and Advances in Ancient Metagenomics: A community of ancient metagenomics researchers
BLAST	Basic Local Alignment Search Tool: A software used to match DNA sequences to taxa based on sequence similarity
LCA	Lowest Common Ancestor: An algorithm used to identify the lowest common ancestor of assigned taxa in a phylogeny
Bold =	glossary terms*

1. Introduction

The ability to extract and sequence DNA from ancient specimens has revolutionised the fields of archaeology, human evolution and paleoecology over the past 40 years. DNA contamination issues have been well documented since the infancy of the field (Austin et al., 1997; Zischler et al., 1995), leading to the establishment of standard criteria of authenticity for ancient DNA (aDNA) research (Cooper and Poinar, 2000), which has helped establish aDNA as a credible and robust information source for studying the past (Orlando et al., 2021).

The study of aDNA from archaeological artefacts has demonstrated promising findings. For example, aDNA techniques have been applied to study leather manuscripts (Anava et al., 2020), leather clothing (O'Sullivan et al., 2016), bison hide moccasins (Shirazi et al., 2022), woollen clothing (Olivieri et al., 2012), feathered cloaks (Hartnup et al., 2011), ceramic jars (Foley et al., 2012), ritual terracotta items (Robinson et al., 2017), chewed birch pitch (Jensen et al., 2019) and ornamental jewellery that has even included the recovery of ancient human DNA–likely from the wearer or maker (Essel et al., 2023). The potential to associate artefacts with their makers or users represents one of the many unique advantages of using aDNA in archaeological studies, where such connections are otherwise only ever contextually implied. However, the application of aDNA analyses to archaeological artefacts must be carefully considered, as it often involves destroying irreplaceable material with significant cultural value (Orlando et al., 2021) and can often yield little or no useful DNA (Green and Speller, 2017). This emphasises further the need for minimally destructive approaches, which may include delaying analysis of exciting samples until the protocols are sufficiently developed and benchmarked. Thus, while the prospects of genetic analyses are intriguing, it is equally important to consider the challenges and pitfalls of this type of research. Most importantly, the authentication of DNA as ancient remains a fundamental requirement in any paleogenomics study. Communicating how aDNA methods can be effectively applied to complement the study of unique and precious archaeological finds is essential for proper integration of genetic analyses into the archaeological field.

We are a group of ancient DNA researchers with research directions that include past human demography and cultures, animal and plant evolution and conservation and palaeoenvironmental reconstruction. In recent years, the rapid interest in ancient DNA has resulted in application of these technical methods to the study of archaeological artefacts. These cases have ranged in their robustness of applying ancient DNA methods. This has motivated us to write the present article with the goal of communicating to the archaeological community the importance of adhering to established ancient DNA practices; the key one being the authentication of DNA as ancient. Our intended audience is archaeologists who are interested in applying paleogenomic methods in conjunction with archaeological approaches to study ancient artefacts. Importantly, we do not want to imply that such work is the sole realm of paleogeneticists, but advocate that these techniques must be applied correctly to ensure scientifically robust results. We strongly endorse local capacity building for paleogenomics and encourage increased cross-pollination between paleogenomic and archaeological disciplines, and in particular the combined training of individuals in aspects of both fields.

Different from studying modern DNA (Alberts et al., 2002), a fundamental challenge of aDNA is that it is degraded. The most common forms of post-mortem **DNA damage** include (1) breakage of DNA molecules due to depurination (i.e., loss of purine bases): that produces a short **fragment size distribution**—with median lengths often less than 100 base pairs; and (2) chemical changes to DNA molecules due to deamination of cytosines (i.e., loss of amino groups of cytosines), which are converted into uracils and then misincorporated as thymines during sequencing, producing the typical 'C-to-T' and corresponding 'G-to-A' misincorporation patterns, herein referred to as **DNA damage signal** (Briggs et al., 2007; Dabney et al., 2013; Orlando et al., 2021). Before attempting to work with a sample, it is important to consider its thermal age—the effect of temperature on DNA degradation over time—as this can significantly affect the preservation and integrity of the aDNA within the sample (Briggs et al., 2007; Robinson et al., 2017). It should be noted that thermal age estimates are based on temperature-dependent rates of depurination in ancient bones and thus may not translate to other substrates (Smith et al., 2003). Moreover, due to the relatively low abundance of target aDNA molecules in ancient samples, aDNA is extremely susceptible to contamination from external modern DNA that saturates all environments (Llamas et al., 2017). Given that modern DNA is essentially everywhere, researchers should presume that any DNA found is modern contamination until proven otherwise by ancient DNA authentication, i.e. by identifying a **DNA damage signal** and a short **fragment size distribution**. These challenges are thoroughly addressed in the comprehensive review by Orlando et al. (2021), which covers the entire process of working with ancient samples, including ethical research, sample considerations, laboratory protocols, computational methods, authentication criteria, and reporting of findings (Orlando et al., 2021). We highly recommend this review to archaeologists who may be considering implementing ancient DNA techniques into their own work.

DNA recovered from an ancient sample can never be assumed to be ancient simply because ancient DNA laboratory protocols have been followed. Because of these limitations, we strongly recommend bioinformatic authentication together with combining aDNA findings with other proxies to complement and independently cross-validate the results when possible. Integrating approaches such as lipid analysis, protein analysis, palynology, isotopes, aDNA, and archaeological context into studies can provide a more comprehensive understanding of the overall archaeological record. For example, Gutarowska et al. (2015) incorporated molecular, microscopic, and chemical methods to reveal insights into the biodeterioration processes affecting historical objects from the Auschwitz II–Birkenau concentration and extermination camp in Oświecim, Poland (Gutarowska et al., 2015). In another example, Braadbaart et al. (2020) applied a range of techniques to study the physical and chemical properties indicative of heat alteration as well as aDNA to investigate the use of 25–40 kya Paleolithic fireplace hearths in France (Braadbaart et al., 2020).

Given the wide diversity of archaeological materials, we advise that common standardised aDNA protocols may not always apply, and therefore, researchers may need to develop protocols specifically for their study. Researchers may also be limited by available funding and resources, which can influence study design, but these factors should not undermine scientific rigour. Consultation and collaboration with aDNA specialists will provide the best approaches to answer the research questions robustly and effectively (Fig. 1). Several useful resources to understand aDNA research are now available to the archaeological

