An extensive archaeological dental calculus dataset spanning 5000 years for ancient human oral microbiome research

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Abstract

Archaeological dental calculus can provide detailed insights into the ancient human oral microbiome. We offer a multi-period, multi-site, ancient shotgun metagenomic dataset consisting of 174 samples obtained primarily from archaeological dental calculus derived from various skeletal collections in the United Kingdom. This article describes all the materials used including the skeletons' historical period and burial location, biological sex, and age determination, data accessibility, and additional details associated with environmental and laboratory controls. In addition, this article describes the laboratory and bioinformatic methods associated with the dataset development and discusses the technical validity of the data following quality assessments, damage evaluations, and decontamination procedures. Our approach to collecting, making accessible, and evaluating bioarchaeological

metadata in advance of metagenomic analysis aims to further enable the exploration of archaeological science topics such as diet, disease, and antimicrobial resistance (AMR).

Background

Ancient oral microbiomes have provided a wealth of information about past lives by analysing dental calculus (mineralised plaque) —a common archaeological material recovered from skeletons spanning most pre-historic and historical periods (Forshaw 2022). Dental calculus is a mineralized biofilm, formed initially by an acquired enamel pellicle (AEP) on the tooth surface, followed by bacterial colonisers, and a series of microbial succession. As dental calculus mineralises, it entombs and preserves biomolecules associated with oral microbiota (Adler et al. 2013; Huynh et al. 2016; Jersie-Christensen et al. 2018; Granehäll et al. 2021), environmental and dietary micro debris (Warinner et al. 2014b; Cristiani et al. 2016; Weyrich et al. 2017; Sawafuji et al. 2020), and the human host —although present in consistent but low quantities (Mann et al. 2018). Previous metagenomic analyses of dental calculus have demonstrated a range of archaeological applications, including: microbiota changes with ancient dietary shifts (Adler et al. 2013); sugar intake in archaeological populations (Chidimuro et al. 2022); early domesticated food consumption (Cristiani et al. 2016); historical human migratory events (Eisenhofer et al. 2019); the presence of various pathogens (Huynh et al. 2016; Fotakis et al. 2020; Jacobson et al. 2020; Neukamm et al. 2020); the identification of dietary sources indicated through ancient proteins (Hendy et al. 2018); the tracing of agricultural transitions (Ottoni et al. 2021); and evidence of ancient dairy consumption (Warinner et al. 2014a).

This dataset is intended for research capitalising on the abundant microbial aDNA encapsulated in ancient dental calculus (Metcalf et al. 2014; Brealey et al. 2020) to gain potential insights into millennia of oral microbial evolution. Our sizable dataset allows for the comparison of oral microbiomes across multiple historic time periods in England (Bronze/Iron Age, Roman, Anglo-Saxon/Viking, Medieval, Industrial, Post-Industrial, modern) spanning 2500 BC to 1900 AD (and 20th century). This time span encompasses significant historical events such as the agricultural revolution, industrialisation, and medical

discoveries, which permit the analysis of changes in dietary, demographic, environmental, and socioeconomic aspects over time. For example, recent studies have identified the Industrial Revolution as a period of shifting community function (Gancz et al. 2023) and diversity (Skelly 2019); yet datasets including ancient oral metagenomes dating to this period are sparse. Therefore, this dataset focuses on expanding the availability of metagenomes dating to the period of cultural and environmental transition during the Industrial Revolution in England, as well as samples from earlier and later periods that contextualise the Industrial Era. The inclusion of populations from both urban centres and rural communities enables research into the impact of geographic differences on ancient oral microbiomes, as well as other environmental factors associated with industrialisation.

Our published dataset facilitates the study of oral microbiomes of burial populations, and/or single individuals, which can contribute to debates in funerary archaeology, social construction, religious history, and health disparities between different social classes. For example, previous research has demonstrated important population-level data that can be retrieved from sites like Spitalfields, London (Adams and Reeve 1987; Molleson et al. 1993; Nitsch et al. 2011; Humphrey et al. 2012), Iron Age and Romano-British settlements (Oliver 1992; Pearce 1999; Watts 2001; Redfern et al. 2015), cemeteries such as the *Cillinis* that entomb groups of unbaptized children (Finlay 2000), and plague pits (Papagrigorakis et al. 2007; Harbeck et al. 2013; Wagner et al. 2014; Barbieri et al. 2017; Spyrou et al. 2018). Nevertheless, every ancient individual is a unique entity of time deserving of their own exploration, and previous research has also demonstrated novel insights that can be obtained from the analysis of solitary individuals, for example: Otzi the Iceman (Acs et al. 2005; Keller et al. 2012; Seiler et al. 2013; Lugli et al. 2017; Larcher 2019; Pires de Souza et al. 2021); the human genome that was retrieved from a piece of prehistoric Scandinavian birch pitch (Jensen et al. 2019); and 'bog bodies' (Nielsen et al. 2020; van Beek et al. 2023).

Our dental calculus dataset also makes a thorough analysis of the interactions between various microbial groups possible. Diseases in the oral cavity are caused by the dysbiosis of a diverse array of microbiome members, rather than by a single pathogen (Almeida et al. 2020). For example, the correlation of carbohydrate diets with *Streptococcus mutans* formation and its visible effects on ancient teeth is intriguing (Adler et al. 2017); but

holobiont units are considerably more complex than that. While *S. mutans* are metabolising sugars, producing acids, and decaying teeth, they form plaque biofilms that bind other hazardous bacteria together and spread to other areas of the oral cavity and body (Zayed et al. 2021). *S. mutans* is just an example, but our oral microbiome data offers the chance to investigate the relationships of other possible multiple microbial communities that have the potential to reveal links between past diseases and dietary patterns, in addition to current health challenges.

In addition to ancient dental calculus, our dataset also includes metagenomic data retrieved from associated archaeological tooth roots and skeletal elements which act as controls to ensure robust and reliable data can be obtained. The inclusion of environmental controls, such as soil from the burial site or bone or tooth root from a skeleton from the same archaeological site, allows for ancient and modern environmental contaminants to be identified and removed (Kazarina et al. 2021). There is currently a scarcity of literature exploring the specific impact of environmental controls on ancient oral metagenomes, as well as very few examples of their use in ancient dental calculus studies. Preliminary studies have indicated that ancient environmental contaminants can be a particular issue in metagenomic studies as their DNA damage patterns can mirror those of the targeted oral microbial DNA (Kazarina et al. 2021). Failure to thoroughly decontaminate ancient oral metagenomes is known to artificially inflate alpha diversities (Farrer et al. 2021) and therefore the inclusion of environmental control is an important consideration. Further research into the impact of environmental controls is necessary.

Methodology

Summary of all materials

All our samples have been archived in the European Nucleotide Archive (ENA 2023) as fastq files in projects PRJEB1716, PRJEB12831, and PRJEB75938 (see **Table S1**). They encompass 174 samples (348 fastq files using paired end reads) from modern (*n=10*) and archaeological dental calculus (*n=133*) and tooth (*n=2*) samples, environmental controls (bone; *n=7*), and laboratory controls (extraction and library blanks; *n=22*). The ancient samples were excavated from multiple archaeological sites across the UK spanning 5000 years, including

the Bronze Age (ca. 2300 BC–700 BC), Iron Age (ca. 700 BC–43 AD), Roman Period (ca. 43 AD–410 AD), Anglo-Saxon Period (ca. 410 AD–1066 AD), Viking Age (ca. 793 AD–1066 AD), Medieval Period (ca. 1066–1485), Industrial (ca. 1750–1850), Post-industrial (ca. 1837–1901). Samples from modern human remains are derived from the University of Tennessee donated skeletal collection within the Forensic Anthropology Centre Anthropology Research Center (20th – 21st century). As an ectopic growth, dental calculus is not considered a human tissue, and analysis is not subject to the legislation of the Human Tissue Act. All samples have received ethical approval for destructive analysis and data publication from relevant repositories. All archaeological sites and time periods are displayed in **Figure 1**. **Table S1** is a summary table with all the necessary information on the individuals in our dataset including: sample code and type; skeletal ID, age, and biological sex; ENA project and number; type of tooth and weight of calculus sampled; adapter sequence; location and time period of archaeological site; and current repository and skeletal report/reference associated with individual skeletons. Proteomic analysis of a subset of these samples have also been published in Hendy et al. (2018) (see **Table S1**).

Laboratory method

Sample preparation and contamination controls

Samples of dental calculus were removed from the teeth using sterilised dental picks and stored in individual 2.0 mL Eppendorf tubes. Sample preparation and DNA extractions were conducted in the BioArCh ancient DNA laboratory at the University of York, and the Ancient DNA and Proteins facility at the University of British Columbia. Both labs are dedicated to the analysis of ancient biomolecules, and the introduction of contamination into the workspace is minimised by the use of protective clothing, including Tyvek suits, gloves, masks, and hairnets. The labs are also equipped with UV filtered ventilation and positive airflow, as well as with dedicated equipment and bench UV lights; countertops and other surfaces in the lab are routinely wiped down with dilute sodium hypochlorite. All reagents and equipment in the ancient DNA laboratory are dedicated solely to the study of degraded DNA. Multiple blank DNA extractions and negative PCR controls are run alongside the ancient samples to identify potential contamination at each stage of the procedure.

aDNA extraction

DNA was extracted in batches, with two extraction blanks prepared alongside each batch. Samples of dental calculus and bone were UV-sterilised for 1 minute on each side. After crushing to a powder, samples were pre-digested for 5 minutes with 1 mL of 0.5M EDTA to remove possible surface contamination. This pre-digestion supernatant was removed, and a further 1.1 mL of 0.5M EDTA added and rotated at room temperature for seven days to fully demineralize. Samples were centrifuged at 13,000 RPM for 2 minutes and 1 mL of supernatant transferred into fresh tubes. To the supernatant, 20 mg/ml Proteinase K was added and rotated at 37 °C for 24 hours. For the majority of samples, DNA was extracted from the dental calculus and bone samples using a protocol based on Dabney et al. (2013) with DNA eluted in 60 μL of EB following a five-minute incubation step. For the Manchester, Cross Street samples (C028–C027), DNA extraction followed a modified silica-spin column protocol (Yang et al. 1998), with DNA concentrated in Amicon® 10K Ultra-4 Centrifugal Filter Devices (Millipore) and purified with QiaQuick MinElute kits (QIAGEN, Hilden, Germany) before being eluted in 25 μL of Qiagen EB Buffer. All DNA extracts were quantified via Qubit® 2.0 Fluorometer using a High-Sensitivity DNA Assay.

