PROTOCOL: Illumina Barcoded Paired-End Whole Genome Shotgun Library Preparation

This protocol provides instructions for preparing barcoded paired-end whole genome shotgun (WGS) libraries for sequencing by Illumina platforms (GAII, HiSeq and MiSeq). It involves using the Covaris S2 system for shearing DNA samples, using the NEBNext End Repair, A-Tailing, and Ligation Modules for DNA modification, as well as using the 2X Phusion High Fidelity PCR Master Mix for ligation-mediated PCR (LM-PCR). Quality control standard or criteria for each major procedural step is also addressed here.

The whole process described in this protocol includes the following steps:
WGS Library preparation
DNA fragmentation
DNA end repair
3'-end adenylation
Ligation with Illumina multiplexing PE adaptor
LM-PCR, purification and QC
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Reagents

Item
NEBNext End-Repair Module, Cat# E6050L, NEB
NEBNext dA-Tailing Module, Cat# E6053L, NEB
NEBNext Ligation Module, Cat# E-6056L, NEB
Illumina Multiplexing PE Adaptor Oligos Note: All these primer oligos need to be HPLC purified. Illumina multiplexing PE adaptor oligo A 5'-ACACTCTTTCCCTACACGACGCTCTTCCGATCT Illumina multiplexing PE adaptor oligo B 5'-phospho-GATCGGAAGAGCACACGTCT
LM-PCR primer IMUX-P1.0 (working concentration 50uM) 5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATC*T HPLC purified; *addition of phosphothioate bond.

LM-PCR barcoded primers (working concentration 50uM)

Note: All these primer oligos need to be HPLC purified. Addition of **phosphothioate bond** before addition of the last "T" as a preventative measure against degradation in storage.

6-bp barcode set (the barcodes were designed by Illumina Inc.)

IBC1	CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IBC2	CAAGCAGAAGACGGCATACGAGATACATCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IBC3	CAAGCAGAAGACGGCATACGAGATGCCTAAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IBC4	CAAGCAGAAGACGGCATACGAGATTGGTCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IBC5	CAAGCAGAAGACGGCATACGAGATCACTGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IBC6	CAAGCAGAAGACGGCATACGAGATATTGGCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IBC7	CAAGCAGAAGACGGCATACGAGATGATCTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IBC8	CAAGCAGAAGACGGCATACGAGATTCAAGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IBC9	CAAGCAGAAGACGGCATACGAGATCTGATCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IBC10	CAAGCAGAAGACGGCATACGAGATAAGCTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IBC11	CAAGCAGAAGACGGCATACGAGATGTAGCCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IBC12	CAAGCAGAAGACGGCATACGAGATTACAAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT

9-bp barcode set (the barcodes were designed by Adey et. al , Genome Biol. 2010; 11: R119)

IDMB1	CAAGCAGAAGACGGCATACGAGATTACGAAGTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB2	CAAGCAGAAGACGGCATACGAGATGACGAGATTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB3	CAAGCAGAAGACGGCATACGAGATACCGTAAGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB4	CAAGCAGAAGACGGCATACGAGATTAGTGGCAAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB5	CAAGCAGAAGACGGCATACGAGATCATTAACGCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB6	CAAGCAGAAGACGGCATACGAGATTCGTTGAAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB7	CAAGCAGAAGACGGCATACGAGATTAGTACGCTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB8	CAAGCAGAAGACGGCATACGAGATCTCAGATCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB9	CAAGCAGAAGACGGCATACGAGATTTCACCGTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB10	CAAGCAGAAGACGGCATACGAGATGTCATGCATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB11	CAAGCAGAAGACGGCATACGAGATAGGACAGTTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB12	CAAGCAGAAGACGGCATACGAGATATGGTGTCTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB13	CAAGCAGAAGACGGCATACGAGATGGATGTTCTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB14	CAAGCAGAAGACGGCATACGAGATCTTATCCAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB15	CAAGCAGAAGACGGCATACGAGATGTAAGTCACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB16	CAAGCAGAAGACGGCATACGAGATTTCAGTGAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB17	CAAGCAGAAGACGGCATACGAGATCTCGTAATGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB18	CAAGCAGAAGACGGCATACGAGATCATGTCTCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB19	CAAGCAGAAGACGGCATACGAGATAATCGTGGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB20	CAAGCAGAAGACGGCATACGAGATGTATCAGTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB21	CAAGCAGAAGACGGCATACGAGATAGCAGATGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB22	CAAGCAGAAGACGGCATACGAGATTCCTAACGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB23	CAAGCAGAAGACGGCATACGAGATAACAGTCCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB24	CAAGCAGAAGACGGCATACGAGATCCTTGAGAAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB25	CAAGCAGAAGACGGCATACGAGATTTAAGCCTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT

IDMB26	CAAGCAGAAGACGGCATACGAGATTTAGACCACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB27	CAAGCAGAAGACGGCATACGAGATTGTCTAGTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB28	CAAGCAGAAGACGGCATACGAGATTAGATCGAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB29	CAAGCAGAAGACGGCATACGAGATTGAATGCCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB30	CAAGCAGAAGACGGCATACGAGATGTGCAATGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB31	CAAGCAGAAGACGGCATACGAGATAGTGGCATAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB32	CAAGCAGAAGACGGCATACGAGATATGATCGGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB33	CAAGCAGAAGACGGCATACGAGATAGTCTACCTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB34	CAAGCAGAAGACGGCATACGAGATGATCAACTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB35	CAAGCAGAAGACGGCATACGAGATATCGGTAGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB36	CAAGCAGAAGACGGCATACGAGATCGTATGATGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB37	CAAGCAGAAGACGGCATACGAGATTTACTGACGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB38	CAAGCAGAAGACGGCATACGAGATCTGTCGTAAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB39	CAAGCAGAAGACGGCATACGAGATTCAACTGGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB40	CAAGCAGAAGACGGCATACGAGATATCGATCTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB41	CAAGCAGAAGACGGCATACGAGATGCAACTATGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB42	CAAGCAGAAGACGGCATACGAGATGATGACTTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB43	CAAGCAGAAGACGGCATACGAGATGACGTTACAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB44	CAAGCAGAAGACGGCATACGAGATCATCTGCTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB45	CAAGCAGAAGACGGCATACGAGATATTAGTCGGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB46	CAAGCAGAAGACGGCATACGAGATTAGCGTACTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB47	CAAGCAGAAGACGGCATACGAGATCCAAGCAATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB48	CAAGCAGAAGACGGCATACGAGATCCGTAATTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB49	CAAGCAGAAGACGGCATACGAGATAGAATTGCCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
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IDMB51	CAAGCAGAAGACGGCATACGAGATCATCAGTGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB52	CAAGCAGAAGACGGCATACGAGATGAATCCTCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB53	CAAGCAGAAGACGGCATACGAGATGCTGTATACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
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IDMB57	CAAGCAGAAGACGGCATACGAGATTGACGCATTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB58	CAAGCAGAAGACGGCATACGAGATCACGATTCTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB59	CAAGCAGAAGACGGCATACGAGATTATTGCCTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB60	CAAGCAGAAGACGGCATACGAGATAAGTCAGAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB61	CAAGCAGAAGACGGCATACGAGATATAGCTGAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
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IDMB64	CAAGCAGAAGACGGCATACGAGATTTGCCGATTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
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IDMB69	CAAGCAGAAGACGGCATACGAGATCTGAACTGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
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IDMB71	CAAGCAGAAGACGGCATACGAGATCCATCTTAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
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IDMB73	CAAGCAGAAGACGGCATACGAGATTCCAACATGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB74	CAAGCAGAAGACGGCATACGAGATGCTATCATCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB75	CAAGCAGAAGACGGCATACGAGATACAGCTTCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB76	CAAGCAGAAGACGGCATACGAGATAGTCATTGCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
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IDMB88	CAAGCAGAAGACGGCATACGAGATTGCCATTAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB89	CAAGCAGAAGACGGCATACGAGATACTAACGCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB90	CAAGCAGAAGACGGCATACGAGATATGTAGCACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB91	CAAGCAGAAGACGGCATACGAGATGGTCGATATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB92	CAAGCAGAAGACGGCATACGAGATGCGAGTTATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB93	CAAGCAGAAGACGGCATACGAGATGACTGAGTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB94	CAAGCAGAAGACGGCATACGAGATAGATACTCCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB95	CAAGCAGAAGACGGCATACGAGATGCTAGAGTTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
IDMB96	CAAGCAGAAGACGGCATACGAGATAATGTAGCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Agencourt AMPure XP beads(60ml), Cat# A63882, Beckman Coulter	
2X Phusion High Fidelity PCR Master Mix with HF buffer, Cat# M0531, NEB	