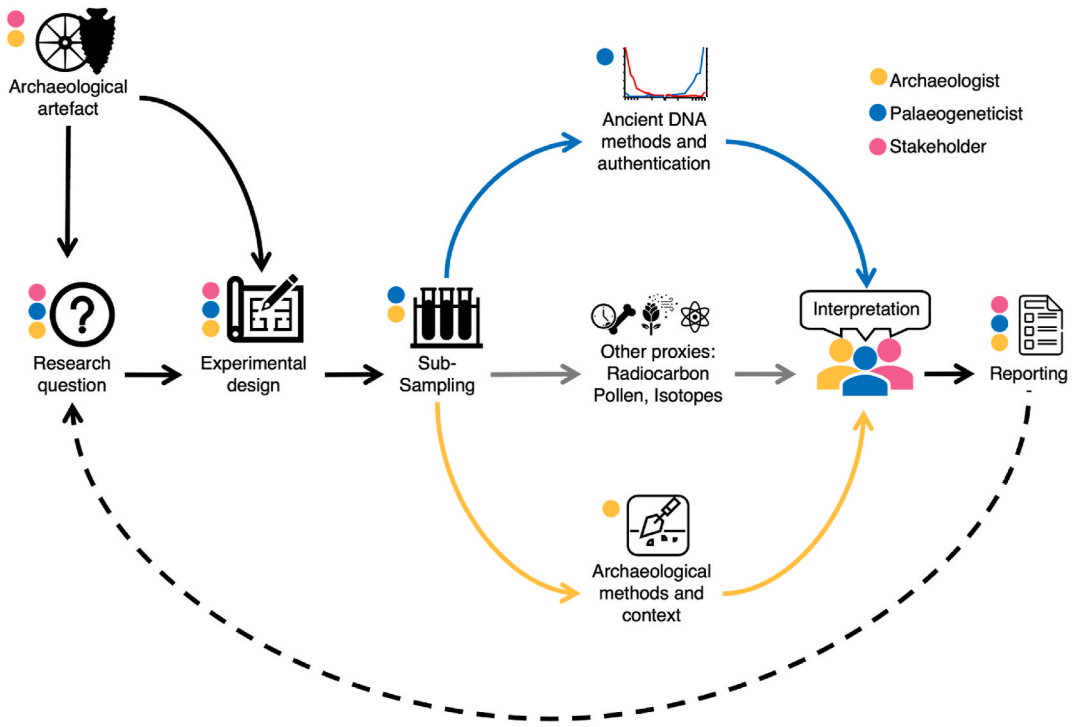

Fig. 1. A flowchart representing the optimal recommended study design for ancient DNA studies of archaeological artefacts. Stakeholders may refer to local cultural community stakeholders, and/or museum curators where such representative groups are lacking.

community. These include initiatives such as the Standards and Precautions and Advances in Ancient Metagenomics (SPAAM community), an international group of researchers in the field who maintain a database of information about aDNA researchers from different laboratories worldwide that can facilitate the process of finding suitable collaborators. Additionally, the SPAAM community has written a textbook, Introduction to Ancient Metagenomics, a resource that encapsulates all the information archaeologists may need to utilise this discipline (Fellows Yates et al., 2024).

Here, we discuss several peer-reviewed studies to illustrate some of the opportunities and pitfalls of using aDNA methodologies to study archaeological artefacts. We discuss each stage of study design and recommend a general approach to choosing specific protocols and validating results (Table 1). Finally, we recommend a workflow for conducting a paleogenomic study of an archaeological artefact and highlight the role of archaeologists, paleogeneticists and cultural stakeholders at each stage in the process (Fig. 1).

2. Best practices when designing a study of archaeological artefacts

2.1. Sampling

The sampling of archaeological artefacts must be carefully considered due to the destructive nature of typical **aDNA extraction** protocols and also the preciousness of these unique and culturally significant artefacts. Ideally, all stakeholders involved with the artefact—including cultural custodians, legal authorities, community representatives, private owners, and researchers—should fully understand and consent to the potential risks and benefits of the study before any sampling takes place (Fig. 1). Stakeholders may also have focus areas or specific questions they want to be answered, often guided by the cultural and historical context of the samples. Understanding the nuances of aDNA research on artefacts is integral to both scientific protocol development and stakeholder engagement, wherein the potential advantages of aDNA research should be explained without overpromising (Lewis et al., 2023). This can avoid the setting of unrealistic expectations from stakeholders as to what insights aDNA may be able to provide. When sampling is performed, established best practices should be followed to minimise modern DNA contamination (Llamas et al., 2017), including use of specialised facilities, protocols, and control checks. Poor adherence to these established standards undermines the quality of scientific work and degrades the essential trust between stakeholders and researchers.

Recent studies have set a framework for working with descendent communities of archaeological artefacts and remains from archaeological sites (Pinotti et al., 2025; Way et al., 2025). Pinotti et al. (2025) present genomic data from ancient individuals dating to 900 CE from remains at Chaco-associated sites in the San Juan River Basin on the Colorado Plateau in Northwestern New Mexico, USA. While the laboratory and computational component of the study was conducted at the University of Copenhagen, Denmark, the study was initiated and the research questions driven by the Tribal leaders of the Picuris Pueblo people, including a two-year consultation period before research began. Here, the data sovereignty of the Picuris Pueblo people is ensured through the involvement of Tribal leadership in formulating research questions, crafting key findings, and retaining the right of the leadership to curtail the research at any point. Furthermore, the genomic data generated as part of the study is controlled by the Picuris Pueblo Tribal Council, ultimately ensuring that the owners of the data can decide its future applications as well. Similarly, findings from archaeological artefacts from the Last Glacial Maximum (LGM) in Australia were authored in partnership with the local descendent communities from surrounding regions, thus preserving the right of descendant communities to be involved in sharing the story of their people (Way et al., 2025).

Regarding studies where aDNA is studied from archaeological artefacts, Robinson et al. (2017) sampled from precious 6th-14th century terracotta objects from Northern Ghana (Robinson et al., 2017). The artefacts were obtained in collaboration with local archaeologists and museum curators. Before commencing aDNA work, the authors calculated the thermal age to determine the viability of DNA surviving in the

Table 1
Examples of optimal, acceptable and not recommended approaches to study design, methods, analysis and reporting of ancient DNA data from archaeological artefacts.

	Optimal	Acceptable	Not Recommended
Study Design	Co-design or collaboration with descendant communities, paleogeneticists, archaeologists	Consultation with stakeholders and paleogeneticists	No collaboration or consultation
Subsampling	Minimally destructive	Necessarily destructive	Unnecessarily destructive
DNA Extraction	Developing and benchmarking protocol for the specific substrate under study or that is demonstrated to work in the literature.	Select protocol used for similar substrate	Inappropriate protocol for the substrate
Damage repair	● Partial DNA repair ● No DNA repair		Full DNA repair
Library Preparation	Shotgun & target taxon enrichment	● Shotgun only ● Metabarcoding (supported with other non-DNA proxies)	● Metabarcoding only ● Target taxon enrichment only
Sequencing	Short read		Long Read
Analysis	Alignment-based taxonomic classification with Lowest Common Ancestor (LCA) refinement & Alignment of enriched library to target reference genome	Alignment-based taxonomic classification with Lowest Common Ancestor (LCA) refinement	● Alignment-based taxonomic classification only. ● Alignment to target reference only
Authentication	Clear DNA damage signal, short DNA sequence length (<100bp) and multiple proxies to confirm ancient taxa	Plausible DNA damage signal and short DNA sequence length	No damage pattern, long DNA sequences
Interpretation	Conservative, hypothesis driven and multiple lines of evidence considered	Conservative and hypothesis driven	Overinterpretation and/or confirmation bias
Reporting	Comprehensive and transparent (including controls and negative results)	Comprehensive (only positive results included)	Incomplete, incorrect or obscure.

artefact, acknowledging the objects were "close to the likely threshold for aDNA recovery", showing clear understanding and communication of risks with the stakeholders involved. In contrast, recent studies involving culturally significant artefacts housed in overseas institutions or collections have been sampled without clearly documented engagement with stakeholders local to where the objects originated. For example, Arbøll et al. (2023) sampled from a unique 2900-year-old clay brick artefact originating from present-day Iraq, accessed through the National Museum of Denmark (Arbøll et al., 2023). While the study represents an important effort to recover aDNA from novel substrates, proactive consultation with Iraqi museum curators, Iraqi historians, local government representatives, or culturally descendant communities could have provided valuable perspectives on the artefact's cultural context, the framing of research questions, and the interpretation of results. Such engagement fosters ethical research practice and offers wider benefits to local communities, while enriching scientific and cultural insights derived from the study (Lewis et al., 2023).