Metagenomic sequencing

For each DNA extract, double-stranded whole genome shotgun Illumina libraries were prepared using a protocol based on Meyer and Kircher (2010). Each dental calculus library was built using between 200-400 ng of DNA; extraction blanks were prepared with 25 μL of DNA extract; library blanks were built using 25 μL of nuclease free water. The libraries were constructed using a double-barcoding approach as described in Fortes and Paijman (2015) which serves as an additional means to filter chimeric sequences from the dataset, and thus increase the confidence in assigning the sequences to a particular library. Individual P7 indexes were ligated through an indexing PCR step using a proof-reading taq polymerase (AccuPrime Pfx Supermix) with the following cycling conditions were: 95˚C for 5 minutes, and cycles of (95˚C for 15s, 60˚C for 30s, 68˚C for 30s), and a final extension of 68˚C for 5 min. Optimal cycle numbers for library indexing were determined through the use of quantitative PCR (qPCR) using Fast SYBR (Gansauge and Meyer 2013). Amplified libraries were subsequently purified using Qiagen MinElute spin columns, the size distribution of the

amplified libraries was determined using an Agilent 2100 Bioanalyzer. The dental calculus libraries were pooled in equimolar concentration and subjected to paired-end sequencing on multiple HiSeq2500 lanes at the Wellcome Trust Sanger Institute (WTSI) or on a NextSeq platform, PE 150 + 150 bp Integrated Microbiome Resource (IMR) at Dalhousie (Manchester Cross Street). In accordance with WTSI protocols, human DNA sequences were removed from dental calculus datasets prior to deposition in the ENA. For samples sequenced at the WTSI (**Table S1**), the raw metagenomic reads were mapped against the human reference genome (GRCh37) using *bwa aln* with default settings; reads mapping to the human genome reads were extracted from the dataset and only unmapped reads uploaded to the ENA. Samples sequences at the IMR (Manchester Cross Street) did not have human reads filtered prior to deposition.

Quality control

FastQC v0.11.9 (Babraham Bioinformatics 2019) was used to assess raw digital data quality. FastQC is a quality control tool for raw sequencing data that provides a modular collection of analyses used to gain insight into any flaws in the data before performing further analysis (Babraham Bioinformatics 2019). The preprocessing programmer Fastp v0.23.2 (Chen *et al*. 2018) was then utilised with default parameters. Fastp is a tool used to filter and trim poor quality reads, cut adapters, repair mismatched base pairs, and produce overall quality. It also provides results that include both pre- and post-filtering data, allowing for a direct comparison on the filtering impact (Chen *et al*. 2018).

Decontamination

To minimise the impact of laboratory and environmental contamination, BBduk (Joint Genome Institute 2023) was used with default parameters to decontaminate samples using Kmers. This procedure involved identifying and removing homologous sequences found in extraction and library blanks, and bone blanks when they were available (see **Table 1** for which samples were paired with which controls). After decontamination, paired read counts did not match because the tool removed a different number of sequences it considered to be contaminants from forward and reverse reads, resulting in unequal counts. Therefore, the

samples were subsequently processed with Trimmomatic v0.39 (Bolger et al. 2014) to re-pair reads.

aDNA authentication

Only samples (105 samples; refer to **Table 3** for this exact list) that were provided with laboratory controls (**Table 1**) were used to authenticate aDNA. The reasoning for this was to avoid potential confusion arising from modern contamination of otherwise ancient samples. Centrifuge v1.0.3 (Kim et al. 2016) was used, with default parameters, to assign taxonomic labels by mapping sequences against the human genome, prokaryotic genomes, and viral genomes including 106 SARS-CoV-2 complete genomes. Human reads from Centrifuge outputs were retained and seqtk 'subseq' was used to convert them into fastq files which were then mapped to the human genome (hg38) (NCBI 2013) using BWA mem v0.7.17 (Li and Durbin 2009). SAMtools v1.12 (Danecek et al. 2021) (-view -rmdup -flagstat -sort -index) was then utilised for alignment formatting and were sorted into BAM files which were run through mapDamage2 v2.2.2 with default parameters (Jónsson et al. 2013).

Data records

We recognize the importance of facilitating easy access to archaeological and scientific data. Analogue and digital repositories, reports and references related to skeletal individuals, and ENA projects associated with digital data in this publication can be found in **Table S1**.

Technical validation

There are a number of concerns regarding the feasibility of our aDNA data that need to be addressed. These include absent controls, short read length, and damage authentication issues that may cause issues for potential future research projects.

Experimental controls

It was not always possible to obtain environmental controls (such as soil, bone, or tooth root blanks) or laboratory controls (such as a library or extraction blanks) (see **Table 1**); therefore, depending on the biological question, some researchers may wish to exclude these samples from downstream analysis. It is beneficial to use environmental controls in projects that analyse the microbial composition of human microbiomes through methods such as diversity or AMR analysis. Environmental controls allow researchers to screen for and eliminate environmental contamination from their findings. These environmental operational taxonomic units (OTUs) may include the modern, living microbes at the grave site, or even the DNA from extinct microorganisms, such as ancient soil-dwelling species or those that hail from other animals and plants, that could distort the findings of a human microbiome study.

Quality assessment

All raw and trimmed data quality, as well as read counts after trimming and decontamination can be viewed in **Table 2** and in **Figure 2**. FastQC and Fastp reports are accessible in the **supporting data (gzip folders)**.

Reads

A subset of 49 samples showed high-quality base calls that had previously been trimmed prior to this research and were 25 base pairs (bp) long. Of the remaining samples, 18 samples were 75 bp, 85 were 125 bp, and 22 were 150 bp (**Table 2**). Short reads are characteristic of aDNA; however, those samples that are 25 bp are not likely to be as practical for certain research such as damage analysis (see **damage analysis** section below), screening for functional genes, contig assembly and metagenomic assembled genome creation, since they may not provide sufficient quality DNA for these type of analyses. Apart from those 25 bp samples that had already been assessed for quality, the remaining reads were trimmed and filtered for post-processing and downstream analysis. These can be viewed in the 'per base sequence' graphs where the X-axis shows the individual bases for reads that have been called, and the Y-axis shows the distribution values (see Fastqc reports in **supporting data**). Samples with good quality scores exhibit a blue line that continually remains high and above a distribution value of 20, which is considered a good bp call according to fastqc reports. **Figure 4** shows overall good mean sequence quality scores (see

full multiQC interactive chart in **supporting data** - **compressed zip folder 3** containing **FastQC reports for trimmed data).**

The dataset initially contained a total of 3,371,978,784 raw reads which were reduced to 3,236,478,044 reads post-filtering. An average of 6% reads per sample (median of 1.84% and interquartile range [IQR] of 4.05%) were lost in the filtering process (see **Table 2** for reads lost per sample). Within the previously trimmed 49 samples, 100% of reads passed the filtering process in two library blanks (BL10 and BL11) and the rest passed >99% of reads. With the exception of laboratory controls, the remaining samples that were not previously trimmed passed >90% of reads.

32 samples could not be decontaminated as they were not assigned a laboratory blank. Samples (*n = 89*) that did not have an environmental blank were still subject to decontamination protocols using laboratory controls, but future research should take caution using these samples for further analysis as ancient and modern environmental contaminants may be present. Alternatively, using a tool like Sourcetracker (Knights et al. 2011) may help understand environmental composition in these samples to an extent. 120 remaining samples (2 tooth root, 7 bone, and 111 dental calculus) comprising 2,339,228,254 reads were all decontaminated with laboratory controls and 30 of these calculus samples were decontaminated with bone and 16 with tooth root (**Table 1**). Following decontamination, 2,125,556,400 (an average of 7.3%, median of 4.47%, and IQR of 9.99%) reads were removed (**Table 2; Figure 3**).

Damage analysis

mapDamage plots (all 104 mapDamage plots are accessible in the **supplementary data** and a summary of C-T frequency transition data is shown in **Table 3**) showed varying base frequencies of C-T transitions on the 5' ends of fragments: 36 samples produced empty plots; 59 samples show misincorporation curves below 0.025; eight showed above 0.025 but below 0.05; and one showed more than 0.05 but less than 0.10, which showed the highest levels of deamination in our sample set (**Figure 5**).

The 36 samples that returned empty plots can all be attributed to short read length (25bp). Current research does not provide a minimum nucleotide substitution frequency threshold to identify DNA as ancient, but it is clear that many of our samples (n=59) show low levels of deamination (<0.025) in contrast to other studies with C-T transition frequency curves as high as 0.3 (Andrades Valtueña et al. 2017).

There are many reasons for the low deamination levels in our ancient samples. In addition to having human reads pre-filtered by the WTSI prior to deposition in the ENA, the majority of samples contained extremely low frequencies of human genomic data because of the nature of metagenomic samples; thus, few human sequences were available to map to the human genome. Second, when mapping short, fragmented and taxonomically diverse sequences to a large and complete reference, like the human genome, mapping tools like BWA are likely to produce low coverage alignments. This observation is evidenced in **Figure 5** by the notable orange line, which would not be visible were it a high coverage alignment. These low coverage alignments make it difficult for authentication tools to identify miscoded lesions in nucleotide sequences. For example, it has been argued that reference-based mapping approaches like mapDamage (Jónsson et al. 2013) and DamageProfiler (Neukamm et al. 2021), and similar limitations in PyDamage (Borry et al. 2021), are impractical approaches for metagenomic samples when handling thousands of unidentified species (Everett and Cribdon 2023).

These tools may be more successful when aligning sequences belonging to bone or other parts of the tooth, such as the pulp, to the human genome. Our strongest damage pattern came from one of our bone samples (**Figure 5**), and other studies have retrieved high damage patterns from bone and teeth using mapDamage (Andrades Valtueña et al. 2017), whereas research that attempts to authenticate shotgun sequences from dental calculus has received results similar to ours (Gancz and Weyrich 2023). We most likely observe this pattern because dental calculus is understood to contain less endogenous human aDNA than other skeletal tissues (Ziesemer et al. 2019). Authenticating aDNA in dental calculus is still possible and has proven to be effective, for example, a 16S rRNA study by (Ziesemer et al. 2015) produced mapDamage results showing typical damage patterns in ancient dental calculus sequences. This study, however, had the advantage of using a targeted gene

approach where the complexity of microbial diversity would not have impacted the reference gene alignment.

