Low input Library Preparation for Illumina Sequencing

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Abstract

Protocol for preparation of short single and paired-end libraries from genomic dsDNA starting from low DNA quantity (up to 10 ng) for Illumina sequenincg. Developed by Adriana Alberti at Genoscope.

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Guidelines

Reagents and consumables

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

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 Lo Bind 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

Fragmented DNA	50 µl
10X Phosphorylation Reaction Buffer	7 µl
dNTPs	4 µl
DNA polymerase I, Large (Klenow)	1 µl
T4 DNA Polymerase	2 µl
T4 Polynucleotide Kinase	2 µl
H_2O	4 µl
Total volume	**70 µl**

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

End repaired, blunt DNA	34 µl
NEBuffer2 10X	5 µl
Deoxyadenosine 5'-triphosphate	10 µl
Klenow Fragment Exo	1 µl
Total volume	**50 µl**

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:

dA-tailed DNA	20 µl
2X Quick Ligation Reaction Buffer	25 µl
Diluted barcoded adapter	1 µl
Quick T4 DNA ligase	4 µl
Total volume	**50 µl**

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

DNA	20 µl
Pfx amplification buffer 10x Reaction Buffer	5 µl
P5 primer 50 µM	1 µl
P7 primer 50 µM	1 µl
$MgSO_4$ 50mM	2 µl
dNTP 10mM	2 µl
Pfx Platinum Taq polymerase	0.8 µl
H_2O	18.2 µl
Total volume	**50 µl**

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 Chi
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

Protocol