Although historically museums have acted as custodians of objects in collections, they are increasingly acknowledging a shift from traditional standards and practices toward a more responsible role in light of social change. Voluntary repatriation, restitution, and reparations are now being embraced as part of a new horizon in museum practices (Morgan, 2024). Ethical stakeholder engagement regarding European samples, particularly from old materials (e.g., Essel et al., 2023), has typically not required the same level of cultural consideration as elsewhere in the world. This is because there is generally no direct cultural representation for the people of the periods of interest in these regions, making such engagement less feasible. However, while relations between local communities, scientists, and legal institutions vary across the world, researchers conducting aDNA research must prioritise local stakeholder engagement whenever possible, as previously suggested (Fox and Hawks, 2019; Wagner et al., 2020). For the genetic study of archaeological artefacts, we recommend that all research includes a thorough statement that either describes the efforts made to engage local stakeholders with any ethical permissions that are granted, or justifies why this process is deemed unnecessary in the particular case.

Sub-sampling artefacts for **DNA extraction** aims to obtain a small amount of material from which to extract DNA. As such, this often involves damage to the artefact, which, even when minimal, is irreversible. This places significant responsibility on researchers to carefully consider both the scientific and cultural significance of an artefact prior to sampling. Sub-sampling can involve highly invasive methods, such as breaking or scraping clay or ceramic artefacts (Arbøll et al., 2023; Foley et al., 2012) or cutting fur, leather or feathers from ancient hides, scrolls and cloaks (Anava et al., 2020; Hartnup et al., 2011; Shirazi et al., 2022). In contrast, minimally destructive methods typically involve swabbing critical surfaces of artefacts such as terracotta objects used for food (Robinson et al., 2017) and bricks of historical buildings (Gutarowska et al., 2015), or successive soaking of artefacts in solution, as demonstrated in the recovery of aDNA from a Paleolithic pendant (Essel et al., 2023). Given the irreversibility of destructive sub-sampling, it is essential that all measures are taken to optimise the chance of successfully recovering DNA, including ensuring that sub-sampling is performed within a dedicated aDNA clean room facility, to reduce the risk of contamination from other DNA sources (Epp et al., 2019; Fulton and Shapiro, 2019). Contamination at this early stage not only jeopardises the integrity of the artefact but also invalidates downstream analyses by compromising the authenticity of the aDNA recovered. Such factors have the potential to impact the recovery of authentic aDNA, for example, if the sub-sampling environment and conditions do not meet widely accepted standards eg. Arbøll et al. (2023). It is important to note that modern DNA contamination does not only occur due to direct contact but that aerosol DNA in the space immediately surrounding the work area may also contaminate samples (see (Fantinato et al., 2024, 2023)) for further information on environment DNA of the air and dust), thus highlighting the importance of sampling procedures and the use of sampling controls such as air or surface swab samples. Given the wide variety of archaeological artefacts encountered in aDNA research, it is often beneficial, and sometimes essential, to develop novel sub-sampling methods that minimise damaging the artefact. For instance, Essel et al. (2023) sampled modified bone artefacts and developed a minimally destructive **DNA extraction** protocol so as not to destroy the unique samples, preserving them for future research. Creating specialist extraction techniques for an artefact and checking the integrity of output throughout aDNA analysis are examples of how researchers can build confidence in the research and provide assurance to stakeholders.

2.2. *Laboratory methods, sequencing and ancient DNA authentication*

It is important that the laboratory protocols that are selected are

Table 2
Glossary of terms highlighted in the text.

Term	Definition
DNA damage signal	This commonly refers to the pattern of cytosine deamination, typically plotted as the proportion of C-to-T transitions (between sample and reference genome) (y-axis) and nucleotide position relative to the 5′ end of the DNA fragment (x-axis). In ancient DNA this is characteristically a high proportion on the first position, which steeply drops off with distance from the end of the DNA fragment. Sometimes just the proportion of C-T transitions in the first position can be quoted as a signal of DNA damage.
DNA extraction	The process of obtaining DNA from sample material. In aDNA research this typically involves a sample digestion and cell lysis stage (mechanical, chemical and enzymatic), binding of DNA molecules to silica (either on a solid membrane or in-solution), washing, and elution of DNA from the silica.
DNA sequencing library	DNA that has been processed in preparation for sequencing on a high throughput sequencing platform. In DNA metabarcoding the library will consist of DNA molecules that include the DNA region (barcode) of interest with the flanking primers, sample-specific indexes and sequencing primers. In shotgun sequencing this will typically include entire DNA fragments bounded by sample-specific indexes and sequencing adapters.
Enrichment	The use of custom-designed 'baits' that are complementary to DNA sequences of the species/taxa of interest and through hybridisation, DNA of interest in the DNA sequencing library will anneal to those baits. Baits and their hybridised DNA are then isolated and non-target DNA molecules will be removed, thereby enriching the proportion of target DNA sequences in the sequencing library.
Fragment size distribution	The distribution obtained when plotting DNA fragment length (x axis) against abundance (y axis). In ancient DNA this should characteristically be skewed to the left, with a peak in distribution (median) typically around 50–70 bp and a tail of longer fragments.
Genetic marker	A specific region of the genome that provides some information of interest. This could be an entire chromosome, gene, or a small region of a gene, and could convey information about taxonomic identity or some other aspect of the individual from which the DNA sequence came.
High- throughput sequencing (HTS)	Sequencing platforms from Illumina, Life Technologies and other companies that generate gigabases of data by performing a large number of short reads in parallel. The amount of data produced leads to the challenge of post-processing (Kircher, 2012).
Library Preparation	The laboratory procedures used to transform the extracted DNA into a DNA sequencing library. In DNA metabarcoding this typically involves one or two rounds of PCR to amplify barcodes and add sample-specific indexes and sequencing adapters, in shotgun sequencing this will involve several steps of enzymatic incubation to join sample-specific indexes and sequencing adapters directly onto the ends of DNA fragments.
Long-read sequencing	Sequencing platforms (currently from PacBio and Oxford Nanopore) that are characterised by their capability of producing reads that could exceed 10 kb. This approach is suitable for modern DNA analysis, but not for aDNA due to its characteristic fragmentation (Wilson et al., 2019).
Metabarcoding	PCR with primers designed to target DNA regions that are highly-conserved across high-taxonomic ranks but are adjacent to short variable barcode regions. In this way, a single PCR can amplify DNA barcodes from a wide range of species that might be present in a sample. Commonly used primers for DNA metabarcoding target groups such as bacteria, eukaryotes, metazoa, plants, mammals or arthropods.
Metagenomic classifier	Computational tools for taxonomic assignment of metagenomic sequence data (Velsko et al., 2018). Metagenomic classifiers can be split into two broad categories: alignment (MALT (Herbig et al., 2016)), and k-mer based classifiers (Kraken2 (Wood et al., 2019), KrakenUniq (Breitwieser et al., 2018)).
Mitochondrial DNA	The chromosome of the mitochondrion, a cell organelle in eukaryotes that has its own genome distinct from that of the cell nucleus. Mitochondrial DNA is maternally inherited and the genome is much shorter than the nuclear genome but is present in more copies per cell due to multiple mitochondria per cell.
Nuclear DNA	The genomic component of the nucleus, a cell organelle in eukaryotes that comprises the majority of the DNA within a cell. Nuclear DNA is bi-parentally inherited.
PCR amplification	Polymerase chain reaction (PCR) - the enzyme-facilitated replication of specific regions of DNA molecules that are targeted using primers. Typically performed using multiple cycles of temperature gradients that (1) denature double-stranded DNA, (2) anneal primers, and (3) elongate the new DNA strand. In the exponential phase of PCR amplification the number of DNA molecules present can double every cycle.
Primer	Also known as an oligonucleotide - a custom-designed synthetic single stranded DNA molecule that is complementary to the DNA sequence adjacent to a region of interest (i.e. genetic marker or barcode) and targets it for PCR amplification. Typically two primers, one on either side of the region of interest, are required for PCR amplification.
Reference genome	A representative DNA sequence of the genomic material of the studied organisms. All mutations (variation) are measured relative to this representative sequence.
Reference sequence database	A repository of genetic information containing a body of published DNA sequences and their respective taxa. Can be used to taxonomically assign novel sequences based on the characterised database.
Short-read sequencing	Sequencing platforms that typically provide sequence lengths of up to 500 bp. Widely used examples include Illumina MiSeq, HiSeq, NextSeq and NovaSeq and BGI.
Shotgun sequencing; Metagenomic sequencing	Where all DNA molecules present in a DNA extract are sequenced together and best reflect the DNA present in the sample. There is no bias introduced through targeting certain gene markers, taxa or fragment lengths as happens with metabarcoding.
Single- stranded library; (ssDNA)	A library preparation method that uses heat to denature DNA into single stranded molecules before adapter ligation. These single DNA strands are then copied to create double stranded DNA. The approach overcomes issues where one strand of a DNA molecule is damaged. This may prevent the molecule being sequenced when using a double-stranded library protocol, but the undamaged strand can be sequenced using a single-stranded approach. ssDNA libraries are better suited for extremely degraded ancient DNA.