In summary, the disadvantage of applying ancient shotgun metagenomics to dental calculus, at least here, is that there are insufficient reads to produce damage signals. Although we understand that damage authentication of aDNA is essential, we think that focusing on minimising the impact of modern contamination is more important at this point.

Summary

This article has described the preparation of an established dataset designed for research utilising microbial aDNA identified in ancient dental calculus, enabling the study of oral microbiomes from both burial populations and individuals, contributing valuable insights to archaeological science. It supports the analysis of microbial interactions in the oral cavity and includes metagenomic data from tooth roots and bone to serve as controls, helping to mitigate contamination risks. Future research projects, however, should take note of the challenges in the field of ancient metagenomics, such as absent controls, short read lengths, and damage authentication issues. Overall, our ancient dental calculus dataset offers the potential to deliver detailed insights into ancient human oral microbiomes. By making this data accessible, we aim to help advance the field of ancient DNA research and enable the exploration of archaeological topics such as diet, disease, and AMR.

Code availability

All bioinformatic scripts are available on GitHub (https://github.com/DrATedder/dental_calculus_dataset/tree/main) for all pre-processing (quality control, decontamination) and post-processing (mapDamage) protocols.

Author contributions

Conceptualization: C.S., S.F., M.C. and G.T., with input from ?. Supervision: C.S., A.T., C.J.M., Funding acquisition: C.S., A.T., C.J.M. Resources: M.H., A.M., C.S., G.T., M.C., and ??. Investigation: F.J.S, G D-A, J.H., C.S., S.F., K.M. Formal Analysis: F.J.S, G. D-A., J.H., C.S., S.M., A.T. and J.W. Data Curation, Visualization and Writing - Original Draft: F.J.S, G. D-A. Writing - Review & Editing: all authors.

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Competing interests

We have no competing interests.

Figures

Norton-on-Tees
Fewston
Victoria Gate, Leeds
Wighill
Driffield Terrace, York
Coppergate, York
Cross Street, Manchester
Tickhill
Melton
Oxford Street, Leicester
Leicester
Radcliffe Infirmary, Oxford
St Barnabas
Lower St Brides Churchyard
Christchurch, Spitalfields
Royal London Hospital

Bronze/Iron Age (ca. 2,300 BC–43 AD)

Roman Period (ca. 43 AD–410 AD)

Viking and Anglo-Saxon (ca. 410 AD–1066 AD)

Medieval Period (ca. 1066–1485)

Industrial (ca. 1750–1850)

Post-industrial (ca. 1837–1901)

Figure 1

Trimming 174 samples

49 samples = 25 bp
18 samples = 75 bp
85 samples = 125 bp
22 samples = 150 bp

Raw read count: 3,371,978,784

Trimmed read count: 3,236,478,044

Decontaminating 120 samples

Now excluded from the above:
22 laboratory controls and 32 samples with no laboratory controls

2 tooth root (by laboratory controls)
7 bone (by laboratory controls)
111 dental calculus (by laboratory controls as well as 30 by bone and 16 by tooth root)

Trimmed read count: 2,339,228,254

Decontaminated read count: 2,125,556,400

Figure 2

□ After trimming ■ After decontamination

Figure 3

Figure 4

Figure 5

Figure Legends

Figure 1. Map of dated archaeological sites across the UK from which the samples came from. More information on the exact number of samples from each site, skeletal ID, ENA

accession number, and publication reference can be accessed in the supplementary data. Sample FW53C, originating from the Church of St. Michael and St. Lawrence, was not placed on the map as it could not be accurately dated. While the other samples from the site were determined to be Victorian/post-industrial, the skeletal report for FW53C did not provide a clear date. Map outline was provided by Twinkl's free education resources (Twinkl 2023) and adapted from Hendy *et al*. 2018.

Figure 2. Flow chart of raw, trimmed, and decontaminated read counts.

Figure 3. Box and whisker plots showing proportion of reads lost following trimming and decontamination.

Figure 4. FastQC sequence quality histograms showing the mean quality value across each base position in the read for all trimmed samples. The green line represents high-quality scores (≥Q25) across all bases, the orange line indicates moderate quality scores (≥Q20-Q25), suggesting some uncertainty in the base calls, and the red line signifies low-quality scores (<Q20).

Figure 5. Example of a mapDamage result of C-T (5' end) and G-A (3' end) base substitutions from bone sample OX04B. The orange line shows nucleotides that do not align to the query sequence.

Tables
Table 1

Sample ID	ENA ID	Sample type	Site	Extraction/library blank decontaminated	Bone blank decontaminated
SK1203, FAO1	ERR1659119	calculus			
SK1207, FAO2	ERR1659110	calculus			
SK1215, FAO3	ERR1659111	calculus			
SK1244.1, FAO4	ERR1659112	calculus			
SK1526, FAO5	ERR1659113	calculus			
SK1558, FAO6	ERR1659120	calculus			
SK1641, FAO7	ERR1659114	calculus			
SK1653, FAO8	ERR1659117	calculus			
SK1655, FAO9	ERR1659115	calculus	Lower St Bride's Churchyard, London	no	no
SK1683, FAO10	ERR1659118	calculus			
SK1785, FAO11	ERR1659116	calculus			
SK1799, FAO12	ERR1681541	calculus			
SK1872, FAO13	ERR1681532	calculus			
SK1932, FAO14	ERR1681533	calculus			
SK1999, FAO15	ERR1681542	calculus			
SK2049, FAO16	ERR1681538	calculus			
SK2134, FAO17	ERR1681534	calculus			
SK2296, FAO18	ERR1681535	calculus			
RLH208	ERR1681523	calculus			
RLH349	ERR1681531	calculus			
RLH365	ERR1681525	calculus			
RLH367	ERR1681526	calculus	Royal London Hospital	eBK686	no
RLH386	ERR1681527	calculus			
RLH397	ERR1681530	calculus			

RLH103	ERR9638308	calculus			
RLH131	ERR9638303	calculus			
RLH135	ERR9638304	calculus			
RLH340	ERR9638305	calculus			
RLH421	ERR9638307	calculus			
RLH572	ERR9638300	calculus			
FW68C	ERR9638278	calculus			
FW88C	ERR9638285	calculus			
FW98C	ERR9638274	calculus			
FW192C	ERR9638281	calculus			
FW217C	ERR9638286	calculus	Fewston, North Yorkshire	no	no
FW268C	ERR9638275	calculus			
FW303C	ERR9638276	calculus			
FW331C	ERR9638283	calculus			
FW450C	ERR9638287	calculus			
STB12B	ERR9638259	bone			n/a
STB16B	ERR9638262	bone			
STB26C	ERR9638263	calculus			
STB2C	ERR9638253	calculus			
STB8C	ERR9638254	calculus			
STB10C	ERR9638255	calculus			
STB11C	ERR9638256	calculus			
STB43C	ERR9638257	calculus	West Kensington	eBK497	
STB27C	ERR9638258	calculus			STB16B, STB12B
STB41C	ERR9638261	calculus			
STB9C	ERR9638267	calculus			
STB54C	ERR9638270	calculus			
STB12C	ERR9638277	calculus			
STB16C	ERR9638279	calculus			
STB45C	ERR9638282	calculus			
VG6C	ERR9638265	calculus			
VG11C	ERR9638266	calculus	Victoria Gate, Leeds	eBK679	no

VG12C	ERR9638268	calculus			
VG15C	ERR9638269	calculus			
SF2300B	ERR9638312	bone			n/a
SF2484B	ERR9638302	bone			
SF01 (SP2182, SK2182, SF1)	ERR9638296	calculus			
SF02 (SP2295, SK2295, SF2)	ERR9638297	calculus			
SF03 (SP2300, SK2300, SF3)	ERR9638289	calculus			
SF04C (SP2301, SK2301, SF4C)	ERR9638280	calculus			
SF05 (SP2369, SK2369, SF5)	ERR9638310	calculus	Christ Church, Spitalfields	eBK682	
SF06 (SP2468, SK2468, SF6)	ERR9638298	calculus			SF2300B, SF2484B
SF07 (SP2477, SK2477, SF7)	ERR9638290	calculus			
SF08 (SP2484, SK2484, SF8)	ERR9638291	calculus			
SF09 (SP2647, SK2647, SF9)	ERR9638309	calculus			
SF10 (SP2748, SK2748)	ERR9638292	calculus			
SF11 (SP2860, SK2860)	ERR9638293	calculus			
ML1032C	ERR1329861	calculus			
ML1823C	ERR1329866	calculus			
ML3890C	ERR1329871	calculus	Melton	eBK695	no
ML4075C	ERR1329872	calculus			
ML1483C	ERR1329865	calculus			
OX12C	ERR1329870	calculus			
OX09C	ERR1329862	calculus			OX12B, OX04B
OX04C	ERR1329853	calculus			
OX05C	ERR1329857	calculus	Oxford St, Leicester	eBK691	
OX12B	ERR1329867	bone			n/a
OX04B	ERR1407510	bone			
3DT26C	ERR1329842	calculus			
3DT21C	ERR1329834	calculus			
6DT7C	ERR1329825	calculus	Driffield Terrace, York	eBK691	no
6DT21C	ERR1329829	calculus			
3DT54C	ERR1329846	calculus			
JV15548C	ERR1329827	calculus	Coppergate, York	eBK695	no

JV30944C	ERR1329830	calculus			
NEM18C	ERR1329851	calculus		no	
NEM093C	ERR1329841	calculus	Norton-on-Tees, East Mill, Durham		no
NEM099C	ERR1329847	calculus			
NBS410C	ERR1329838	calculus			
NBS262C	ERR1329832	calculus		eBK695	
NBS325C	ERR1329836	calculus			
TKAC	ERR1329826	calculus			
TKDC	ERR1329831	calculus	St Mary's Church, Tickhill,South Yorkshire	BL711	no
TKEC	ERR1329835	calculus			
TKFC	ERR1329839	calculus			
WG1688B	ERR1422702	bone		n/a	n/a
WG1705C	ERR1329868	calculus		no	
WG1252C	ERR1329848	calculus			
WG1566C	ERR1329856	calculus	Wighill, North Yorkshire		no
WG1082C	ERR1329843	calculus		BL711	
WG1585C	ERR1407511	calculus			
WG1688C	ERR1422716	calculus			
FW283T	ERR1329878	tooth			n/a
FW435C	ERR1329875	calculus			
FW177C	ERR1329840	calculus			
FW378C	ERR1329874	calculus			
FW130C	ERR1329833	calculus			
FW238C	ERR1329844	calculus			
FW348C	ERR1407505	calculus			
FW366C	ERR1329869	calculus	Fewston, North Yorkshire	BL708	FW283T
FW077C	ERR1329828	calculus			
FW156C	ERR1329837	calculus			
FW319C	ERR1422707	calculus			
FW339C	ERR1329855	calculus			
FW241C	ERR1329845	calculus			
FW283C	ERR1329849	calculus			

