



Version 2

Jun 15, 2021

A-Z of ancient DNA protocols for shotgun Illumina Next Generation Sequencing V.2

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ABSTRACT

This collection contains established ancient DNA (aDNA) specific protocols for Illumina next generation sequencing. It covers sampling of skeletal remains for regions preserving higher amounts of aDNA, DNA extraction methods designed for retrieval of very short DNA fragments, and various library construction methods (including treatments for the 'removal' of typical aDNA damage). All protocols have been adapted after published protocols (see each for the corresponding original publication), and are used primarily in the Department of Archaeogenetics at the MPI-SHH (Jena, Germany).

All protocols in this collection can be used together, and in some cases swapped out with others. All protocols prior indexing are mostly performed in a dedicated ancient DNA laboratory - please see the guidelines section of each protocol for more information.

The order of protocols is as follows:

1. Sampling for aDNA

1. Sampling of *Pars petrosa (os temporale, 'petrous bones')* for human aDNA extraction
2. Sampling of teeth for human and microbial (e.g. pathogen) aDNA extraction
3. Sampling of dental calculus for oral microbiome aDNA extraction

2. Retrieval of DNA

1. Ancient DNA Extraction from Skeletal Material (based on the [Dabney et al. 2013 Proc Natl Acad Sci USA](#) protocol)
2. Ancient DNA extraction from dental calculus (based on the [Dabney et al. 2013 Proc Natl Acad Sci USA](#) with extra washing steps from [Ozga et al. 2016 Am J Phys Anthropol](#))

3. Library Preparation

1. Library Adapter Preparation for Dual-Index Double Stranded DNA Illumina Sequencing (based on [Meyer and Kircher 2010 Cold Spring Harb. Protoc.](#) protocol)
2. Non-UDG Treated double-stranded ancient DNA library preparation for Illumina sequencing (**requires 3.1**, based on [Meyer and Kircher 2010 Cold Spring Harb. Protoc.](#) protocol)
3. Half-UDG Treated double-stranded ancient DNA library preparation for Illumina sequencing (**Variant of 3.2**, with partial damage removal treatment from [Rohland et al. 2015 Philos Trans R Soc Lond B Biol Sci](#))
4. Full-UDG Treated double-stranded ancient DNA library preparation for Illumina sequencing (**Variant of 3.2**, with complete damage removal treatment from [Briggs et al. 2010 Nucleic Acids Res](#))
5. Dual-Indexing of aDNA libraries for Illumina Sequencing (based on [Meyer and Kircher 2010 Cold Spring Harb. Protoc.](#) protocol)

4. Preparation for Sequencing

1. Amplification and Pooling for dual-indexed Illumina library for sequencing

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COLLECTION CITATION

James A Fellows Yates, Franziska Aron, Gunnar U Neumann, Irina Velsko, Eirini Skourtanioti, Eleftheria Orfanou, Zandra Fagernäs, Raphaela Stahl, Aida Andrades Valtuena, Christina Warinner, Wolfgang Haak, Guido Brandt 2021. A-Z of ancient DNA protocols for shotgun Illumina Next Generation Sequencing.
protocols.io
<https://dx.doi.org/10.17504/protocols.io.bvt9n6r6>
Version created by [James Fellows Yates](#)



KEYWORDS

ancient DNA, aDNA, palaeogenetics, archaeogenetics, next generation sequencing, illumina, UDG treatment, sampling, dental calculus, tooth, teeth, petrous, skeleton, pars petrousa

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IMAGE ATTRIBUTION

Diagram by James Fellows Yates with CC-BY icons from [FontAwesome](#)

CREATED

Jun 15, 2021

LAST MODIFIED

Jun 15, 2021

COLLECTION INTEGER ID

50785

GUIDELINES

Working in an Ancient DNA Laboratory

Some of the protocols in this collection **require** working in dedicated ancient DNA laboratories to limit modern DNA contamination.

- All steps of the protocol should take place in a clean room facility specifically designed for ancient DNA.
- The researcher performing lab work should wear correspondingly suitable lab-wear, such as:
 - full-body suit with hood (e.g., Tyvek)
 - hairnet
 - face mask
 - two pairs of clean gloves
 - clean shoes
 - protective glasses
- Sample processing should be carried out in separated work benches with integrated UV irradiation (e.g. Dead Air PCR work bench)
- Surfaces and equipment should be regularly decontaminated with e.g. bleach solution or Thermofisher's DNA AWAY (or similar) and irradiated with UV.

Please see the following for more detailed guidance:

Llamas, B. et al., 2017. From the field to the laboratory: Controlling DNA contamination in human ancient DNA research in the high-throughput sequencing era. STAR: Science & Technology of Archaeological Research, 3(1), pp.1–14. Available at: <https://doi.org/10.1080/20548923.2016.1258824>.

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4. Preparation for Sequencing



1. Amplification and Pooling for dual-indexed Illumina library for sequencing



FILES



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Minimally-invasive sampling of *pars petrosa* (*os temporale*) for ancient DNA extraction
Version 2
 by Eleftheria Orfanou
- 




Tooth Sampling from the inner pulp chamber for ancient DNA Extraction
Version 2
 by James Fellows Yates, Max Planck Institute for the Science of Human History
- 




Dental Calculus Field-Sampling Protocol (Sabin version)
Version 2
 by Zandra Fagernäs
- 




Dental Calculus Field-Sampling Protocol (Warinner Version)
Version 1
 by James Fellows Yates, Max Planck Institute for the Science of Human History
- 




Ancient DNA Extraction from Skeletal Material
Version 1
 by Eirini Skourtanioti
- 




Ancient DNA Extraction from Dental Calculus
Version 1
 by Zandra Fagernäs
- 


Library Adapter Preparation for Dual-Index Double Stranded DNA Illumina Sequencing
Version 1
 by Franziska Aron
- 


Non-UDG treated double-stranded ancient DNA library preparation for Illumina sequencing
Version 1
 by Gunnar Neumann
- 


Half-UDG treated double-stranded ancient DNA library preparation for Illumina sequencing
Version 1
 by James Fellows Yates, Max Planck Institute for the Science of Human History
- 


Full-UDG treated double-stranded ancient DNA library preparation for Illumina sequencing
Version 1
 by James Fellows Yates, Max Planck Institute for the Science of Human History
- 


Illumina double-stranded DNA dual indexing for ancient DNA
Version 2
 by Christina Warinner, Max Planck Institute for the Science of Human History
- 


Amplification and Pooling
Version 1
 by Franziska Aron

Citation: James A Fellows Yates, Franziska Aron, Gunnar U Neumann, Irina Velsko, Eirini Skourtanioti, Eleftheria Orfanou, Zandra FagernÃs, Raphaela Stahl, Aida Andrades Valtuena, Christina Warinner, Wolfgang Haak, Guido Brandt (06/15/2021). A-Z of ancient DNA protocols for shotgun Illumina Next Generation Sequencing. <https://dx.doi.org/10.17504/protocols.io.bvt9n6r6>

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