appropriate for the sample and DNA substrate, as evidenced by successful application in the literature. For example, using bone **DNA extraction** protocols for bone samples, sediment **DNA extractions** for sedimentary samples, minimally-destructive **DNA extraction** protocols for precious and unique samples, or, optimally, that custom protocols are developed for novel substrates (Table 1). See Table 2 for definitions. Use of inappropriate protocols (e.g., Arbøll et al., 2023) could theoretically lead to loss of authentic aDNA and correspondingly over-represent modern contaminants. To prepare **DNA sequencing libraries**, protocols with partial or no DNA-damage repair are optimal to ensure enough **aDNA damage signal** is retained for ancient authentication. Selecting the correct sequencing platform is also critical with **high throughput short-read sequencing** being preferred due to the fragmented nature of aDNA (Table 1). **Shotgun sequencing** , in which all DNA from the sample is randomly sequenced, is regarded as the gold standard for metagenomic samples because this approach offers an unbiased and comprehensive view of the entire genomic diversity within the sample (Table 1). **Library enrichments** may be used to increase the proportion of target DNA in the **sequencing library**, when a hypothesis informs a taxa of interest, thus reducing relative sequencing costs. However, an initial **shotgun sequencing** run is recommended for understanding the genomic profile of the sample quality before applying any **library enrichment** (Table 1). Moreover, **shotgun sequencing** allows the identification of degraded short aDNA fragments with less **PCR amplification** bias than **library enrichment** methods (Davidson et al., 2023, 2024; Haarkötter et al., 2024).

The careful selection and development of laboratory protocols, tailored to both the substrate and research question, can optimise the preservation of invaluable artefacts while also yielding authentic aDNA. Jensen et al. (2019) analysed a 5700-year old birch pitch from Denmark, using destructive sampling to remove 250 mg from the specimen for DNA extraction. They followed established aDNA protocols, building **double-stranded DNA sequencing libraries** and leaving **damaged DNA** unrepaired, thus allowing later authentication of aDNA. The

libraries were then sequenced using **shotgun sequencing,** providing the closest approximation of the true composition of DNA in the sample. Notably, they detected contamination from *Delftia* spp., a common laboratory contaminant, in their control samples and thus excluded any sub-samples containing the species from downstream analysis, given one of their research questions involved identifying the oral microbiome deposited onto the sample. This demonstrates how, even in carefully controlled clean-room environments, contamination remains a prevalent risk. Further, Essel et al. (2023) worked with highly valuable, modified bone artefacts. They developed a minimally destructive **DNA extraction** method specifically tailored to their human-modified bone samples type. This is a key advancement for the field given the rare and precious nature of such samples. Their approach involved soaking the artefact in a series of chemical solutions and using the DNA washed off in the process, rather than grinding up the sample itself into powder as previous protocols require, and thus preserving the form of the artefact for subsequent research. They built **single-stranded DNA sequencing libraries**, a protocol specialised for extremely short and degraded DNA and, as with Jensen et al. (2019), left **damaged DNA** unrepaired before **shotgun sequencing**. Essel et al. (2023) then performed targeted **enrichments** given the research questions informed by the archaeological context: mammalian **mitochondrial library enrichment** to identify the species of bone, and human **nuclear** and **mitochondrial library enrichment** to identify the ancestry of the wearer or maker of the pendant.

Of note, the chewed 5700-year old birch pitch mentioned above (Jensen et al., 2019) contained sufficient human DNA from the individual who interacted with the artefact to allow characterisation of the chewer's genetic profile through **shotgun sequencing** alone. In contrast, the trace amount of human DNA recovered from the paleolithic pendant required targeted **enrichment** to increase the proportion of human DNA, enabling insights into the ancestry of its wearer. Collectively, these analyses underscore the importance of tailoring sampling and sequencing strategies to the characteristics of each artefact, as similar research questions may require different approaches depending on the context. They also highlight the benefits of preparing samples in a manner that minimises the risk of sample contamination. In these contexts, accidental contamination of samples with modern human DNA may have left any authentic signal of ancient human DNA irretrievable.

An alternative to **shotgun libraries**, DNA **metabarcoding libraries** were an important tool in early aDNA studies. Here, specific gene regions are amplified, using pairs of short single-stranded DNA fragments called **primers** that flank the region of interest, and sequenced. However, this technique has several limitations and challenges, such as **PCR amplification** biases, that can favour longer modern DNA fragments, thus increasing the relative level of contamination (Ziesemer et al., 2015). In **metabarcoding** data analysis, the **DNA damage signal** cannot be identified because the ends of the molecules are lost during the **PCR amplification** of specific gene regions, which also prevents the sample's **DNA fragment size distribution** from being observed. Therefore, we generally advise against the use of **metabarcoding** in archaeological aDNA studies, although **metabarcoding** may still play a role for relatively young or well-preserved artefacts. Where **metabarcoding** analyses are used however, it is essential to complement these analyses with other traditional paleoecological proxies, for example, pollen records when characterising plant communities in a sample (Boast et al., 2023) or morphological analysis when characterising fossil assemblages (Grealy et al., 2015). Such complementary lines of evidence provide an independent means of authentication in the absence of **DNA damage** patterns, and failure to do so (e.g., Arbøll et al., 2023) can leave no way of knowing whether results are authentic or not.