FW351C	ERR1329864	calculus			
FW53C (FW53C)	ERR1329824	calculus			
M116	ERR1343019	calculus	University of Tennessee	LBL589	n/a
M20	ERR1343010	calculus			
M56	ERR1343011	calculus			
M85	ERR1343012	calculus			
M103	ERR1343013	calculus			
M111	ERR1343014	calculus			
M31	ERR1343015	calculus			
M73	ERR1343016	calculus			
M89	ERR1343017	calculus			
M112	ERR1343018	calculus			
LC26C	ERR1407497	calculus	Leicester	eBK695	LC26T
LC26T	ERR1422701	tooth			n/a
C028	ERS20133254	calculus	Cross St Cemetery, Manchester	eBK1, eBK2, LBL_439, LBL_695	no
C029	ERS20133255	calculus			
C030	ERS20133256	calculus			
C031	ERS20133257	calculus			
C034	ERS20133258	calculus			
C035	ERS20133260	calculus			
C036	ERS20133261	calculus			
C037	ERS20133262	calculus			
C038	ERS20133263	calculus			
C039	ERS20133264	calculus			
C040	ERS20133265	calculus			
C041	ERS20133266	calculus			
C042	ERS20133267	calculus			
C043	ERS20133268	calculus			
C044	ERS20133269	calculus			
C045	ERS20133270	calculus			
C046	ERS20133271	calculus			
C047	ERS20133272	calculus			

Table 2

ENA/ERS (specified) number	Previously trimmed	Mismatched sequences (%)	Reads passed filters (%)	Mean bp before filtering	Mean bp after filtering	Read length before trimming	Read length after trimming	Read length after decontamination
1329824	Y	N	99.97	25	25	2583158	2582416	2487082
1329825	Y	N	99.96	25	25	879712	879380	876832
1329826	Y	N	99.97	25	25	409780	409684	409684
1329827	Y	N	99.96	25	25	644972	644748	639120
1329828	Y	N	99.96	25	25	461822	461682	447704
1329829	Y	N	99.93	25	25	605826	605460	604424
1329830	Y	N	99.96	25	25	1369538	1369022	1348176
1329831	Y	N	99.97	25	25	476316	476216	476214
1329832	Y	N	99.97	25	25	892672	892432	882258
1329833	Y	N	99.97	25	25	548974	548856	514522
1329834	Y	N	99.96	25	25	388638	388502	387788
1329835	Y	N	99.98	25	25	239084	239058	239058
1329836	Y	N	99.89	25	25	391314	390916	386706
1329837	Y	N	99.97	25	25	210862	210818	201148
1329838	Y	N	99.94	25	25	198406	198304	196176
1329839	Y	N	99.96	25	25	247038	246944	246944
1329840	Y	N	99.97	25	25	467224	467108	451178
1329841	Y	N	99.93	25	25	74130	74084	No match to blank
1329842	Y	N	99.97	25	25	809948	809716	806872
1329843	Y	N	99.97	25	25	897988	897792	897790
1329844	Y	N	99.97	25	25	152184	152148	147296
1329845	Y	N	99.97	25	25	883494	883276	843806
1329846	Y	N	99.97	25	25	721958	721780	720434
1329847	Y	N	99.87	25	25	125702	125540	No match to blank
1329848	Y	N	99.96	25	25	402624	402492	402492
1329849	Y	N	99.82	25	25	251528	251084	243366
1329851	Y	N	99.97	25	25	491376	491238	No match to blank
1329853	Y	N	99.64	25	25	231618	230790	229678
1329855	Y	N	99.97	25	25	171824	171780	162654
1329856	Y	N	99.95	25	25	78242	78210	78210
1329857	Y	N	99.96	25	25	136876	136824	135554
1329861	Y	N	99.96	25	25	261800	261700	258868

1329862	Y	N	99.96	25	25	181530	181462	179992
1329864	Y	N	99.96	25	25	111654	111616	108182
1329865	Y	N	99.68	25	25	275252	274374	271342
1329866	Y	N	99.85	25	25	321792	321326	319042
1329867	Y	N	99.93	25	25	850018	849476	849112
1329868	Y	N	99.98	25	25	725652	725516	No match to blank
1329869	Y	N	99.97	25	25	269398	269336	256532
1329870	Y	N	99.9	25	25	416958	416556	413234
1329871	Y	N	99.96	25	25	450618	450452	445752
1329872	Y	N	99.96	25	25	287424	287336	283892
1329874	Y	N	99.46	25	25	19568	19464	19006
1329875	Y	N	99.97	25	25	194014	193966	186070
1329878	Y	N	99.97	25	25	394528	394416	392586
1329879	Y	N	99.98	25	25	52032	52026	Blank sample
1329880	Y	N	99.99	25	25	20898	20896	Blank sample
1329881	Y	N	100	25	25	2770	2770	Blank sample
1329882	Y	N	100	25	25	322	322	Blank sample
1343010	N	19.32	98.2	75	65	42560574	41797270	40603602
1343011	N	16.23	97.08	75	67	22883998	22216802	21063068
1343012	N	17.59	98.48	75	67	34442652	33920804	32940382
1343013	N	20.46	97.87	75	67	30978568	30320904	29360946
1343014	N	13.71	96.69	75	64	34561856	33419504	32140738
1343015	N	18.66	97.23	75	66	33328916	32407552	31712270
1343016	N	23.89	94.21	75	68	31635170	29804612	28942244
1343017	N	26.46	98.03	75	68	27706734	27162150	25949236
1343018	N	15.39	98.29	75	66	30454984	29936826	28909058
1343019	N	12.94	98.3	75	66	30692120	30172286	29213134
1343020	N	32.08	94.99	75	65	13175352	12516206	Blank sample
1343021	N	96.03	18.35	75	72	9036794	1658910	Blank sample
1407493	N	83.87	36.28	75	68	359280	130368	Blank sample
1407497	N	10.96	97.01	75	68	58423618	56682086	54393516
1407505	N	28.94	94.83	75	70	58589354	55565860	51893282
1407510	N	8.28	97.91	75	53	47001744	46022816	44928798
1407511	N	4.74	97.25	75	58	48838650	47499126	47498426
1407514	N	41.84	74.56	75	55	1540026	1148350	Blank sample
1422701	N	17.66	96.56	125	109	61646806	59530712	58995200
1422702	N	26.46	93.98	125	104	43051330	40460558	No match to blank

1422707	N	0.88	97.69	125	70	50642508	49474228	46352006
1422716	N	10.8	95.52	125	102	52182752	49847030	49846178
1659110	N	1.46	98.23	125	83	29512290	28992366	No match to blank
1659111	N	1.01	98.47	125	79	26875932	26467410	No match to blank
1659112	N	4.8	98.49	125	91	26969988	26565434	No match to blank
1659113	N	2.5	97.83	125	85	29055384	28427660	No match to blank
1659114	N	15.01	85.62	125	86	24805462	21238676	No match to blank
1659115	N	2.05	98.28	125	85	25821896	25378038	No match to blank
1659116	N	0.8	98.41	125	76	47146778	46400438	No match to blank
1659117	N	1.98	98.38	125	76	28774712	28310064	No match to blank
1659118	N	4.63	98.31	125	86	32731048	32180872	No match to blank
1659119	N	1.38	98.27	125	78	24592154	24166868	No match to blank
1659120	N	2.66	98.26	125	82	35486302	34872316	No match to blank
1681523	N	3.26	96.22	125	72	49411398	47545688	47156130
1681525	N	1.26	97.75	125	70	36920280	36093176	34991188
1681526	N	1.3	97.38	125	76	57625826	56120936	53873302
1681527	N	3.26	96.04	125	84	46975236	45116958	41743542
1681530	N	1.63	97.38	125	78	65612894	63896898	62081334
1681531	N	2.05	96.59	125	65	42871320	41409526	39056832
1681532	N	1.42	98.16	125	63	47032120	46171356	No match to blank
1681533	N	1.11	98.17	125	71	35660320	35009952	No match to blank
1681534	N	3.51	98.2	125	82	33354436	32756626	No match to blank
1681535	N	2.69	97.55	125	81	24320390	23726436	No match to blank
1681538	N	3.44	97.98	125	82	30385320	29773404	No match to blank
1681541	N	4.35	97.99	125	85	37920560	37159360	No match to blank
1681542	N	2.39	98.13	125	83	24833088	24370008	No match to blank
9638253	N	3.2	99.01	125	78	24674054	24431924	21710018
9638254	N	12.35	98.89	125	102	28260842	27947766	19212448
9638255	N	7.18	98.81	125	90	27501276	27174018	21299116
9638256	N	8.77	98.75	125	95	25797650	25487318	16667600
9638257	N	5.7	98.71	125	82	29957450	29572208	27105134
9638258	N	4.51	98.68	125	84	24253730	23935210	21708146
9638259	N	7.26	98.14	125	82	24643382	24185674	23720532
9638260	N	90.18	53.44	125	117	724146	387022	Blank sample
9638261	N	6.51	98.59	125	91	27624128	27235894	20130754
9638262	N	14.97	86.89	125	77	26588560	23103962	22367356
9638263	N	7.45	98.32	125	93	27257322	26799464	18403542

