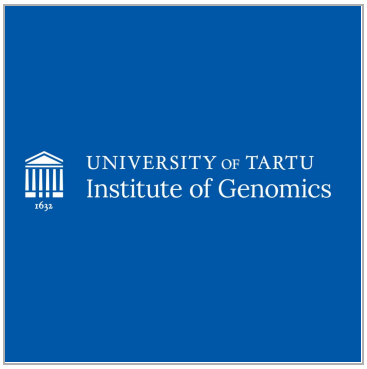

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Ancient DNA protocol collection – University of Tartu, Institute of Genomics

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ABSTRACT

Ancient DNA protocols from sampling to library preparation for shotgun Illumina sequencing - aDNA lab UTIG. All protocols except the indexing PCR and purification need to be performed in a dedicated ancient DNA laboratory (see guidelines).

The individual steps are respective protocols are:

1. Sampling
1.a tooth roots

2. Decontamination of samples (optional)

3. Ancient DNA extraction
3.a high volume (chunk samples)

4. Purification of DNA extracts

6. Library preparation
6.a dsDNA, double indexing, non-UDG, 2x split
6.b dsDNA, single indexing, non-UDG
6.c dsDNA, double indexing, full-UDG, 2x split
6.d dsDNA, single indexing, full-UDG

7. Indexing PCR and purification

GUIDELINES

Ancient DNA facility ("aDNA lab")
All steps from sampling to library preparation need to be performed in a dedicated ancient DNA facility. The ancient DNA facility, accessible only to instructed personnel, consists of a cascade of rooms with HEPA-filtrated air and positive air pressure from the laboratories towards the entry rooms to avoid the introduction of

Keywords: ancient DNA, aDNA, archeogenetics, archaeogenetics, paleogenetics, palaeogenetics

modern DNA.

Every person entering the ancient DNA facility needs to put on protective wear to minimize the risk of contaminating samples with their own DNA. This includes:
- A separate set of clothes only used in the aDNA facility
- first pair of gloves
- hair net
- mask
- Tyvek suit
- second pair of gloves (change regularly)
- clogs (entry area) and boots (laboratories)
- safety glasses when working with NaOCl (bleach) or other harmful chemicals (see warnings).

To avoid the introduction of amplified DNA, showering and wearing fresh clothes are mandatory after entering a modern molecular biology lab.

All workspaces need to be decontaminated with DNA Exitus or a similar product before starting and after finishing lab work. Floors need to be cleaned with diluted bleach once per week or whenever there was a risk of contamination (e.g. while drilling samples). Thorough cleaning of all equipment and furniture should be performed once per month.

All steps involving samples, extracts, or reagents need to be performed in a laminar flow hood (except during sample decontamination).

All equipment and consumables introduced into the aDNA facility need to be decontaminated with NaOCl (6% v/v), DNA Exitus or a similar product, irradiation with UV light, or a combination of the aforementioned.

Amplification of DNA, the processing of modern DNA (e.g. of modern reference samples), or the introduction of amplified DNA into the ancient DNA facility is forbidden.

Negative controls (using PCR-grade water) need to be taken along with extractions and library preparations to check for systematic contaminations.

Molecular biology laboratory ("modern lab")

Amplification of libraries during the indexing PCR and all further steps must be performed in a physically separated molecular biology lab.

The regular rules for protective wear in molecular biology labs apply; gloves need to be changed whenever getting in contact with libraries or reagents.

All steps involving libraries or reagents need to be performed in a dead air hood.

Surfaces and equipment need to be decontaminated with DNA Exitus or a similar product.

SAFETY WARNINGS

Reagents

NaOCl (bleach) solution (6%)
- H290 May be corrosive to metals.
- H314 Causes severe skin burns and eye damage.
- H411 Toxic to aquatic life with long lasting effects.
- EUH206 Warning! Do not use together with other products. May release dangerous gases (chlorine). Remove from surface after recommended incubation time with water-soaked tissue.

DNA ExitusPlus
H319 Causes serious eye irritation.

Guanidinium hydrochloride (GuHCl) (in PB buffer of Qiagen MinElute kit)
- H302 Harmful if swallowed.
- H332 Harmful if inhaled.
- H315 Causes skin irritation.
- H319 Causes serious eye irritation.

Ethanol
- H225 Highly flammable liquid and vapor.
- H319 Causes serious eye irritation.

Equipment

UV radiation

- UV radiation can damage eyes and can be carcinogenic in contact with skin. Do not look directly at unshielded UV radiation. Do not expose unprotected skin to UV radiation.
- UV emitters generate ozone during operation. Use only in ventilated rooms.

FILES

Protocol

NAME

Sampling of tooth roots for ancient DNA

VERSION 1

CREATED BY

Christiana L Scheib University of Tartu

OPEN →

Protocol

NAME

Decontamination of tooth roots/petrous bone cores for ancient DNA extraction

VERSION 1

CREATED BY

Marcel Keller Institute of Forensic Medicine, University of Bern

OPEN →

Protocol

NAME

Ancient DNA extraction (chunk samples/high volume)

VERSION 1

CREATED BY

Marcel Keller Institute of Forensic Medicine, University of Bern

OPEN →

Protocol

NAME

Ancient DNA extract purification (chunk samples/high volume)

VERSION 1

CREATED BY

Marcel Keller Institute of Forensic Medicine, University of Bern

OPEN →

Protocol

NAME

Library preparation (dsDNA double indexing, non-UDG, 2x split)

VERSION 1

CREATED BY

Marcel Keller Institute of Forensic Medicine, University of Bern

OPEN →

Protocol

NAME

Library preparation (dsDNA single indexing, non-UDG, no split)

VERSION 1

CREATED BY

Marcel Keller Institute of Forensic Medicine, University of Bern

OPEN →

Protocol

NAME

Library preparation (dsDNA single indexing, full-UDG, no split)

VERSION 1

CREATED BY

Marcel KellerInstitute of Forensic Medicine, University of Bern

OPEN →

Protocol

NAME

Library preparation (dsDNA double indexing, full-UDG, 2x split)

VERSION 1

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Marcel KellerInstitute of Forensic Medicine, University of Bern

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Protocol

NAME

Indexing PCR and purification of dsDNA libraries

VERSION 1

CREATED BY

Marcel KellerInstitute of Forensic Medicine, University of Bern

OPEN →