In contrast, earlier **metabarcoding** studies such as Foley et al. (2012) and Robinson et al. (2017) demonstrate a typical application of **metabarcoding** in appropriate contexts. Foley et al. (2012) apply minimally destructive swabbing methods to the inside of ceramic transport jars (amphoras) from Greek shipwrecks in the Mediterranean sea dated to the 5th-3rd centuries BC (Foley et al., 2012). Similarly, Robinson et al. (2017) also swab the inside of ceramics from Ghana (Robinson et al., 2017). Foley et al. (2012) applied **metabarcoding** techniques tailoring their **primers** to target 96–500 nucleotides (nt) fragments common to land plant chloroplast DNA, and specifically excluding DNA present in algae that would likely be present in their oceanic samples. Similarly, Robinson et al. (2017) target 80–85 nt fragments aiming to find ancient plant DNA and also target larger fragments >125 nt for crops grown around the area such as maize and pearl millet. Both studies discuss the goals and limitations of their **primer** sets and the risk of modern contamination, cautiously concluding that, given its short fragment length, the isolated DNA is likely to be ancient, within the limits of available methodology at the time of the study. Although, we emphasise that standards have changed with the availability of sequencing technologies, and emphasise that in the case of archaeological artefacts, **shotgun sequencing** is preferred.

2.3. Analysis and interpretation

aDNA sampled from artefacts or macrofossils like bone are subject to contamination from microbes, the environment, and people who have handled the samples. Therefore, any aDNA sample must be considered a **metagenomic** sample, i.e. that contains DNA coming from many species, and the sequence data must be analysed appropriately to ensure confirmation bias does not corrupt interpretations. A common method is to use **metagenomic classifiers** to match sequenced DNA to a **reference sequence database** and classify taxa present in the sample. Sequence alignments can be refined by considering properties such as evenness of alignment across the **reference genome**, edit distance between the sequenced reads and the reference, and the number of unique alignments to reduce false positive classifications (Pochon et al., 2023; Wang et al., 2022). For sequences with multiple alignments in the database, a lowest common ancestor (LCA) algorithm is used to refine taxonomic assignment (Huson et al., 2007; Wang et al., 2022). However, these approaches are still being refined within the field, and results are often highly dependent on the choice of **metagenomic classifier**, **reference sequence database**, and parameters used to refine the results (Pochon et al., 2023; Ravishankar et al., 2024)). Therefore, we emphasise the need for caution and collaboration with experts in this area (Fig. 1). Finally, it is recommended to cross-validate any interpretations with other proxies and analyses (Fig. 1).

A multitude of tools and approaches have been used, for instance, Arbøll et al. (2023), Robinson et al. (2017), Foley et al. (2012) and Essel et al. (2023) aligned their sequence data using, BLAST (Zhang et al., 2000), to the NCBI nucleotide **reference sequence database**. However, only Essel et al. (2023) applied LCA to their BLAST alignment to obtain a robust taxonomic profile of their artefact (Vernot et al., 2021). The lack of LCA refinement after BLAST for the other studies doesn't invalidate the results but rather casts doubt over their robustness. It should be noted that Robinson et al. (2017) do discuss the limitations of their approach when interpreting the results and acknowledge that a BLAST alignment of a sequence alone does not necessitate the presence of taxa in the artefact. In the case of **metabarcoding** studies where it is not possible to authenticate aDNA through elevated **DNA damage signals**, studies greatly benefit from incorporating complementary archaeological proxies to validate their results. For example, employing palynological analysis of vegetative matter in the brick from Arbøll et al. (2023) could validate the plants identified from DNA results as there is often a degree of overlap between these assemblages (Boast et al., 2023).

For **shotgun sequencing**, Essel et al. (2023) first authenticated the presence of aDNA through elevated **DNA damage signals** at fragment ends, across the reported taxa of interest. Subsequently they used targeted **enrichments** for **genetic markers** of interest and conservative analyses to genetically identify both the artefact as originating from deer bone, and the maker or wearer of the pendant sample as a human female with Ancient Siberian ancestry. Furthermore, the authors corroborated

their dual-species phylogenetic dating of the sample with radiocarbon dating of charcoal discovered in the proximity of their artefact. We emphasise that the use of strict aDNA contamination protocols during sampling and laboratory work are employed to ensure the greatest likelihood that authentic aDNA can be obtained, but use of such protocols does not guarantee the presence of aDNA nor the absence of modern DNA contamination. Computational authentication of the presence of aDNA in sequencing data by identification of **DNA damage signal** and **short fragment size distribution** must be employed before conducting further analyses and making interpretations.

Jensen et al. (2019) used a variety of approaches for their different research questions, for instance, in order to characterise the microbial profile of the sample they aligned the reads using Bowtie2 (Langmead and Salzberg, 2012) to a MetaPhlan2 (Truong et al., 2015) database containing clade specific markers for more than 17,000 **reference genomes**, before comparing the profile to known microbial profiles from Human Microbiome Project Consortium, allowing them characterise the microbial profile of birth pitch as that of an human oral microbiome. To further characterise the metagenomic reads they used MALT (Megan ALignment Tool) ((Herbig et al., 2016)) to map reads to a custom database they built containing more than 33,000 genomes, before applying LCA for robust taxonomic assignment and reconstruction of entire microbial genomes. Finally, to characterise trace amounts of Metazoa (animals) and Viridiplantae (plants) sequences, they used the metagenomic pipeline 'Holi' (Pedersen et al., 2016) which is specialised in the taxonomic profiling of ancient metagenomic shotgun reads, and ran ngsLCA (Wang et al., 2022) for LCA analysis identifying plant taxa that could have been part of a recent meal. Since Jensen et al. (2019) used **shotgun sequencing** they were able to authenticate the presence of **DNA damage signal** in all taxa they identified, increasing confidence in their results. Furthermore, they present evidence of remains from animal and plant taxa they identify in the archaeological site to corroborate the genetic results.

Interpretation of results varies by the type of methods used and the known limitations or risks. However, this does not preclude any researcher from being transparent about the limitations and making interpretations that are appropriate to the weight of the evidence. As mentioned, though Foley et al. (2012) used **metabarcoding** techniques, which are no longer preferred, they are clear in their discussion that chloroplast DNA sampled from an artefact that was underwater could have interference from modern algae, but given their choice of **primers** and maximum amplicon length of 200 nt they conclude that their DNA is likely ancient within the limitations of their methods. Similarly, Robinson et al. (2017) employed **metabarcoding** to swabs from the inside of ceramics from Ghana. Aware of possible contaminants they also targeted human DNA, corn and other local contemporary crop species. Additionally they took negative control swabs from similar substrates in a different context. Samples where sporadic human DNA was identified were not included in the interpretations of the project. Finally, Arbøll et al. (2023) also found sporadic presence of human and swine DNA throughout their samples when targeting vertebrate DNA and thus exclude interpreting other vertebrate taxa.