9638264	N	15.13	92.23	125	79	4642754	4282398	Blank sample
9638265	N	7.77	98.52	125	89	27644478	27236206	25596208
9638266	N	2.11	98.28	125	76	25996920	25550050	23611344
9638267	N	8.94	98.05	125	83	25031646	24543538	21289618
9638268	N	2.09	98.21	125	78	25575492	25117978	22180726
9638269	N	1.88	98.22	125	79	28529412	28024144	24710314
9638270	N	8.27	98.14	125	85	30716356	30146808	26628622
9638271	N	33.67	73.3	125	87	15952534	11694636	Blank sample
9638272	N	92.6	17.65	125	108	8723184	1539914	Blank sample
9638274	N	1	98.48	125	71	27892400	27470640	No match to blank
9638275	N	5.49	98.59	125	87	25283884	24928344	No match to blank
9638276	N	4.49	98.3	125	84	30013726	29503986	No match to blank
9638277	N	4.93	97.88	125	81	26023962	25473438	22699452
9638278	N	0.61	98.97	125	70	26956626	26681156	No match to blank
9638279	N	0.55	99.06	125	57	25132870	24896936	22752928
9638280	N	1.99	98.49	125	77	25666712	25280914	22733888
9638281	N	4.28	98.66	125	84	28059552	27684056	No match to blank
9638282	N	21.46	85.44	125	90	26409456	22564398	20161446
9638283	N	2.98	97.69	125	77	29453596	28774998	No match to blank
9638284	N	9.02	92.18	125	80	18608440	17154190	Blank sample
9638285	N	3.6	98.92	125	81	27242600	26924196	No match to blank
9638286	N	10.83	89.97	125	84	22855790	20564950	No match to blank
9638287	N	6.21	98.09	125	85	26873758	26362722	No match to blank
9638288	N	77	46.89	125	104	924370	433452	Blank sample
9638289	N	2.32	96.19	125	67	44031332	42353852	35461384
9638290	N	2.57	96.72	125	56	32380974	31321288	24602766
9638291	N	3.39	96.66	125	74	33505890	32388386	28026322
9638292	N	3.4	95.72	125	80	39558620	37867974	29699936
9638293	N	1.9	97.03	125	63	35925288	34858864	22648130
9638294	N	73.42	32.86	125	95	2888654	949470	Blank sample
9638295	N	87.54	377.77	125	106	1222796	377772	Blank sample
9638296	N	87.54	30.89	125	106	41936516	40682502	33970538
9638297	N	2.02	97	125	77	34318286	33304306	28638038
9638298	N	3.93	97.04	125	77	34510834	33516594	25277366
9638300	N	3.64	94.59	125	66	33321126	31521220	30483986
9638301	N	90.36	53.6	125	115	1428434	765698	Blank sample
9638302	N	13.32	96.2	125	104	33809420	32526006	32438428

9638303	N	2.15	96.39	125	65	34867684	33609404	31310986
9638304	N	5.01	96.89	125	85	38289660	37099046	33449992
9638305	N	2.18	96.82	125	77	35541750	34414316	32141250
9638306	N	12.14	91.74	125	81	3605416	3307704	Blank sample
9638307	N	3	97.04	125	77	36222640	35152254	32784300
9638308	N	1.62	97.81	125	76	37429770	36613212	35064998
9638309	N	3.33	95.3	125	69	36701602	34979784	28690092
9638310	N	1.9	96.85	125	65	34990762	33889492	26642234
9638312	N	1.86	97.25	125	52	37248422	36226502	36085818
ERS20133254	N	1.75	13.45	151	78	13540920	11801754	10129902
ERS20133255	N	4.11	9.59	151	113	9650924	9160164	8583678
ERS20133256	N	13.05	7.23	151	92	7279134	6762296	4985224
ERS20133257	N	1.13	10.5	151	72	10566408	9186790	7749908
ERS20133258	N	2.58	14.29	151	96	14393380	13572660	12758236
ERS20133260	N	1.65	12.01	151	76	12086216	10929198	9706028
ERS20133261	N	2.95	11.03	151	89	11101120	10242942	9237776
ERS20133262	N	1.76	11.92	151	86	11993372	10904066	9815586
ERS20133263	N	1.92	13.11	151	82	13209018	11221758	9713430
ERS20133264	N	2.38	11.64	151	88	11712516	10121162	8946904
ERS20133265	N	6.78	11.33	151	103	11412902	9722646	8385754
ERS20133266	N	6.78	9.12	151	104	9186792	8712604	7620040
ERS20133267	N	7.79	17.64	151	102	17916774	16828530	15356984
ERS20133268	N	2.42	12.66	151	83	12760300	11460324	10016358
ERS20133269	N	3.23	8.64	151	72	8724436	7620118	6275740
ERS20133270	N	2.06	12	151	94	12083728	11220760	10547040
ERS20133271	N	3.14	94	151	20.15	20271626	18933902	17435362
ERS20133272	N	7.82	14.55	151	116	14657670	14051062	13095506
ERS20133273	N	85.45	98.97	151	140	2072654	2013310	Blank sample
ERS20133275	N	33.43	99.13	151	101	1259734	985856	Blank sample
ERS20133276	N	99.93	98.67	151	150	4493018	4433302	Blank sample
ERS20133277	N	99.87	98.32	151	150	667232	655950	Blank sample

Table 3

ENA/sample ID	Sample type	Average bp length	Frequency
ERR1329825	Calculus	25bp	empty
ERR1329827	Calculus	25bp	empty
ERR1329829	Calculus	25bp	empty

ERR1329830	Calculus	25bp	empty
ERR1329832	Calculus	25bp	empty
ERR1329834	Calculus	25bp	empty
ERR1329836	Calculus	25bp	empty
ERR1329838	Calculus	25bp	empty
ERR1329842	Calculus	25bp	empty
ERR1329846	Calculus	25bp	empty
ERR1329853	Calculus	25bp	empty
ERR1329857	Calculus	25bp	empty
ERR1329861	Calculus	25bp	empty
ERR1329862	Calculus	25bp	empty
ERR1329865	Calculus	25bp	empty
ERR1329866	Calculus	25bp	empty
ERR1329867	Bone	25bp	empty
ERR1329870	Calculus	25bp	empty
ERR1329871	Calculus	25bp	empty
ERR1329872	Calculus	25bp	empty
ERR1407510	Bone	75bp	>0.05 <0.10
ERR1329826	Calculus	25bp	Empty
ERR1329831	Calculus	25bp	Empty
ERR1329835	Calculus	25bp	Empty
ERR1329839	Calculus	25bp	Empty
ERR1329843	Calculus	25bp	Empty
ERR1329848	Calculus	25bp	Empty
ERR1329856	Calculus	25bp	Empty
ERR1407497	Calculus	75bp	<0.025 >0
ERR1407511	Calculus	75bp	<0.026 >0
ERR1422701	Tooth	125bp	>0.025 <0.05
ERR1422716	Calculus	125bp	<0.026 >0
ERR1329833	Calculus	25bp	Empty
ERR1329844	Calculus	25bp	Empty
ERR1329849	Calculus	25bp	Empty
ERR1329864	Calculus	25bp	Empty
ERR1329874	Calculus	25bp	Empty
ERR9638253	Calculus	125bp	<0.025 >0
ERR9638254	Calculus	125bp	<0.025 >0
ERR9638255	Calculus	125bp	<0.025 >0

ERR9638256	Calculus	125bp	<0.025 >0
ERR9638257	Calculus	125bp	<0.025 >0
ERR9638258	Calculus	125bp	>0.025 <0.05
ERR9638259	Bone	125bp	<0.025 >0
ERR9638261	Calculus	125bp	>0.025 <0.05
ERR9638262	Bone	125bp	<0.025 >0
ERR9638263	Calculus	125bp	<0.025 >0
ERR9638265	Calculus	125bp	<0.025 >0
ERR9638266	Calculus	125bp	<0.025 >0
ERR9638267	Calculus	125bp	<0.025 >0
ERR9638268	Calculus	125bp	<0.025 >0
ERR9638269	Calculus	125bp	<0.025 >0
ERR9638270	Calculus	125bp	<0.025 >0
ERR9638277	Calculus	125bp	<0.025 >0
ERR9638279	Calculus	125bp	<0.025 >0
ERR9638282	Calculus	125bp	<0.025 >0
ERR1329828	Calculus	25bp	Empty
ERR1329840	Calculus	25bp	Empty
ERR1329845	Calculus	25bp	Empty
ERR1329875	Calculus	25bp	Empty
ERR1407505	Calculus	75bp	<0.025 >0
ERR1681523	Calculus	125bp	<0.025 >0
ERR1681525	Calculus	125bp	>0.025 <0.05
ERR1681526	Calculus	125bp	<0.025 >0
ERR1681527	Calculus	125bp	<0.025 >0
ERR1681530	Calculus	125bp	<0.025 >0
ERR1681531	Calculus	125bp	>0.025 <0.05
ERR9638280	Calculus	125bp	<0.025 >0
ERR9638289	Calculus	125bp	<0.025 >0
ERR9638290	Calculus	125bp	<0.025 >0
ERR9638291	Calculus	125bp	<0.025 >0
ERR9638292	Calculus	125bp	<0.025 >0
ERR9638293	Calculus	125bp	<0.025 >0
ERR9638296	Calculus	125bp	<0.025 >0
ERR9638297	Calculus	125bp	>0.025 <0.05
ERR9638298	Calculus	125bp	<0.025 >0
ERR9638300	Calculus	125bp	<0.025 >0

ERR9638302	Bone	125bp	<0.025 >0
ERR9638303	Calculus	125bp	>0.025 <0.05
ERR9638304	Calculus	125bp	<0.025 >0
ERR9638305	Calculus	125bp	<0.025 >0
ERR9638307	Calculus	125bp	>0.025 <0.05
ERR9638308	Calculus	125bp	<0.025 >0
ERR9638309	Calculus	125bp	<0.025 >0
ERR9638310	Calculus	125bp	<0.025 >0
ERR9638312	Bone	125bp	<0.025 >0
ERS20133254	Calculus	150bp	<0.025 >0
ERS20133255	Calculus	150bp	<0.025 >0
ERS20133256	Calculus	150bp	<0.025 >0
ERS20133257	Calculus	150bp	<0.025 >0
ERS20133258	Calculus	150bp	<0.025 >0
ERS20133260	Calculus	150bp	<0.025 >0
ERS20133261	Calculus	150bp	<0.025 >0
ERS20133262	Calculus	150bp	<0.025 >0
ERS20133263	Calculus	150bp	<0.025 >0
ERS20133264	Calculus	150bp	<0.025 >0
ERS20133265	Calculus	150bp	<0.025 >0
ERS20133266	Calculus	150bp	<0.025 >0
ERS20133267	Calculus	150bp	<0.025 >0
ERS20133268	Calculus	150bp	<0.025 >0
ERS20133269	Calculus	150bp	<0.025 >0
ERS20133270	Calculus	150bp	<0.025 >0
ERS20133271	Calculus	150bp	<0.025 >0
ERS20133272	Calculus	150bp	<0.025 >0

Table legends

Table S1. Skeletal information, proveniences and ENA accession numbers for the archaeological and modern dental calculus samples collected in this study.