Consumables

Item	Source	Catalog #
Agilent Chip 7500	Agilent	5067-1506
2.2% FLASH DNA gel cassette	Lonza/VWR	57023
Flash Gel Loading Dye (5X)	Lonza/VWR	50462
Flash Gel Marker 100 – 4kb	Lonza/VWR	50473
EtOH, 200 proof	Aaper	Stock room
Clear 12-strip 0.2ml PCR tubes w/ caps	Phenix	MPC-665
Caps Only 12-Strip Clear for MPC-655	Phenix	MPC-660

1.7 ml microtubes (MCT-175-B)	VWR	10011-720
10 ul Pipet tips	VWR	47449-868
200 ul pipet tips	VWR	22234-016
1000 ul pipet tips	VWR	16466-008
Shearing microtube	Covaris	520045
Nuclease-free Water	Qiagen	129114
Flashgel Starter Kit	VWR	95015-612

Equipments and Devices

Item	Source	Catalog #
Covaris S2 Acoustic Shearer	Covaris	600028
P1000 pipette	Rainin	L-1000
P200 pipette	Rainin	L-200
P10 pipette	Rainin	L-10
Gel Imager PC 2000	BioRad	1708195
Thermomixer	Eppendorf	0022670000
Agilent Bioanalyzer 2100	Agilent	G2940CA
Microcentrifuge 5424	Eppendorf	5424000410
AB GeneAmp Thermocycler	Applied Biophysics	N8050200
Thermocycler Sample Block Module	Applied Biophysics	4314443
DynaMag2 particle concentration rack	Invitrogen	123-21D

Before you start, make sure that you read carefully the following safety information:

- Wear lab coat, gloves, and protective goggles.

- Protect bench working area with absorbent bottom plasticized pad.

- Discard all materials in Biohazard bag. Discard all liquid in a Biohazard labeled plastic bottle that should be tightly closed and disposed of in the Biohazard bag when full.

References:

- Illumina Multiplexing_SamplePrep_Guide_1005361_D.

WGS Library Preparation

1. DNA Fragmentation

a) Fill a Covaris™ water bath to the level of 12 in the Covaris S2 device and degas for 30-45 minutes prior to shearing your gDNA in the S2. When a microtube is placed in the tube holder, the water level should be at the base of the cap and the glass portion of the tube should be completely submerged. The water bath temperature should be between 6 ºC and 8 ºC.

b) Dilute 500ng of DNA (based on pico-green or Qubit) into 100 µl of low-TE water in a microcentrifuge tube.

c) Place a Covaris™ microTube (6X16mm) into the loading station. Keep the cap on the tube and use a tapered pipette tip to slowly transfer the 100 µL of DNA sample through the pre-split septa. Be careful not to introduce a bubble into the bottom of the tube. Bubbles will interfere with the acoustic field.

d) Shear the DNA using the following Covaris™ S2 System shearing conditions.
   1. Number of Cycles: **2 (40 seconds per cycle)** (if sample quality is poor, start with 1 cycle and check on a gel).
   2. Batch Temperatue: **6-8 ºC**
   3. Bath Temperature Limit: **<15 ºC**
   4. Mode: **Frequency sweeping**
   5. Water Quality Testing Function: **Off**
   6. Duty cycle: **10%**
   7. Intensity: **4**
   8. Cycles/burst: **200**
   9. Time: **80 s**

e) Place the Covaris™ microtube into the loading station. While keeping the snap-cap on, insert a pipette tip through the pre-split septa, slowly remove the sheared DNA and transfer it into a new 1.7 ml tube.

f) Load 1 µl of each sample and 2 µl of 100 bp – 4000 bp marker on a 2.2% FlashGel DNA Cassette (Lonza, Cat#: 57023). Gel running condition set as the following: Voltage 270, running time: 4 minutes. Gel imager (Bio-Rad) setting should be uniformed to the condition as described here. Condition of Bio-Rad gel imager: Filter 1 for Eth Bromide, auto expose of 0.6 s. Image transform: Higher: 3000, low: 200, Gamma: 1.00, Min: 79, Max: 4095.

Note: The average size should be approximately 300 bp and the majority of fragment sizes should be smaller than 500 bp (see Figure 1 gel picture for example). If majority size of sheared fragments is larger than 800 bp, re-shear the sample with 20 s increment each time for up to 2 additional cycles. The negative control should give no product.