2.4. *Reporting, reproducibility and replicability*

Methodological reporting and data sharing are essential components of the scientific method that enable reproducibility and replicability—especially in aDNA studies where the analysis of genetic material commonly relies on co-analysis with published data. Clear and transparent reporting of the methods employed in the study ensures a comprehensive understanding of the conducted research and allows proper interpretations of findings for the reader. Reporting is also essential to enable replicability of the methods. Reporting should also include negative results to inform the research community about artefacts that do not yield ancient DNA and protocols that were unsuccessful in specific cases and reduce unnecessary destructive sampling of unique artefacts. Reproducibility is the ability of other researchers to conduct the same study design with different equipment and find similar results. This is rarely applicable in an archaeological DNA context as studies pertain to one or a few artefacts and their specific provenance. However, it may also apply to broad contexts, such as the study of many artefacts from a specific material culture across a region. Replicability is the ability of other researchers to obtain the same findings by repeating the same study with the same samples and methodology. In aDNA studies, samples are unique, so replicability from the beginning is often technically difficult as the sub-sample is usually partially-destroyed during processing. This emphasises the need for minimally-destructive protocols wherever possible, and for ethical public data sharing and analyses protocols used to enable replicability, at least from the processing of DNA sequencing data onwards.

Accurate and transparent reporting of methodologies is essential to ensure the integrity and utility of aDNA studies. Incomplete documentation of **primer** sequences, gene regions targeted, or not making sequence datasets publicly available (e.g., Arbøll et al., 2023) when ethically permitted, limit others' ability to access, replicate or build upon published findings. In contrast, studies that provide comprehensive reporting, including detailed descriptions of assay development, laboratory protocols, contamination controls, and data processing steps, strengthens the transparency and reproducibility of aDNA research. Examples such as Essel et al. (2023), Robinson et al. (2017) and Jensen et al. (2019) demonstrate the benefits of this approach, enabling robust reproduction and interpretation of results and extending the research value of unique and irreplaceable artefacts.

3. On the limitation of resources

While we strongly endorse the development of local aDNA research capacity building and the further training and education of researchers in adjacent disciplines, we also acknowledge that not all researchers have access to the capacity or funding to conduct extensive protocol development, such as that done by Essel et al. (2023). Nonetheless, it is of the utmost importance that aDNA research is conducted robustly, given the irreplaceability of such rare and unique artefacts, and that the interpretations drawn from this research are often highly impactful to the lives and identity of contemporary peoples, and the global human story. Thus, it is paramount to carefully consider and justify appropriate laboratory protocol selection, data processing and aDNA validation, and data analysis methods. Well funded research does not inherently make the research robust or the DNA authentic, nor is lower funded research an excuse to conduct poor scientific work or unnecessary destruction of samples. Researchers of all means are responsible for ensuring their projects follow scientific rigour and established best-practice protocols. Therefore, we underscore the importance of collaboration and consultation with aDNA experts by archaeologists who wish to apply the methods and to develop an appropriately curated study design for the samples in question (Fig. 1).

4. Conclusion

In summary, while applying aDNA methods to archaeological artefacts is a promising avenue of research, many technical mistakes may befall researchers looking to apply these approaches in novel contexts such as unique archaeological artefacts. Ultimately, authentication of DNA as ancient is absolutely essential to the integrity of results and studies should be designed with this in mind.

CRediT authorship contribution statement

Roberta Davidson: Writing – review & editing, Writing – original draft, Visualization, Project administration, Investigation, Conceptualization. **Shyamsundar Ravishankar:** Writing – review & editing, Writing – original draft, Visualization, Project administration,

Investigation, Conceptualization. **Yassine Souilmi:** Writing – review & editing, Project administration, Conceptualization. **Xavier Roca-Rada:** Writing – review & editing, Conceptualization. **Colin Sobek:** Writing – review & editing. **Leonard Taufik:** Writing – review & editing, Writing – original draft, Conceptualization. **Siobhan Evans:** Writing – review & editing, Writing – original draft, Conceptualization. **Gludhug A. Purnomo:** Writing – review & editing, Writing – original draft. **Adam B. Rohrlach:** Writing – review & editing, Writing – original draft. **Thomas Harvey:** Writing – review & editing, Writing – original draft. **Christian Haarkötter:** Writing – review & editing, Writing – original draft. **Karina Pfeiler:** Writing – review & editing, Writing – original draft. **Dawn Lewis:** Writing – review & editing, Writing – original draft, Visualization, Conceptualization. **Bastien Llamas:** Writing – review & editing, Writing – original draft, Project administration, Conceptualization. **Jamie R. Wood:** Writing – review & editing, Writing – original draft, Conceptualization. **Vilma Pérez:** Writing – review & editing, Writing – original draft, Project administration, Investigation, Conceptualization.

Declaration of competing interest

None.

Acknowledgements

We would like to acknowledge that the University of Adelaide and the Australian Center for Ancient DNA are located on the unceded lands of the Kaurna people, we extend our gratitude and acknowledgement to elders past and present. We sincerely thank our colleagues at the Australian Centre for Ancient DNA for their valuable feedback on the manuscript. We thank the reviewers of this manuscript for their insight and free labour in the pursuit of science.

References

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., Walter, P., 2002. The Structure and Function of DNA. Garland Science.

Anava, S., Neuhof, M., Gingold, H., Sagy, O., Munters, A., Svensson, E.M., Afshinnekoo, E., Danko, D., Foox, J., Shor, P., Riestra, B., Huchon, D., Mason, C.E., Mizrahi, N., Jakobsson, M., Rechavi, O., 2020. Illuminating genetic mysteries of the dead Sea scrolls. Cell 181, 1218–1231.e27.

Arbøll, T.P., Rasmussen, S.L., de Jonge, N., Hansen, A.H., Pertoldi, C., Nielsen, J.L., 2023. Revealing the secrets of a 2900-year-old clay brick, discovering a time capsule of ancient DNA. Sci. Rep. 13, 13092.

Austin, J.J., Ross, A.J., Smith, A.B., Fortey, R.A., Thomas, R.H., 1997. Problems of reproducibility–does geologically ancient DNA survive in amber-preserved insects? Proc. Biol. Sci. 264, 467–474.

Boast, A.P., Wood, J.R., Bolstridge, N., Perry, G.L.W., Wilmshurst, J.M., 2023. Ancient and modern scats record broken ecological interactions and a decline in dietary breadth of the critically endangered kākāpō parrot (Strigops habroptilus). Front. Ecol. Evol. 11. https://doi.org/10.3389/fevo.2023.1058130.

Braadbaart, F., Reidsma, F.H., Roebroeks, W., Chiotti, L., Slon, V., Meyer, M., Théry-Parisot, I., van Hoesel, A., Nierop, K.G.J., Kaal, J., van Os, B., Marquer, L., 2020. Heating histories and taphonomy of ancient fireplaces: a multi-proxy case study from the Upper Palaeolithic sequence of Abri Pataud (Les Eyzies-de-Tayac, France). J. Archaeol. Sci. Rep 33, 102468.

Breitwieser, F.P., Baker, D.N., Salzberg, S.L., 2018. KrakenUniq: confident and fast metagenomics classification using unique k-mer counts. Genome Biol. 19, 198.

Briggs, A.W., Stenzel, U., Johnson, P.L.F., Green, R.E., Kelso, J., Prüfer, K., Meyer, M., Krause, J., Ronan, M.T., Lachmann, M., Pääbo, S., 2007. Patterns of damage in genomic DNA sequences from a Neandertal. Proc. Natl. Acad. Sci. U. S. A 104, 14616–14621.

