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

In 2 collections

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We use this protocol and it's working

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ABSTRACT

Adapter preparation protocol for double-stranded ancient DNA libraries for Illumina Next-Generation-Sequencing, based on Meyer and Kircher *et al.* (2010) *Cold Spring Harb. Protoc.* (doi: [10.1101/pdb.prot5448](https://doi.org/10.1101/pdb.prot5448)).

The protocol describes the production of an adapter mix for 2000 libraries.

This protocol is used in conjunction with Meyer and Kircher-based **ancient DNA libraries** and is described accordingly (see library construction like: Non-UDG treated double-stranded ancient DNA library preparation for Illumina sequencing).

IMAGE ATTRIBUTION

Matthäus Rest

Keywords: ancient DNA, palaeogenetics, archaeogenetics, DNA, Illumina, aDNA, nucleic acids, paleogenetics, archeogenetics, library preparation, genomic DNA, genomics

GUIDELINES

Working in an Ancient DNA Laboratory

- 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.
- All home-made buffers should be prepared in a separate dedicated PCR-free ultra-clean room and UV-irradiated for 30 min.

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>.

All steps of the protocol should take place in a clean room facility specifically designed for ancient DNA.

Protocol Specific Guidelines

This protocol requires the use of a two rooms - a buffer preparation room and a library preparation room.

MATERIALS

MATERIALS

✂ 2 ml LoBind Tubes **Eppendorf Catalog #0030108078**

✂ 0.2 ml PCR Tube strips **Eppendorf Catalog #0030124359**

✂ 1.5 ml LoBind tubes **Eppendorf Catalog #0030108051**

✂ EDTA (0.5 M) pH 8.0 **Life Technologies Catalog #AM9261**

✂ 5 M Sodium chloride (NaCl) **Sigma Aldrich Catalog #S5150-1L**

✂ Tris-HCL **Life Technologies Catalog #15568025**

✂ Water HPLC Plus **Merck Millipore Sigma Catalog #34877-2.5L-M**

Lab Equipment

PCR Thermocycler (e.g. Eppendorf Mastercycler Nexus)

UV irradiation box or cross linker (e.g. Vilber Lourmat Bio-Link BLX-254)

Additional Reagents

Oligos (e.g. SigmaAldrich Custom DNA Oligos)

Oligo ID	Sequence (5'-3')	Concentration
IS1_adapter.P5.F	A*C*A*C*TCTTTCCCTACACGACGCTCTTCCG*A *T*C*T	500 µM
IS2_adapter.P7.F	G*T*G*A*CTGGAGTTCAGACGTGTGCTCTTCCG* A*T*C*T	500 µM
IS3_adapter.P5+P7.R	A*G*A*T*CGGAA*G*A*G*C	500 µM

* indicates a PTO bond

SAFETY WARNINGS

Reagents

Household bleach solution (2-6%) diluted to a working concentration of 0.2-0.5 % NaClO in total

- 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 AWAY

- H314 Causes severe skin burns and eye damage.

Note: Both bleach solutions and DNA AWAY are used for decontamination. DNA AWAY is less corrosive than bleach and should be preferred for decontamination of sensitive equipments such as surfaces of electric devices.

EDTA

- H373 May cause damage to organs through prolonged or repeated exposure.

Sodium Chloride

-H290 May be corrosive to metal

-H314 Causes severe skin burns and eye damage

-H400 Very toxic to aquatic life

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.

BEFORE START INSTRUCTIONS

Planning

This protocol takes around 3 hours including the cleaning process of the workspace afterwards.

Preparation of Reagents

Only the oligo hybridisation buffer can be prepared within buffer preparation room, with a DNA-free hood. All other steps are performed in the the library preparation room.

HPLC-Water should be decontaminated with a **30 min UV irradiation** before use.

Equipment

Make sure all necessary equipment is available (see Materials)

Abbreviations

EDTA = Ethylendiaminetetraacetic acid

HPLC = High Performance Liquid Chromatography (-Grade Water)

NaCl = Sodium chloride

Tris-HCl = Tris hydrochloride

UV = Ultraviolet (radiation)

Hybridization buffer preparation (Buffer Preparation Room)

- 1 Prepare oligo hybridization buffer (10× concentration, 1 mL for 50 reactions)

Reagent	Stock concentration [M]	Final Concentration [M]	1× Volume [μl]
NaCl	5	0.5	100
Tris-HCl	1	0.01	10
EDTA	0.5	0.001	2
UV HPLC-water			888

Total			1000
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Note

The oligo hybridization buffer can be stored at room temperature for up to one year.

- Irradiate the buffer with UV for 00:30:00 without the lid or with an open lid.

Adapter Preparation (Library Preparation Room)

- Prepare P5 adapter** (100 μ L per reaction)

Use one tube of a 0.2 ml PCR strip to set up the P5 adapter mix.

Reagent	Stock concentration	Final concentration	1× Volume [μ l]
Oligo Hybridization Buffer	10 ×	1 ×	10
IS1_adapter.P5	500 μ M	200 μ M	40
IS3_adapter.P5+P7	500 μ M	200 μ M	40
UV HPLC-water			10
Total			100

Gently pipette up and down to mix. Split the 100 μ L reaction into two tubes of the 0.2ml PCR strip with 50 μ L each .

- Prepare P7 adapter** (100 μ L per reaction)

Use one tube of a 0.2 ml PCR strip to set up the P7 adapter mix.

Reagent	Stock concentration	Final concentration	1× Volume [μ l]
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Oligo Hybridization Buffer	10 ×	1 ×	10
IS2_adapter.P7	500 µM	200 µM	40
IS3_adapter.P5+P7	500 µM	200 µM	40
UV HPLC-water			10
Total			100

Split the 100 µL reaction into two tubes of the 0.2ml PCR strip with 50 µL each .

5 Prepare ready-to-use adapter mix.

- ### 5.1
- Combine one P5 adapter reaction with one P7 adapter reaction in one tube, mix thoroughly by flicking the tubes with a finger, spin down briefly.

Repeat with the second P5 and P7 adapter reaction.

Note

In the end you should have two 0.2 ml tubes with a total of 100 µL volume, each containing 50 µL of the P5 adapter and 50 µL of the P7 adapter.

- ### 5.2
- Incubate both reactions in a thermocycler with a heated lid at 95 °C for 00:00:10 ,

followed by a cool down ramp from 95 °C to 12 °C at a rate of 0.1 °C per sec .

Combine and Dilute

- 6 Combine both reactions into a 2 ml tube to obtain a ready-to-use double-stranded library adapter mix with 100 micromolar (μM) adapter (each) .

- 7 Add 1800 μL of UV HPLC-water to dilute the double-stranded library adapter mix to 10 micromolar (μM) and aliquot the dilution in 10 $\times$ 1.5 ml LoBind tubes, each containing 200 μL . Briefly vortex and spin down before freezing.

- 8 Store the adapter mix at -20 $^{\circ}\text{C}$

Note

Each aliquot contains sufficient double-stranded library adapter mix for 200 reactions (overall 2000 libraries).

The adapter mix aliquot can be thawed and re-frozen with no detriment to the reagent quality.