Low input Library Preparation for Illumina sequencing
Developed by Adriana Alberti at Genoscope

Protocol for preparation of short single and paired-end libraries from genomic dsDNA. starting from low DNA quantity (up to 10 ng)

Reagents and consumables

<table>
<thead>
<tr>
<th>Reagent / consommable</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-mm × 16-mm AFA microtubes and snap caps</td>
<td>Covaris</td>
</tr>
<tr>
<td>LoBind tubes, 1.5 mL</td>
<td>Eppendorf</td>
</tr>
<tr>
<td>Agencourt AmPure XP beads</td>
<td>Beckman Coulter</td>
</tr>
<tr>
<td>NEBNext™ DNA Sample Prep Reagent Set 1</td>
<td>New England Biolabs</td>
</tr>
<tr>
<td>Nucleospin Gel and PCR Cleanup kit</td>
<td>Macherey-Nagel</td>
</tr>
<tr>
<td>Platinum Pfx Taq Polymerase kit</td>
<td>Life Technologies</td>
</tr>
<tr>
<td>Agilent DNA HS kit</td>
<td>Agilent</td>
</tr>
<tr>
<td>Quant-iT dsDNA HS assay kit</td>
<td>Life Technologies</td>
</tr>
<tr>
<td>Illumina adapters</td>
<td>Bioo Scientific</td>
</tr>
<tr>
<td>Illumina Library quantification kit</td>
<td>KAPA Biosystems</td>
</tr>
</tbody>
</table>

STEP 1: DNA fragmentation using Covaris

Fragment DNA using S2 or E210 systems. Follow Covaris protocol recommendations except for the sample volume in the microtube.

a) Allow the Covaris chiller to reach 4 °C, and degas for at least 30 min (for S2) or 1h (for E210).

b) During this time, prepare the DNA sample:
   Dilute 10-50 ng DNA to 50 μl with EB buffer and transfer the DNA sample to a 100-μl Covaris microtube, keeping the cap on the tube

c) Insert the microtube into the holder (S2) or the rack (E210), and run the Covaris with the following settings:
   - Duty cycle: 5%
   - Intensity: 3
   - Cycles per burst: 200
   - Time: 80 sec.

d) Transfer processed sample to a LoBind microfuge tube and proceed to step 2

STEP 2: End Repair

Use NEBNext DNA sample Prep Reagent Set 1 from New England Biolabs
a) Combine and mix the following components in a LoBind tube

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fragmented DNA</td>
<td>50 µl</td>
</tr>
<tr>
<td>10X Phosphorylation Reaction Buffer</td>
<td>7 µl</td>
</tr>
<tr>
<td>dNTPs</td>
<td>4 µl</td>
</tr>
<tr>
<td>DNA polymerase I, Large (Klenow)</td>
<td>1 µl</td>
</tr>
<tr>
<td>T4 DNA Polymerase</td>
<td>2 µl</td>
</tr>
<tr>
<td>T4 Polynucleotide Kinase</td>
<td>2 µl</td>
</tr>
<tr>
<td>H2O</td>
<td>4 µl</td>
</tr>
<tr>
<td><strong>Total volume</strong></td>
<td>70 µl</td>
</tr>
</tbody>
</table>

b) Incubate for 30 minutes at 20 °C.
c) Purify on one Nucleospin column using the Nucleospin Gel and PCR Cleanup Kit and protocol. Elute in 34 µl of NE or EB in a LoBind tube.

**STEP 3: dA-tailing**

Use NEBNext DNA sample Prep Reagent Set 1 from New England Biolabs

a) Combine and mix the following components in the tube containing repaired DNA

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>End repaired, blunt DNA</td>
<td>34 µl</td>
</tr>
<tr>
<td>NEBuffer2 10X</td>
<td>5 µl</td>
</tr>
<tr>
<td>Deoxyadenosine 5’-triphosphate</td>
<td>10 µl</td>
</tr>
<tr>
<td>Klenow Fragment Exo’</td>
<td>1 µl</td>
</tr>
<tr>
<td><strong>Total volume</strong></td>
<td>50 µl</td>
</tr>
</tbody>
</table>

b) Incubate for 30 min at 37 °C.
c) Purify on one Nucleospin column using the Nucleospin Gel and PCR Cleanup Kit and protocol. Elute in 20 µL of NE or EB in a LoBind tube.