Cooper, A., Poinar, H.N., 2000. Ancient DNA: do it right or not at all. Science 289, 1139.

Dabney, J., Meyer, M., Pääbo, S., 2013. Ancient DNA damage. Cold Spring Harbor Perspect. Biol. 5. https://doi.org/10.1101/cshperspect.a012567.

Davidson, R., Roca-Rada, X., Ravishankar, S., Taufik, L., Haarkötter, C., Collen, E., Williams, M., Webb, P., Irfan Mahmud, M., Djami, E.N.I., Purnomo, G.A., Santos, C., Malgosa, A., Manzanilla, L.R., Silva, A.M., Tereso, S., Matos, V., Carvalho, P.C., Fernandes, T., Maurer, A.-F., Teixeira, J.C., Tobler, R., Fehren-Schmitz, L., Llamas, B., 2024. Optimised in-solution Enrichment of over a Million Ancient Human Snps. bioRxiv. https://doi.org/10.1101/2024.05.16.594432.

Davidson, R., Williams, M.P., Roca-Rada, X., Kassadjikova, K., Tobler, R., Fehren-Schmitz, L., Llamas, B., 2023. Allelic bias when performing in-solution enrichment of ancient human DNA. Mol. Ecol. Resour. 23, 1823–1840.

Epp, L.S., Zimmermann, H.H., Stoof-Leichsenring, K.R., 2019. Sampling and extraction of Ancient DNA from sediments. In: Shapiro, B., Barlow, A., Heintzman, P.D., Hofreiter, M., Paijmans, J.L.A., Soares, A.E.R. (Eds.), Ancient DNA: Methods and Protocols. Springer, New York, New York, NY, pp. 31–44.

Essel, E., Zavala, E.I., Schulz-Kornas, E., Kozlikin, M.B., Fewlass, H., Vernot, B., Shunkov, M.V., Derevianko, A.P., Douka, K., Barnes, I., Soulier, M.-C., Schmidt, A., Szymanski, M., Tsanova, T., Sirakov, N., Endarova, E., McPherron, S.P., Hublin, J.-J., Kelso, J., Pääbo, S., Hajdinjak, M., Soressi, M., Meyer, M., 2023. Ancient human DNA recovered from a Palaeolithic pendant. Nature 618, 328–332.

Fantinato, C., Fonneløp, A.E., Bleka, Ø., Vigeland, M.D., Gill, P., 2023. The invisible witness: air and dust as DNA evidence of human occupancy in indoor premises. Sci. Rep. 13, 19059.

Fantinato, C., Gill, P., Fonneløp, A.E., 2024. Investigative use of human environmental DNA in forensic genetics. Forensic Sci. Int. Genet. 70, 103021.

Fellows Yates, J.A., Warinner, C., Andrades Valtueña, A., Borry, M., Guellil, M., Herbig, A., Hiß, A., Hübner, A., Kocher, A., Lamnidis, T.C., Michel, M.E., Nota, K., Oskolkov, N., Pach, A.L., Pérez, V., Schmid, C., Velsko, I.M., Warner, R., Zampirolo, G., Zeibig, T., 2024. Introduction to Ancient Metagenomics. Zenodo. https://doi.org/10.5281/ZENODO.13784555.

Foley, B.P., Hansson, M.C., Kourkoumelis, D.P., Theodoulou, T.A., 2012. Aspects of ancient Greek trade re-evaluated with amphora DNA evidence. J. Archaeol. Sci. 39, 389–398.

Fox, K., Hawks, J., 2019. Use ancient remains more wisely. Nature 572, 581–583.

Fulton, T.L., Shapiro, B., 2019. Setting up an Ancient DNA laboratory. In: Shapiro, B., Barlow, A., Heintzman, P.D., Hofreiter, M., Paijmans, J.L.A., Soares, A.E.R. (Eds.), Ancient DNA: Methods and Protocols. Springer, New York, New York, NY, pp. 1–13.

Grealy, A.C., McDowell, M.C., Scofield, P., Murray, D.C., Fusco, D.A., Haile, J., Prideaux, G.J., Bunce, M., 2015. A critical evaluation of how ancient DNA bulk bone metabarcoding complements traditional morphological analysis of fossil assemblages. Quat. Sci. Rev. 128, 37–47.

Green, E.J., Speller, C.F., 2017. Novel substrates as sources of ancient DNA: prospects and hurdles. Genes (Basel) 8, 180.

Gutarowska, B., Celikkol-Aydin, S., Bonifay, V., Otlewska, A., Aydin, E., Oldham, A.L., Brauer, J.I., Duncan, K.E., Adamiak, J., Sunner, J.A., Beech, I.B., 2015. Metabolomic and high-throughput sequencing analysis-modern approach for the assessment of biodeterioration of materials from historic buildings. Front. Microbiol. 6, 979.

Haarkötter, C., Roca-Rada, X., Saiz, M., Vinueza-Espinosa, D.C., Gálvez, X., Medina-Lozano, M.I., Díaz-Ruiz, D., Álvarez, J.C., Llamas, B., Lorente, J.A., Austin, J., 2024. Exploring the potential of genome-wide hybridization capture enrichment for forensic DNA profiling of degraded bones. Genes (Basel) 16, 23.

Hartnup, K., Huynen, L., Te Kanawa, R., Shepherd, L.D., Millar, C.D., Lambert, D.M., 2011. Ancient DNA recovers the origins of Māori feather cloaks. Mol. Biol. Evol. 28, 2741–2750.

Herbig, A., Maixner, F., Bos, K.I., Zink, A., Krause, J., Huson, D.H., 2016. MALT: fast alignment and analysis of metagenomic DNA sequence data applied to the Tyrolean Iceman. bioRxiv. https://doi.org/10.1101/050559.

Huson, D.H., Auch, A.F., Qi, J., Schuster, S.C., 2007. MEGAN analysis of metagenomic data. Genome Res. 17, 377–386.

Jensen, T.Z.T., Niemann, J., Iversen, K.H., Fotakis, A.K., Gopalakrishnan, S., Vågene, Å. J., Pedersen, M.W., Sinding, M.-H.S., Ellegaard, M.R., Allentoft, M.E., Lanigan, L.T., Taurozzi, A.J., Nielsen, S.H., Dee, M.W., Mortensen, M.N., Christensen, M.C., Sørensen, S.A., Collins, M.J., Gilbert, M.T.P., Sikora, M., Rasmussen, S., Schroeder, H., 2019. A 5700 year-old human genome and oral microbiome from chewed birch pitch. Nat. Commun. 10, 5520.

Kircher, M., 2012. Analysis of high-throughput Ancient DNA sequencing data. In: Shapiro, B., Hofreiter, M. (Eds.), Ancient DNA: Methods and Protocols. Humana Press, Totowa, NJ, pp. 197–228.

Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with Bowtie 2. Nat. Methods 9, 357–359.

Lewis, D.A., Simpson, R., Hermes, A., Brown, A., Llamas, B., 2023. More than dirt: sedimentary ancient DNA and Indigenous Australia. Mol. Ecol. Resour. https://doi.org/10.1111/1755-0998.13835.

Llamas, B., Valverde, G., Fehren-Schmitz, L., Weyrich, L.S., Cooper, A., Haak, W., 2017. From the field to the laboratory: controlling DNA contamination in human ancient DNA research in the high-throughput sequencing era. STAR 3, 1–14.