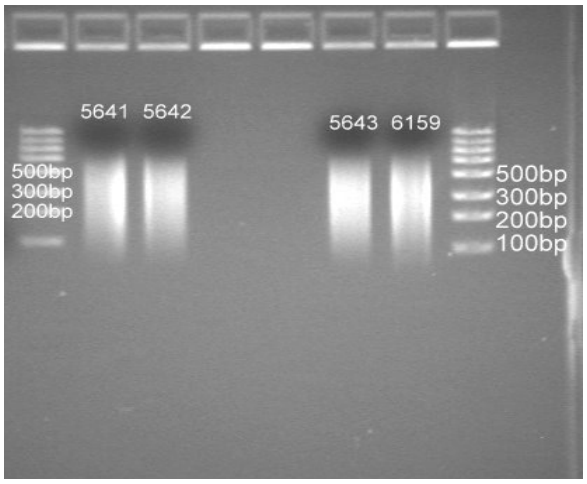

Figure 1 Post-Covaris DNA size distribution on 2.2% Flash Gel. Marker lane: FlashGel DNA marker (100 bp – 4000 bp, Cat. No. 50473, Lonza).

g) Purify by adding 1.8X Agencourt AMPure XP beads and binding by rotating on a thermomixer for about 5 minutes at RT before placing into the magnetic particle concentrator (MPC). Remove the supernatant (and save). Wash 3 times with freshly prepared 70% ethanol. Keep the sample tube in MPC during the wash. Add ethanol slowly to avoid disturbing the bead pellet. After wash, briefly dry the beads in the air. Elute DNA in 52 µl of nuclear-free H2O.

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2. DNA End Repair

a) Combine and mix the following components in a 1.7 ml tube:

Component	Volume (µL)
Sheared DNA	50.0
End-repair 10× buffer*	9.0
End-repair enzyme mix*	5.0
Nuclease-free water	26.0
Total	90.0

*From NEBNext End-Repair Module (Cat. No. E6050L).

b) Incubate the mixture at 20°C for 30 minutes at bench top cooler.

c) Purify by adding 1.8X Agencourt AMPure XP beads and binding by rotating on a thermomixer for about 5 minutes at RT before placing into the magnetic particle concentrator (MPC). Remove the supernatant (and save). Wash 3 times with freshly prepared 70% ethanol. Keep the sample tube in MPC during the wash.

Add ethanol slowly to avoid disturbing the bead pellet. After wash, briefly dry the beads in the air.Elute DNA in 42 µl of nuclear-free H2O.

3. 3'-end Adenylation

a) Combine and mix the following components in a 1.7 ml tube:

Component	Volume (µL)
End-repaired DNA	40.0
NEBNext™ dA-Tailing Reaction Buffer (10X)*	6.0
Klenow Fragment (3'-5' exo⁻)*	3.0
Nuclease-free water	11.0
Total	60.0

*From NEBNext dA-Tailing Module (Cat. No. E6053L).

b) Incubate the mixture at 37ºC for 30 min in a calibrated thermomixer.

4) Purify by adding 1.8X Agencourt AMPure XP beads and binding by rotating on a thermomixer for about 5 minutes at RT before placing into the magnetic particle concentrator (MPC). Remove the supernatant (and save). Wash 3 times with freshly prepared 70% ethanol. Keep the sample tube in MPC during the wash. Add ethanol slowly to avoid disturbing the bead pellet. After wash, briefly dry the beads in the air.Elute DNA in 42 µl of nuclear-free H2O.

4. Ligate Adaptors to the DNA

Oligonucleotide annealing:

Illumina multiplexing PE adapter oligo A and Illumina multiplexing PE adapter oligo B should be ordered as HPLC purified.

Mix the paired oligonuclieotides at final concentration of 300uM in 1x Ligase buffer (from NEBNext Ligation Module, Cat# E6056-L) and run with the following annealing program on thermocycler: 95°C for 5 min, 80°C for 3 min, 70°C for 3 min, 60°C for 3 min, 50°C for 3 min, 40°C for 3 min, 30°C for 3 min, 20°C for 3 min and 4°C hold. Dilute 20x to obtain 15uM working concentration. Aliquot annealed adaptor into eppendorf tubes and store them in -20°C freezer.

a) Combine and mix the components below

Component	Volume (µL)

Nuclease-free water	22.0
Illumina Multiplexing PE Adaptors (15 µM concentration)	5.0
Quick Ligase 5X buffer*	18.0
A-Tailed DNA	40.0
Quick Ligase Enzyme*	5.0
Total	90.0

- From NEB (Cat. No. E-6056L).

b) Incubate at room temperature for 30 minutes.

c) Purify by adding 1.0X Agencourt AMPure XP beads and binding by rotating on a thermomixer for about 5 minutes at RT before placing into the magnetic particle concentrator (MPC). Remove the supernatant (and save). Wash 3 times with freshly prepared 70% ethanol. Keep the sample tube in MPC during the wash. Add ethanol slowly to avoid disturbing the bead pellet. After wash, briefly dry the beads in the air.Elute DNA in 58 µl of nuclear-free H2O.