**STEP 4: Adapter ligation**

Use NEBNext DNA sample Prep Reagent Set 1 from New England Biolabs and barcoded Illumina compatible adapters (they can be home made or purchased from various suppliers as Bioo Scientific)

**Note: depending on the initial adapter concentration, dilute the adapters with EB buffer to adjust for the small quantity of DNA. Excess adapters can interfere with sequencing. The adapters may have to be titrated relative to starting material. For example: 50 µM adapters have to be diluted 1:150 if the input DNA quantity is around 10-15 ng.**
a) Combine and mix the following components:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>dA-tailed DNA</td>
<td>20 µl</td>
</tr>
<tr>
<td>2x Quick Ligation Reaction Buffer</td>
<td>25 µl</td>
</tr>
<tr>
<td>Diluted barcoded adapter</td>
<td>1 µl</td>
</tr>
<tr>
<td>Quick T4 DNA ligase</td>
<td>4 µl</td>
</tr>
<tr>
<td><strong>Total volume</strong></td>
<td>50 µl</td>
</tr>
</tbody>
</table>

b) Incubate for 30 min at 25 °C.

c) Clean up the reaction using AmPure XP beads (Agencourt).

Add 50 µL (1 volume) AmPure XP beads, mix by short vortexing. Incubate for 5 minutes, then bind the beads and remove the supernatant. Add 500 µL 70% ethanol (made fresh each time), incubate 30 seconds and remove. Repeat once. Let the pellet dry completely (5-10 minutes), then elute in 40 µL EB.

**STEP 5: PCR enrichment**

Perform two independent PCR reactions using Platinum Pfx Taq Polymerase (Life Technologies) and P5 and P7 primers

P5
5’ AATGATACGGCGACCACCGAG

P7
5’ CAAGCAGAAGACGGCATACGAG

a) Combine and mix the following components in two sterile 0.2 ml tubes:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>20 µl</td>
</tr>
<tr>
<td>Pfx amplification buffer 10x Reaction Buffer</td>
<td>5 µl</td>
</tr>
<tr>
<td>P5 primer 50 µM</td>
<td>1 µl</td>
</tr>
<tr>
<td>P7 primer 50 µM</td>
<td>1 µl</td>
</tr>
<tr>
<td>MgSO₄ 50mM</td>
<td>2 µl</td>
</tr>
<tr>
<td>dNTP 10mM</td>
<td>2 µl</td>
</tr>
<tr>
<td>Pfx Platinum Taq polymerase</td>
<td>0.8 µl</td>
</tr>
<tr>
<td>H₂O</td>
<td>18.2 µl</td>
</tr>
<tr>
<td><strong>Total volume</strong></td>
<td>50 µl</td>
</tr>
</tbody>
</table>

b) Amplify using the following PCR cycling conditions:

30 sec at 98 °C
[10 sec at 98 °C, 30 sec at 60 °C, 30 sec at 72 °C] 12 cycles total
5 min at 72 °C
Hold at 4 °C
c) Clean up the reaction using AmPure XP beads (Agencourt).

   Add 30 μL (0.6 volume) AmPure XP beads, mix by short vortexing. Incubate for 5 minutes, then bind the beads and remove the supernatant. Add 500 μL 70% Ethanol (made fresh each time), incubate 30 seconds and remove. Repeat once. Let the pellet dry completely (5-10 minutes), then elute in 20 μL EB.

STEP 6: Quantitative and qualitative assessment of the library

The sample must be accurately quantified in order to optimize yield. This step is absolutely crucial to the success of any experiment.

a) Measure the concentration using the Qubit using the HS kit.
b) Run 1 ng of the sample on the Bioanalyzer High Sensitivity DNA Chip

c) Quantify the library by qPCR The unknown library is compared to a previously analyzed library for which the optimal cluster density has been achieved