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Protocol status: Working
 We use these protocols and it works for us

Created: Aug 24, 2020

A-Z of ancient DNA protocols for shotgun Illumina Next Generation Sequencing V.1

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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

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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 U S A*](#) protocol)
2. Ancient DNA extraction from dental calculus (based on the [Dabney et al. 2013 *Proc Natl Acad Sci U S A*](#) 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

IMAGE ATTRIBUTION

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

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>.

FILES

Protocol

NAME

Minimally-invasive sampling of pars petrosa (os temporale) for ancient DNA extraction

VERSION 2

CREATED BY

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OPEN →

Protocol

NAME

Tooth Sampling from the inner pulp chamber for ancient DNA Extraction

VERSION 2

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[OPEN](#) →

Protocol

NAME

Dental Calculus Field-Sampling Protocol (Sabin version)

VERSION 2

CREATED BY

Zandra Fagerås

[OPEN](#) →

Protocol

NAME

Dental Calculus Field-Sampling Protocol (Warinner Version)

VERSION 1

CREATED BY

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[OPEN](#) →

Protocol

NAME

Ancient DNA Extraction from Skeletal Material

VERSION 1

CREATED BY

Eirini Skourtanioti Max Planck Institute for Evolutionary Anthropology

[OPEN](#) →

Protocol

NAME

Ancient DNA Extraction from Dental Calculus

VERSION 1

CREATED BY

Protocol

NAME

Library Adapter Preparation for Dual-Index Double Stranded DNA Illumina Sequencing

VERSION 1

CREATED BY

Franziska AronFriedrich-Schiller Universität Jena

[OPEN](#) →

Protocol

NAME

Non-UDG treated double-stranded ancient DNA library preparation for Illumina sequencing

VERSION 1

CREATED BY

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Protocol

NAME

Half-UDG treated double-stranded ancient DNA library preparation for Illumina sequencing

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Protocol

NAME

Full-UDG treated double-stranded ancient DNA library preparation for Illumina sequencing

VERSION 1

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Protocol

NAME

Illumina double-stranded DNA dual indexing for ancient DNA

VERSION 1

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OPEN →

Protocol

NAME

Amplification and Pooling

VERSION 1

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