Note: (Optional) Assess yield and quality by running Agilent Bioanalyzer 2100 DNA 7500 Chip. Make sure there is no excess free adaptor's peak in the eluted DNA sample.

5. LM-PCR Amplification

a) Prepare the following PCR mix: using only A or B column instructed as follow for Invitrogen enzyme mix or NEB phusion enzyme mix accordingly

Component	Volume (µL)
DNA ligation product	56.0
2X Phusion High Fidelity PCR Master Mix with HF buffer	60.0
LM-PCR primer IMUX-P1.0 (50 µM)	2.0
LM-PCR barcoded primer IBCn (n=1-12) or IDMBn (n=1-96) (50 µM)	2.0
Total	120.0

b) Vortex gently and transfer mix into a 0.2 ml PCR strip tubes. Seal the wells with caps.

c) Place them in the ABI GeneAmp PCR System 9700/Veriti for amplification enrichment. PCR condition:

(1) 30'' @ 98ºC

(2) 10" @ 98ºC
(3) 30" @ 60ºC
(4) 30'' @ 72ºC
(5) Repeat step (2) to (4) 6 times for total 6 cycles
(6) 5' @ 72ºC
(7) HOLD @ 4ºC

d) Load 1 µl of sample into one lane of a 2.2% FlashGel to check amplification.
e) Purify by adding 1.0X Agencourt AMPure XP beads and binding by rotating on a thermomixer for about 5 minutes at RT before placing into the magnetic particle concentrator (MPC). Remove the supernatant (and save). Wash 3 times with freshly prepared 70% ethanol. Keep the sample tube in MPC during the wash. Add ethanol slowly to avoid disturbing the bead pellet. After wash, briefly dry the beads in the air.Elute DNA in 40 µl of nuclear-free H2O.
f) Run an Agilent Bioanalyzer 2100 DNA Chip 7500 to check size distribution and quantity of PCR product. Make sure there is no excess primer left in the eluted sample (See Figure 2 for example). The yield should be at least >1ug.

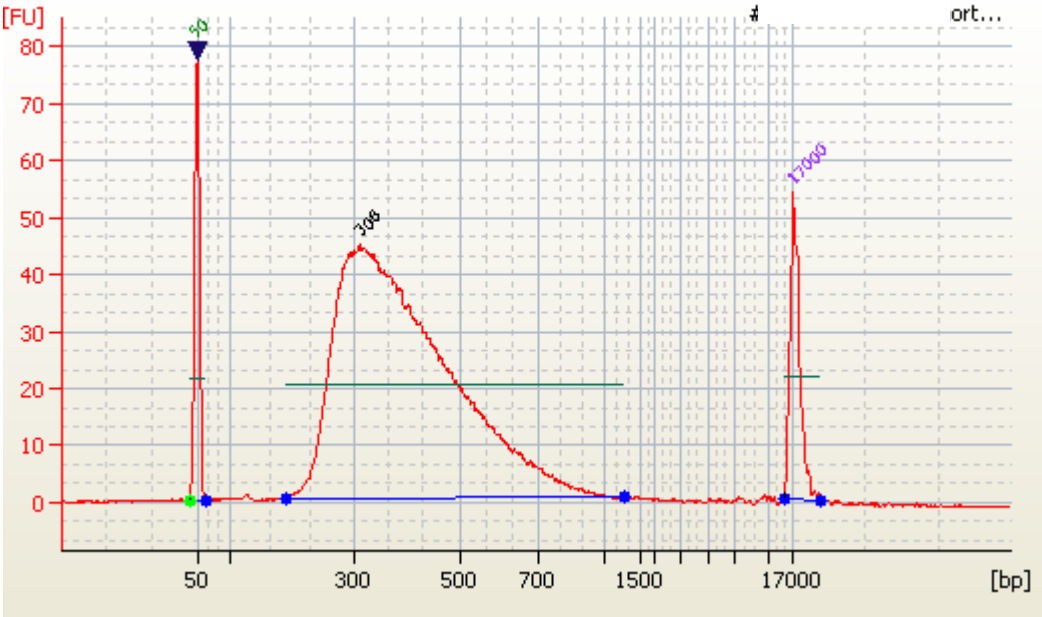

Figure 2 Example of Illumina barcoded paired end WGS library on Agilent Bioanalyzer 2100 DNA 7500 Chip.

(end)