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

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ABSTRACT


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
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 ultraclean room and UV-irradiated for 30 min.

Please see the following for more detailed guidance:


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

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)

<table>
<thead>
<tr>
<th>Oligo ID</th>
<th>Sequence (5'-3')</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS1_adapter.P5.F</td>
<td>A<em>C</em>A<em>C</em>TCTTTCCCTACACGACGCTCTTCCG<em>A</em>T<em>C</em>T</td>
<td>500 µM</td>
</tr>
<tr>
<td>IS2_adapter.P7.F</td>
<td>G<em>T</em>G<em>A</em>CTGGAGTTACAGCTGTGCTCTTCCG<em>A</em>T<em>C</em>T</td>
<td>500 µM</td>
</tr>
</tbody>
</table>

* 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 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 = Ethylenediaminetetraacetic acid
HPLC = High Performance Liquid Chromatography (-Grade Water)
NaCl = Sodium chloride
Tris-HCl = Tris hydrochloride
UV = Ultraviolet (radiation)

Hybridization buffer preparation (Buffer Preparation Room)

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Stock concentration [M]</th>
<th>Final Concentration [M]</th>
<th>1x Volume [µl]</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>5</td>
<td>0.5</td>
<td>100</td>
</tr>
<tr>
<td>Tris-HCl</td>
<td>1</td>
<td>0.01</td>
<td>10</td>
</tr>
<tr>
<td>EDTA</td>
<td>0.5</td>
<td>0.001</td>
<td>2</td>
</tr>
<tr>
<td>UV HPLC-water</td>
<td></td>
<td></td>
<td>888</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>1000</td>
</tr>
</tbody>
</table>

Note
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.

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**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.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Stock concentration</th>
<th>Final concentration</th>
<th>1× Volume [µl]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligo Hybridization Buffer</td>
<td>10 ×</td>
<td>1 ×</td>
<td>10</td>
</tr>
<tr>
<td>IS1_adapter.P5</td>
<td>500 µM</td>
<td>200 µM</td>
<td>40</td>
</tr>
<tr>
<td>IS3_adapter.P5+P7</td>
<td>500 µM</td>
<td>200 µM</td>
<td>40</td>
</tr>
<tr>
<td>UV HPLC-water</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>100</td>
</tr>
</tbody>
</table>

   Gently pipette up and down to mix. Split the 100 µL reaction into two tubes of the 0.2 ml 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.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Stock concentration</th>
<th>Final concentration</th>
<th>1× Volume [µl]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligo Hybridization Buffer</td>
<td>10 ×</td>
<td>1 ×</td>
<td>10</td>
</tr>
<tr>
<td>IS2_adapter.P7</td>
<td>500 µM</td>
<td>200 µM</td>
<td>40</td>
</tr>
<tr>
<td>IS3_adapter.P5+P7</td>
<td>500 µM</td>
<td>200 µM</td>
<td>40</td>
</tr>
<tr>
<td>UV HPLC-water</td>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>100</td>
</tr>
</tbody>
</table>
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.

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
and aliquot the dilution in 10 × 1.5 ml LoBind tubes, each containing 200 µL. Briefly vortex and spin down before freezing.

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.