Morgan, B.Y.B.M., 2024. Museum as way station: reframing repositories as part of object journeys. https://www.aam-us.org/wp-content/uploads/2024/11/HorizonPapers-Morgan.pdf, 6.23.25.

Olivieri, C., Ermini, L., Rizzi, E., Corti, G., Luciani, S., Marota, I., De Bellis, G., Rollo, F., 2012. Phylogenetic position of a copper age sheep (Ovis aries) mitochondrial DNA. PLoS One 7, e33792.

Orlando, L., Allaby, R., Skoglund, P., Der Sarkissian, C., Stockhammer, P.W., Ávila-Arcos, M.C., Fu, Q., Krause, J., Willerslev, E., Stone, A.C., Warinner, C., 2021. Ancient DNA analysis. Nature Reviews Methods Primers 1, 1–26.

O'Sullivan, N.J., Teasdale, M.D., Mattiangeli, V., Maixner, F., Pinhasi, R., Bradley, D.G., Zink, A., 2016. A whole mitochondria analysis of the Tyrolean Iceman's leather provides insights into the animal sources of Copper Age clothing. Sci. Rep. 6, 31279.

Pedersen, M.W., Ruter, A., Schweger, C., Friebe, H., Staff, R.A., Kjeldsen, K.K., Mendoza, M.L.Z., Beaudoin, A.B., Zutter, C., Larsen, N.K., Potter, B.A., Nielsen, R., Rainville, R.A., Orlando, L., Meltzer, D.J., Kjær, K.H., Willerslev, E., 2016. Postglacial viability and colonization in North America's ice-free corridor. Nature 537, 45–49.

Pinotti, T., Adler, M.A., Mermejo, R., Bitz-Thorsen, J., McColl, H., Scorrano, G., Feizabadifarahani, M., Gandy, D., Boulanger, M., Gaunitz, C., Stenderup, J., Ramsøe, A., Korneliussen, T., Demeter, F., Santos, F.R., Vinner, L., Sikora, M., Meltzer, D.J., Moreno-Mayar, J.V., Quanchello, C., Willerslev, E., 2025. Picuris Pueblo oral history and genomics reveal continuity in US Southwest. Nature 642, 125–132.

Pochon, Z., Bergfeldt, N., Kırdök, E., Vicente, M., Naidoo, T., van der Valk, T., Altınışık, N.E., Krzewińska, M., Dalén, L., Götherström, A., Mirabello, C., Unneberg, P., Oskolkov, N., 2023. aMeta: an accurate and memory-efficient ancient metagenomic profiling workflow. Genome Biol. 24, 242.

Ravishankar, S., Perez, V., Davidson, R., Roca-Rada, X., Lan, D., Souilmi, Y., Llamas, B., 2024. Filtering out the noise: metagenomic classifiers optimize ancient DNA mapping. Briefings Bioinf. 26. https://doi.org/10.1093/bib/bbae646.

Robinson, H.A., Insoll, T., Kankpeyeng, B.W., Brown, K.A., Brown, T.A., 2017. Ritual complexity in a past community revealed by ancient DNA analysis of pre-colonial terracotta items from Northern Ghana. J. Archaeol. Sci. 79, 10–18.

Shirazi, S., Broomandkhoshbacht, N., Oppenheimer, J., Metcalfe, J.Z., Found, R., Ives, J. W., Shapiro, B., 2022. Ancient DNA-based sex determination of bison hide moccasins indicates promontory cave occupants selected female hides for footwear. J. Archaeol. Sci. 137, 105533.

Smith, C.I., Chamberlain, A.T., Riley, M.S., Stringer, C., Collins, M.J., 2003. The thermal history of human fossils and the likelihood of successful DNA amplification. J. Hum. Evol. 45, 203–217.

Truong, D.T., Franzosa, E.A., Tickle, T.L., Scholz, M., Weingart, G., Pasolli, E., Tett, A., Huttenhower, C., Segata, N., 2015. MetaPhlAn2 for enhanced metagenomic taxonomic profiling. Nat. Methods 12, 902–903.

Velsko, I.M., Frantz, L.A.F., Herbig, A., Larson, G., Warinner, C., 2018. Selection of appropriate metagenome taxonomic classifiers for Ancient microbiome research. mSystems 3. https://doi.org/10.1128/mSystems.00080-18.

Vernot, B., Zavala, E.I., Gómez-Olivencia, A., Jacobs, Z., Slon, V., Mafessoni, F., Romagné, F., Pearson, A., Petr, M., Sala, N., Pablos, A., Aranburu, A., de Castro, J.M. B., Carbonell, E., Li, B., Krajcarz, M.T., Krivoshapkin, A.I., Kolobova, K.A., Kozlikin, M.B., Shunkov, M.V., Derevianko, A.P., Viola, B., Grote, S., Essel, E., Herráez, D.L., Nagel, S., Nickel, B., Richter, J., Schmidt, A., Peter, B., Kelso, J., Roberts, R.G., Arsuaga, J.-L., Meyer, M., 2021. Unearthing Neanderthal population history using nuclear and mitochondrial DNA from cave sediments. Science 372. https://doi.org/10.1126/science.abf1667.

Wagner, J.K., Colwell, C., Claw, K.G., Stone, A.C., Bolnick, D.A., Hawks, J., Brothers, K. B., Garrison, N.A., 2020. Fostering responsible research on Ancient DNA. Am. J. Hum. Genet. 107, 183–195.

Wang, Y., Korneliussen, T.S., Holman, L.E., Manica, A., Pedersen, M.W., 2022. *ngs*LCA—A toolkit for fast and flexible lowest common ancestor inference and taxonomic profiling of metagenomic data. Methods Ecol. Evol. 13, 2699–2708.

Way, A.M., Piper, P.J., Chalker, R., Wilkins, D., Wilkins, E., Watson Redpath, L., Glass, P., Carroll, M.R., Nutman, E., Kononenko, N., Spate, M., Barrows, T.T., Wright, D., Brennan, W., 2025. The earliest evidence of high-elevation ice age occupation in Australia. Nat. Hum. Behav. 1–9.

Wilson, B.D., Eisenstein, M., Soh, H.T., 2019. High-fidelity nanopore sequencing of ultra-short DNA targets. Anal. Chem. 91, 6783–6789.

Wood, D.E., Lu, J., Langmead, B., 2019. Improved metagenomic analysis with Kraken 2. Genome Biol. 20, 257.

Zhang, Z., Schwartz, S., Wagner, L., Miller, W., 2000. A greedy algorithm for aligning DNA sequences. J. Comput. Biol. 7, 203–214.

Ziesemer, K.A., Mann, A.E., Sankaranarayanan, K., Schroeder, H., Ozga, A.T., Brandt, B. W., Zaura, E., Waters-Rist, A., Hoogland, M., Salazar-García, D.C., Aldenderfer, M., Speller, C., Hendy, J., Weston, D.A., MacDonald, S.J., Thomas, G.H., Collins, M.J., Lewis, C.M., Hofman, C., Warinner, C., 2015. Intrinsic challenges in ancient microbiome reconstruction using 16S rRNA gene amplification. Sci. Rep. 5, 16498.

Zischler, H., Höss, M., Handt, O., von Haeseler, A., van der Kuyl, A.C., Goudsmit, J., 1995. Detecting dinosaur DNA. Science 268, 1192–1193 author reply 1194.