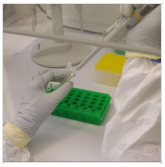

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Library Adapter Preparation for Dual-Index Double Stranded DNA Illumina Sequencing

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1 Works for me dx.doi.org/10.17504/protocols.io.bem5jc86

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ABSTRACT

Adapter preparation protocol for doubble-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).

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).

DOI

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PROTOCOL CITATION

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KEYWORDS

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

IMAGE ATTRIBUTION

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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)

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- 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 seperate decidated 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

NAME	CATALOG #	VENDOR
2 ml LoBind Tubes	0030108078	Eppendorf
0.2 ml PCR Tube strips	0030124359	Eppendorf
1.5 ml LoBind tubes	#0030108051	Eppendorf
EDTA (0.5 M) pH 8.0	AM9261	Life Technologies
5 M Sodium chloride (NaCl)	S5150-1L	Sigma Aldrich
Tris-HCL	15568025	Life Technologies
Water HPLC Plus	34877-2.5L-M	Merck Millipore Sigma

MATERIALS TEXT

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.

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DNA AWAY
- H314 Causes severe skin burns and eye damage.

Note: Both bleach solutions and DNA AWAY are used for decontamintation. 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 STARTING

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 chroride
Tris-HCl = Tris hydrochloride
UV = Ultraviolet (radiation)

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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

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

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

Adapter Preparation (Library Preparation Room)

3 **Prepare P5 adapter (100 µl 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 [µl]
Oligo Hybridization Buffer	10 ×	1 ×	10
IS1_adapter.P5	500 µM	200 µM	40
IS3_adapter.P5+P7	500 µM	200 µM	40
UV HPLC-water			10
Total			**100**

Gently pipette up anddown to mix. Split the **100 µl** reaction into two tubes of the 0.2ml PCR strip with **50 µl each** .

4 **Prepare P7 adapter (100 µl 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 [µl]
Oligo Hybridization Buffer	10 ×	1 ×	10
IS2_adapter.P7	500 µM	200 µM	40

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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 adpater reaction.

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 thermocyler 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 × 1.5 ml LoBind tubes, each containing **200 µl** . Briefly vortex and spin down before freezing.

8 Store the adapter mix at **-20 °C**

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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.

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