Table 1. Decontamination information on samples and their matching laboratory/environmental blanks.

Table 2. Quality control results showing percentage of mismatches sequences and reads past filters, and bp and read length pre and post-trimming and pre and post-decontamination.

Table 3. Cytosine-Thymine frequency transition values for damage authentication interpretation.

Supporting data

Table S1 (large metadata table)

Compressed zip folder 1: FastQC reports for raw data
Compressed zip folder 2: Fastp reports
Compressed zip folder 3: FastQC reports for trimmed data
Compressed zip folder 4: mapDamage plots

References

Acs, P., Wilhalm, T. and Oeggl, K. (2005) Remains of grasses found with the Neolithic Iceman "Ötzi". *Vegetation History and Archaeobotany* 14 (3), 198-206.

Adams, M. and Reeve, J. (1987) Excavations at Christ Church, Spitalfields 1984–6. *Antiquity* 61 (232), 247-256.

Adler, C. J., Browne, G. V., Sukumar, S. and Hughes, T. (2017) Evolution of the Oral Microbiome and Dental Caries. *Current Oral Health Reports* 4 (3), 264-269.

Adler, C. J., Dobney, K., Weyrich, L. S., Kaidonis, J., Walker, A. W., Haak, W., Bradshaw, C. J. A., Townsend, G., Sołtysiak, A., Alt, K. W., Parkhill, J. and Cooper, A. (2013) Sequencing ancient calcified dental plaque shows changes in oral microbiota with dietary shifts of the Neolithic and Industrial revolutions. *Nature Genetics* 45 (4), 450-455.

Almeida, V. D. S. M., Azevedo, J., Leal, H. F., Queiroz, A. T. L. D., Da Silva Filho, H. P. and Reis, J. N. (2020) Bacterial diversity and prevalence of antibiotic resistance genes in the oral microbiome. *PLOS ONE* 15 (9), e0239664.

Andrades Valtueña, A., Mittnik, A., Key, F. M., Haak, W., Allmäe, R., Belinskij, A., Daubaras, M., Feldman, M., Jankauskas, R., Janković, I., Massy, K., Novak, M., Pfrengle, S., Reinhold, S., Šlaus, M., Spyrou, M. A., Szécsényi-Nagy, A., Tõrv, M., Hansen, S., Bos, K. I., Stockhammer, P. W., Herbig, A. and Krause, J. (2017) The Stone Age Plague and Its Persistence in Eurasia. *Current Biology* 27 (23), 3683-3691.e8.

Barbieri, R., Mekni, R., Levasseur, A., Chabrière, E., Signoli, M., Tzortzis, S., Aboudharam, G. and Drancourt, M. (2017) Paleoproteomics of the Dental Pulp: The plague paradigm. *PLOS ONE* 12 (7), e0180552.

Bliujienė, A., Skipitytė, R., Garbaras, A., Miliauskienė, Ž., Šapolaitė, J., Ežerinskis, Ž., Čeponkus, J., Masiulienė, I., Simčenka, E., Minkevičius, K. and Piličiauskienė, G. (2020) The first data on the human diet in Late Roman and Early Migration period western Lithuania: Evidence from stable isotope, archaebotanical and zooarchaeological analyses. *Journal of archaeological science, reports* 33, 102545.

Bolger, A. M., Lohse, M. and Usadel, B. (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30 (15), 2114-2120.

Borry, M., Hübner, A., Rohrlach, A. B. and Warinner, C. (2021) PyDamage: automated ancient damage identification and estimation for contigs in ancient DNA <i>de novo</i> assembly. *PeerJ* 9, e11845.

Bos, K. I., Herbig, A., Sahl, J., Waglechner, N., Fourment, M., Forrest, S. A., Klunk, J., Schuenemann, V. J., Poinar, D., Kuch, M., Golding, G. B., Dutour, O., Keim, P., Wagner, D. M., Holmes, E. C., Krause, J. and Poinar, H. N. (2016) Eighteenth century Yersinia pestis genomes reveal the long-term persistence of an historical plague focus. *eLife* 5, e12994-e12994.

Bos, K. I., Schuenemann, V. J., Golding, G. B., Burbano, H. A., Waglechner, N., Coombes, B. K., McPhee, J. B., DeWitte, S. N., Meyer, M., Schmedes, S., Wood, J., Earn, D. J. D., Herring, D. A., Bauer, P., Poinar, H. N. and Krause, J. (2011) A draft genome of Yersinia pestis from victims of the Black Death. *Nature* 478 (7370), 506-510.

Brealey, J. C., Leitão, H. G., van der Valk, T., Xu, W., Bougiouri, K., Dalén, L. and Guschanski, K. (2020) Dental Calculus as a Tool to Study the Evolution of the Mammalian Oral Microbiome. *Mol. Biol. Evol.* 37 (10), 3003-3022.

Caselli, E., Fabbri, C., D'Accolti, M., Soffritti, I., Bassi, C., Mazzacane, S. and Franchi, M. (2020) Defining the oral microbiome by whole-genome sequencing and resistome analysis: the complexity of the healthy picture. *BMC Microbiology* 20 (1).

Chidimuro, B., Mundorff, A., Speller, C., Radini, A., Boudreault, N., Lucas, M., Holst, M., Lamb, A., Collins, M. and Alexander, M. (2022) Isotope analysis of human dental calculus $\delta^{13}CO_3^{2-}$: Investigating a potential new proxy for sugar consumption. *Rapid Communications in Mass Spectrometry* 36 (11).

Cristiani, E., Radini, A., Edinborough, M. and Borić, D. (2016) Dental calculus reveals Mesolithic foragers in the Balkans consumed domesticated plant foods. *Proceedings of the National Academy of Sciences* 113 (37), 10298-10303.

Curtis, M. J., Beaumont, J., Elamin, F., Wilson, A. S. and Koon, H. E. C. (2022) Method of micro-sampling human dentine collagen for stable isotope analysis. *Rapid Communications in Mass Spectrometry* 36 (13).

Dabney, J., Knapp, M., Glocke, I., Gansauge, M.-T., Weihmann, A., Nickel, B., Valdiosera, C., García, N., Pääbo, S., Arsuaga, J.-L., Meyer, M., 2013. Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proc. Natl. Acad. Sci. U. S. A*. 110, 15758–15763.

Danecek, P., Bonfield, J. K., Liddle, J., Marshall, J., Ohan, V., Pollard, M. O., Whitwham, A., Keane, T., McCarthy, S. A., Davies, R. M. and Li, H. (2021) Twelve years of SAMtools and BCFtools. *GigaScience* 10 (2).

Eisenhofer, R., Anderson, A., Dobney, K., Cooper, A. and Weyrich, L. S. (2019) Ancient Microbial DNA in Dental Calculus: A New method for Studying Rapid Human Migration Events. *The Journal of Island and Coastal Archaeology* 14 (2), 149-162.

ENA, E. N. A. (2023) *European Nucleotide Archive.* https://www.ebi.ac.uk/ena/browser/home Accessed 22 September.

Everett, R. and Cribdon, B. (2023) MetaDamage tool: Examining post-mortem damage in sedaDNA on a metagenomic scale. *Frontiers in Ecology and Evolution* 10.

Faerman, M., Filon, D., Kahila, G., Greenblatt, C. L., Smith, P. and Oppenheim, A. (1995) Sex identification of archaeological human remains based on amplification of the X and Y amelogenin alleles. *Gene* 167 (1-2), 327-332.

Fagernäs, Z., Salazar-García, D. C., Avilés, A., Haber, M., Henry, A., Maurandi, J. L., Ozga, A., Velsko, I. M. and Warinner, C. (2021) Understanding the microbial biogeography of ancient human dentitions to guide study design and interpretation. Cold Spring Harbor Laboratory.

Farrer, A. G., Bekvalac, J., Redfern, R., Gully, N., Dobney, K., Cooper, A. and Weyrich, L. S. (2018) Biological and cultural drivers of oral microbiota in Medieval and Post-Medieval London, UK. Cold Spring Harbor Laboratory.

Farrer, A. G., Wright, S. L., Skelly, E., Eisenhofer, R., Dobney, K. and Weyrich, L. S. (2021) Effectiveness of decontamination protocols when analyzing ancient DNA preserved in dental calculus. Scientific Reports 11 (1), 7456.

Finlay, N. (2000) Outside of life: traditions of infant burial in Ireland from cillin to cist. *World Archaeology* 31 (3), 407-422.

Forshaw, R. (2022) Dental calculus - oral health, forensic studies and archaeology: a review. *British Dental Journal* 233 (11), 961-967.

Fortes, G. G. & Paijmans, J. L. A. 2015 Analysis of Whole Mitogenomes from Ancient Samples. *Methods Mol. Biol*. 1347, 179–195.

Fotakis, A. K., Denham, S. D., Mackie, M., Orbegozo, M. I., Mylopotamitaki, D., Gopalakrishnan, S., Sicheritz-Pontén, T., Olsen, J. V., Cappellini, E., Zhang, G., Christophersen, A., Gilbert, M. T. P. and Vågene, Å. J. (2020) Multi-omic detection of Mycobacterium leprae in archaeological human dental calculus. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 375 (1812), 20190584.

Fuller, B. T., Molleson, T. I., Harris, D. A., Gilmour, L. T. and Hedges, R. E. M. (2006) Isotopic evidence for breastfeeding and possible adult dietary differences from Late/Sub-Roman Britain. *Am. J. Phys. Anthropol.* 129 (1), 45-54.

Gancz, A. S. and Weyrich, L. S. (2023) Studying ancient human oral microbiomes could yield insights into the evolutionary history of noncommunicable diseases. *F1000Research* 12, 109.

Gancz, A.S., Farrer, A.G., Nixon, M.P., Wright, S., Arriola, L., Adler, C., Davenport, E. R., Gully, N., Cooper, A., Britton, K., Dobney, K., Silverman, J. D., Weyrich, L. S. (2023) Ancient dental calculus reveals oral microbiome shifts associated with lifestyle and disease in Great Britain. *Nat Microbiol.* 8, 2315–2325. https://doi.org/10.1038/s41564-023-01527-3.

Gansauge, M.-T., Meyer, M., 2013. Single-stranded DNA library preparation for the sequencing of ancient or damaged DNA. *Nat. Protoc*. 8, 737–748.

Granehäll, L., Huang, K. D., Tett, A., Manghi, P., Paladin, A., O'Sullivan, N., Rota-Stabelli, O., Segata, N., Zink, A. and Maixner, F. (2021) Metagenomic analysis of ancient dental calculus reveals unexplored diversity of oral archaeal Methanobrevibacter. *Microbiome* 9 (1).

Harbeck, M., Seifert, L., Hänsch, S., Wagner, D. M., Birdsell, D., Parise, K. L., Wiechmann, I., Grupe, G., Thomas, A., Keim, P., Zöller, L., Bramanti, B., Riehm, J. M. and Scholz, H. C. (2013) Yersinia pestis DNA from Skeletal Remains from the 6th Century AD Reveals Insights into Justinianic Plague. *PLoS Pathogens* 9 (5), e1003349.

Hendy, J., Warinner, C., Bouwman, A., Collins, M. J., Fiddyment, S., Fischer, R., Hagan, R., Hofman, C. A., Holst, M., Chaves, E., Klaus, L., Larson, G., Mackie, M., McGrath, K., Mundorff, A. Z., Radini, A., Rao, H., Trachsel, C., Velsko, I. M. and Speller, C. F. (2018) Proteomic evidence of dietary sources in ancient dental calculus. *Proceedings of the Royal Society B: Biological Sciences* 285 (1883), 20180977.

Humphrey, L., Bello, S. and Rousham, E. (2012) SEX DIFFERENCES IN INFANT MORTALITY IN SPITALFIELDS, LONDON, 1750–1839. *J. Biosoc. Sci.* 44 (1), 95-119.

Huynh, H. T. T., Nkamga, V. D., Signoli, M., Tzortzis, S., Pinguet, R., Audoly, G., Aboudharam, G. and Drancourt, M. (2016) Restricted diversity of dental calculus methanogens over five centuries, France. *Scientific Reports* 6 (1), 25775.

Jacobson, D. K., Honap, T. P., Monroe, C., Lund, J., Houk, B. A., Novotny, A. C., Robin, C., Marini, E. and Lewis, C. M. (2020) Functional diversity of microbial ecologies estimated from ancient human coprolites and dental calculus. *Philosophical Transactions of the Royal Society B: Biological Sciences* 375 (1812), 20190586.

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. and Schroeder, H. (2019) A 5700 year-old human genome and oral microbiome from chewed birch pitch. *Nature Communications* 10 (1).

Jersie-Christensen, R. R., Lanigan, L. T., Lyon, D., Mackie, M., Belstrøm, D., Kelstrup, C. D., Fotakis, A. K., Willerslev, E., Lynnerup, N., Jensen, L. J., Cappellini, E. and Olsen, J. V. (2018) Quantitative metaproteomics of medieval dental calculus reveals individual oral health status. *Nature Communications* 9 (1).

Jónsson, H., Ginolhac, A., Schubert, M., Johnson, P. L. F. and Orlando, L. (2013) mapDamage2.0: fast approximate Bayesian estimates of ancient DNA damage parameters. *Bioinformatics* 29 (13), 1682-1684.

Kazarina, A., Petersone-Gordina, E., Kimsis, J., Kuzmicka, J., Zayakin, P., Griškjans, Ž., Gerhards, G. and Ranka, R. (2021) The Postmedieval Latvian Oral Microbiome in the Context of Modern Dental Calculus and Modern Dental Plaque Microbial Profiles. *Genes* 12 (2).

Keller, A., Graefen, A., Ball, M., Matzas, M., Boisguerin, V., Maixner, F., Leidinger, P., Backes, C., Khairat, R., Forster, M., Stade, B., Franke, A., Mayer, J., Spangler, J., McLaughlin, S., Shah, M., Lee, C., Harkins, T. T., Sartori, A., Moreno-Estrada, A., Henn, B., Sikora, M., Semino, O., Chiaroni, J., Rootsi, S., Myres, N. M., Cabrera, V. M., Underhill, P. A., Bustamante, C. D., Vigl, E. E., Samadelli, M., Cipollini, G., Haas, J., Katus, H., O'Connor, B. D., Carlson, M. R. J., Meder, B., Blin, N., Meese, E., Pusch, C. M. and Zink, A. (2012) New insights into the Tyrolean Iceman's origin and phenotype as inferred by whole-genome sequencing. *Nature communications* 3 (1), 698-698.

Kilian, M., Chapple, I. L. C., Hannig, M., Marsh, P. D., Meuric, V., Pedersen, A. M. L., Tonetti, M. S., Wade, W. G. and Zaura, E. (2016) The oral microbiome - An update for oral healthcare professionals. *British dental journal* 221 (10), 657-666.

Kim, D., Song, L., Breitwieser, F. P. and Salzberg, S. L. (2016) Centrifuge: rapid and sensitive classification of metagenomic sequences. Genome Research 26(12), 1721–1729.

Knights, D., Kuczynski, J., Charlson, E. S., Zaneveld, J., Mozer, M. C., Collman, R. G., Bushman, F. D., Knight, R. and Kelley, S. T. (2011) Bayesian community-wide culture-independent microbial source tracking. *Nature Methods* 8 (9), 761-763.

Larcher, A. (2019) How Isotopes Traced Ötzi's Origins. 40(4), 16-20. *https://go.exlibris.link/88vYKVDY*

Leach, S., Eckardt, H., Chenery, C., Müldner, G. and Lewis, M. (2010) A Lady of York: migration, ethnicity and identity in Roman Britain. *Antiquity* 84 (323), 131-145.

Li, H. (2024) *seqtk.* GitHub: GitHub, Inc. *https://github.com/lh3/seqtk* Accessed 26 August.

Li, H. and Durbin, R. (2009) Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* 25 (14), 1754-1760.

Loreille, O., Ratnayake, S., Bazinet, A. L., Stockwell, T. B., Sommer, D. D., Rohland, N., Mallick, S., Johnson, P. L. F., Skoglund, P., Onorato, A. J., Bergman, N. H., Reich, D. and Irwin, J. A. (2018) Biological Sexing of a 4000-Year-Old Egyptian Mummy Head to Assess the Potential of Nuclear DNA Recovery from the Most Damaged and Limited Forensic Specimens. *Genes* 9 (3).

Lugli, F., Di Rocco, G., Vazzana, A., Genovese, F., Pinetti, D., Cilli, E., Carile, M. C., Silvestrini, S., Gabanini, G., Arrighi, S., Buti, L., Bortolini, E., Cipriani, A., Figus, C., Marciani, G., Oxilia, G., Romandini, M., Sorrentino, R., Sola, M. and Benazzi, S. (2019) Enamel peptides reveal the sex of the Late Antique 'Lovers of Modena'. *Sci. Rep.* 9 (1), 13130.

Lugli, G. A., Milani, C., Mancabelli, L., Turroni, F., Ferrario, C., Duranti, S., Van Sinderen, D. and Ventura, M. (2017) Ancient bacteria of the Ötzi's microbiome: a genomic tale from the Copper Age. *Microbiome* 5 (1).

Mann, A. E., Sabin, S., Ziesemer, K., Vågene, Å. J., Schroeder, H., Ozga, A. T., Sankaranarayanan, K., Hofman, C. A., Fellows Yates, J. A., Salazar-García, D. C., Frohlich, B., Aldenderfer, M., Hoogland, M., Read, C., Milner, G. R., Stone, A. C., Lewis, C. M., Krause, J., Hofman, C., Bos, K. I. and Warinner, C. (2018) Differential preservation of endogenous human and microbial DNA in dental calculus and dentin. *Scientific Reports* 8 (1).

Makarewicz, C. A. and Sealy, J. (2015) Dietary reconstruction, mobility, and the analysis of ancient skeletal tissues: Expanding the prospects of stable isotope research in archaeology. *Journal of archaeological science* 56, 146-158.

Metcalf, J. L., Ursell, L. K. and Knight, R. (2014) Ancient human oral plaque preserves a wealth of biological data. *Nature Genetics* 46 (4), 321-323.

Meyer, M. and Kircher, M. (2010) Illumina sequencing library preparation for highly multiplexed target capture and sequencing. Cold Spring Harbor Protocols 6, 1–20.

Molleson, T., Cox, M., Waldron, T. and Whittaker, D. K. (1993) *The Spitalfields Project Volume 2: The Anthropology; the Middling Sort.* Council for British Archaeology York.

Nath, S., Handsley-Davis, M., Weyrich, L. S. and Jamieson, L. M. (2021) Diversity and bias in oral microbiome research: A commentary. *EClinicalMedicine* 36, 100923-100923.

NCBI (2013) *Genome assembly GRCh38.* *https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_000001405.26/* Accessed 31 July.

Neukamm, J., Peltzer, A. and Nieselt, K. (2021) DamageProfiler: fast damage pattern calculation for ancient DNA. *Bioinformatics* 37 (20), 3652-3653.

Neukamm, J., Pfrengle, S., Molak, M., Seitz, A., Francken, M., Eppenberger, P., Avanzi, C., Reiter, E., Urban, C., Welte, B., Stockhammer, P. W., Teßmann, B., Herbig, A., Harvati, K., Nieselt, K., Krause, J. and Schuenemann, V. J. (2020) 2000-year-old pathogen genomes reconstructed from metagenomic analysis of Egyptian mummified individuals. *BMC Biol.* 18 (1), 108.

Nielsen, B. H., Christensen, T. and Frei, K. M. (2020) New insights from forgotten bog bodies: The potential of bog skeletons for investigating the phenomenon of deposition of human remains in bogs during prehistory. *Journal of archaeological science* 120, 105166.

Nitsch, E. K., Humphrey, L. T. and Hedges, R. E. M. (2011) Using stable isotope analysis to examine the effect of economic change on breastfeeding practices in Spitalfields, London, UK. *Am. J. Phys. Anthropol.* 146 (4), 619-628.

Oliver, M. (1992) Excavation of an Iron Age and Romano-British settlement site at Oakridge, Basingstoke, Hampshire 1965--6. *Proceedings of Hampshire Field Club and Archaeological Society* 48, 55-94.

Ottoni, C., Borić, D., Cheronet, O., Sparacello, V., Dori, I., Coppa, A., Antonović, D., Vujević, D., Price, T. D., Pinhasi, R. and Cristiani, E. (2021) Tracking the transition to agriculture in Southern Europe through ancient DNA analysis of dental calculus. *Proceedings of the National Academy of Sciences* 118 (32), e2102116118.

Papagrigorakis, M. J., Synodinos, P. N. and Yapijakis, C. (2007) Ancient typhoid epidemic reveals possible ancestral strain of Salmonella enterica serovar Typhi. *Infect. Genet. Evol.* 7 (1), 126-127.

Parker, G. J., Yip, J. M., Eerkens, J. W., Salemi, M., Durbin-Johnson, B., Kiesow, C., Haas, R., Buikstra, J. E., Klaus, H., Regan, L. A., Rocke, D. M. and Phinney, B. S. (2019) Sex estimation using sexually dimorphic amelogenin protein fragments in human enamel. *Journal of Archaeological Science* 101, 169-180.

Pearce, J. (1999) The Dispersed Dead: Preliminary Observations on Burial and Settlement in Rural Roman Britain. *Theoretical Roman Archaeology Journal* 0 (1998), 151.

Pires de Souza, G. A., Rolland, C., Nafeh, B., La Scola, B. and Colson, P. (2021) Giant virus-related sequences in the 5300-year-old Ötzi mummy metagenome. *Virus genes* 57 (2), 222-227.

Preus, H. R., Marvik, O. J., Selvig, K. A. and Bennike, P. (2011) Ancient bacterial DNA (aDNA) in dental calculus from archaeological human remains. *Journal of archaeological science* 38 (8), 1827-1831.

Proctor, D. M., Fukuyama, J. A., Loomer, P. M., Armitage, G. C., Lee, S. A., Davis, N. M., Ryder, M. I., Holmes, S. P. and Relman, D. A. (2018) A spatial gradient of bacterial diversity in the human oral cavity shaped by salivary flow. *Nature Communications* 9 (1).

Quagliariello, A., Modi, A., Innocenti, G., Zaro, V., Conati Barbaro, C., Ronchitelli, A., Boschin, F., Cavazzuti, C., Dellù, E., Radina, F., Sperduti, A., Bondioli, L., Ricci, S., Lognoli, M., Belcastro, M. G., Mariotti, V., Caramelli, D., Mariotti Lippi, M., Cristiani, E., Martino, M. E., Muntoni, I. M. and Lari, M. (2022) Ancient oral microbiomes support gradual Neolithic dietary shifts towards agriculture. *Nature Communications* 13 (1).

Rascovan, N., Sjögren, K.-G., Kristiansen, K., Nielsen, R., Willerslev, E., Desnues, C. and Rasmussen, S. (2019) Emergence and Spread of Basal Lineages of Yersinia pestis during the Neolithic Decline. *Cell* 176 (1-2), 295-305.e10.

Redfern, R. C., DeWitte, S. N., Pearce, J., Hamlin, C. and Dinwiddy, K. E. (2015) Urban-rural differences in Roman Dorset, England: A bioarchaeological perspective on Roman settlements. *American Journal of Physical Anthropology* 157 (1), 107-120.

Sanctis, B., Money, D., Pedersen, M. W. and Durbin, R. (2022) A theoretical analysis of taxonomic binning accuracy. *Molecular ecology resources* 22 (6), 2208-2219.

Sawafuji, R., Saso, A., Suda, W., Hattori, M. and Ueda, S. (2020) Ancient DNA analysis of food remains in human dental calculus from the Edo period, Japan. *PLOS ONE* 15 (3), e0226654.

Seiler, R., Spielman, A. I., Zink, A. and Rühli, F. (2013) Oral pathologies of the Neolithic Iceman, c.3,300 bc. *European journal of oral sciences* 121 (3pt1), 137-141.

Skelly, E. J. (2019) Processes of industrialisation influencing the human oral microbiome. https://hdl.handle.net/2440/121332. Accessed 14 May 2024.

Skoglund, P., Storå, J., Götherström, A. and Jakobsson, M. (2013) Accurate sex identification of ancient human remains using DNA shotgun sequencing. *J. Archaeol. Sci.* 40 (12), 4477-4482.

Smith, J. S. (2019) Archaeological oral health: a comparison of post-medieval and modern-day dental caries exposure of adults in East London. *British Dental Journal* 227 (8), 721-725.

Spyrou, M. A., Tukhbatova, R. I., Wang, C.-C., Valtueña, A. A., Lankapalli, A. K., Kondrashin, V. V., Tsybin, V. A., Khokhlov, A., Kühnert, D., Herbig, A., Bos, K. I. and Krause, J. (2018) Analysis of 3800-year-old Yersinia pestis genomes suggests Bronze Age origin for bubonic plague. *Nature Communications* 9 (1).

Stewart, N. A., Gerlach, R. F., Gowland, R. L., Gron, K. J. and Montgomery, J. (2017) Sex determination of human remains from peptides in tooth enamel. *Proc. Natl. Acad. Sci. U. S. A.* 114 (52), 13649-13654.

Twinkl (2023) *Blank UK Map.*   https://www.twinkl.co.uk/resource/t-g-162-blank-uk-map Accessed 22 September.

van Beek, R., Quik, C., Bergerbrant, S., Huisman, F., Kama, P., Institutionen för historiska, s., Humanistiska, f., Faculty of, H., Göteborgs, u., Gothenburg, U. and Department of Historical, S. (2023) Bogs, bones and bodies: the deposition of human remains in northern European mires (9000 BC–AD 1900). *Antiquity* 97 (391), 120-140.

Velsko, I. M., Fellows Yates, J. A., Aron, F., Hagan, R. W., Frantz, L. A. F., Loe, L., Martinez, J. B. R., Chaves, E., Gosden, C., Larson, G. and Warinner, C. (2019) Microbial differences between dental plaque and historic dental calculus are related to oral biofilm maturation stage. *Microbiome* 7 (1).

Wagner, D. M. P., Klunk, J. B. S., Harbeck, M. P., Devault, A. M. A., Waglechner, N. M., Sahl, J. W. P., Enk, J. M., Birdsell, D. N. P., Kuch, M. M., Lumibao, C. M., Poinar, D. M. A., Pearson, T. P., Fourment, M. P., Golding, B. P., Riehm, J. M. P., Earn, D. J. D. P., DeWitte, S. P., Rouillard, J.-M. P., Grupe, G. P., Wiechmann, I. P., Bliska, J. B. P., Keim, P. S. P., Scholz, H. C. P., Holmes, E. C. P. and Poinar, H. D. (2014) Yersinia pestis and the Plague of Justinian 541–543 AD: a genomic analysis. *The Lancet infectious diseases* 14 (4), 319-326.

Warinner, C., Hendy, J., Speller, C., Cappellini, E., Fischer, R., Trachsel, C., Arneborg, J., Lynnerup, N., Craig, O. E., Swallow, D. M., Fotakis, A., Christensen, R. J., Olsen, J. V., Liebert, A., Montalva, N., Fiddyment, S., Charlton, S., Mackie, M., Canci, A., Bouwman, A., Rühli, F., Gilbert, M. T. P. and Collins, M. J. (2014a) Direct evidence of milk consumption from ancient human dental calculus. *Scientific Reports* 4 (1), 7104.

Warinner, C., Rodrigues, J. F. M., Vyas, R., Trachsel, C., Shved, N., Grossmann, J., Radini, A., Hancock, Y., Tito, R. Y., Fiddyment, S., Speller, C., Hendy, J., Charlton, S., Luder, H. U., Salazar-García, D. C., Eppler, E., Seiler, R., Hansen, L. H., Castruita, J. A. S., Barkow-Oesterreicher, S., Teoh, K. Y., Kelstrup, C. D., Olsen, J. V., Nanni, P., Kawai, T., Willerslev, E., Von Mering, C., Lewis, C. M., Collins, M. J., Gilbert, M. T. P., Rühli, F. and Cappellini, E. (2014b) Pathogens and host immunity in the ancient human oral cavity. *Nature Genetics* 46 (4), 336-344.

Watts, D. J. (2001) The Silent Minority: Women in Romano-British Cemeteries. *Archaeological Journal* 158 (1), 332-347.

Weyrich, L. S., Duchene, S., Soubrier, J., Arriola, L., Llamas, B., Breen, J., Morris, A. G., Alt, K. W., Caramelli, D., Dresely, V., Farrell, M., Farrer, A. G., Francken, M., Gully, N., Haak, W., Hardy, K., Harvati, K., Held, P., Holmes, E. C., Kaidonis, J., Lalueza-Fox, C., de la

Rasilla, M., Rosas, A., Semal, P., Soltysiak, A., Townsend, G., Usai, D., Wahl, J., Huson, D. H., Dobney, K. and Cooper, A. (2017) Neanderthal behaviour, diet, and disease inferred from ancient DNA in dental calculus. *Nature* 544 (7650), 357-361.

Willmann, C., Mata, X., Hanghoej, K., Tonasso, L., Tisseyre, L., Jeziorski, C., Cabot, E., Chevet, P., Crubézy, E., Orlando, L., Esclassan, R. and Thèves, C. (2018) Oral health status in historic population: Macroscopic and metagenomic evidence. *PloS one* 13 (5), e0196482-e0196482.

Yang, D.Y., Eng, B., Waye, J.S., Dudar, J.C., Saunders, S.R., 1998. Technical note: improved DNA extraction from ancient bones using silica-based spin columns. Am. J. Phys. Anthropol. 105, 539–543.

Zayed, S. M., Aboulwafa, M. M., Hashem, A. M. and Saleh, S. E. (2021) Biofilm formation by Streptococcus mutans and its inhibition by green tea extracts. *AMB Express* 11 (1), 73-73.

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. and Warinner, C. (2015) Intrinsic challenges in ancient microbiome reconstruction using 16S rRNA gene amplification. *Sci. Rep.* 5, 16498.

Ziesemer, K. A., Ramos-Madrigal, J., Mann, A. E., Brandt, B. W., Sankaranarayanan, K., Ozga, A. T., Hoogland, M., Hofman, C. A., Salazar-García, D. C., Frohlich, B., Milner, G. R., Stone, A. C., Aldenderfer, M., Lewis, C. M., Hofman, C. L., Warinner, C. and Schroeder, H. (2019) The efficacy of whole human genome capture on ancient dental calculus and dentin. *American Journal of Physical Anthropology* 168 (3), 496-509.