VAHTS Mate Pair Library Prep Kit for Illumina®

Catalog # ND104

Version 5.1

Vazyme biotech co., ltd.

1. Introduction

VAHTS Mate Pair Library Prep kit for Illumina® is a directed optimized kit for library construction of the high-throughput sequencing platform of Illumina. With this kit, DNA samples can be prepared into a special library for Illumina high-throughput sequencing platform. All the reagents provided in the kit are strictly controlled by quality and function test, which guarantees the stability and repeatability of the library construction in the maximum degree. The mate pair library with size of 2-15 (mainly 3-5) kb of insert fragments can be obtained without gel extraction. With gel extraction, the size of insert fragment in mate pair library depends on the size of the fragment cut from the gel.

2. Package Information

VAHTS Mate Pair Library Prep Kit-Box1

Component	ND104-01(48 rxn)
VAHTS Tagmentase	192 μl
VAHTS Tagmentase Buffer(5×)	1 ml
VAHTS Strand Displacement Polymerase	48 μl
VAHTS Strand Displacement Buffer (5×)	500 μl
dNTP(10 mM each)	48 μl
VAHTS Circulation Ligase	336 μl
VAHTS Circulation Buffer(10×)	1.5 ml
VAHTS Exonuclease	288 μl
VAHTS Turbo End Prep Enzyme Mix	144 μl
VAHTS Turbo End Prep Reaction Buffer (10×)	312 μl
VAHTS Turbo T4 DNA Ligase	96 μl
VAHTS Turbo Ligation Enhancer	2×1 ml

VAHTS Mate Pair Library Prep Kit-Box2

Component	ND104-01(48 rxn)
VAHTS Adapter for Illumina (15 μM)	120 μl
VAHTS Universal PCR Primer for Illumina (25 μM)	50 μl
VAHTS Index 2 Primer for Illumina (25 μM)	20 μl
VAHTS Index 4 Primer for Illumina (25 μM)	20 μl
VAHTS Index 5 Primer for Illumina (25 μM)	20 μl
VAHTS Index 6 Primer for Illumina (25 μM)	20 μl
VAHTS Index 7 Primer for Illumina (25 μM)	20 μl
VAHTS Index 12 Primer for Illumina (25 μM)	20 μl
VAHTS Index 13 Primer for Illumina (25 μM)	20 μl
VAHTS Index 14 Primer for Illumina (25 μM)	20 μl
VAHTS Index 15 Primer for Illumina (25 μM)	20 μl
VAHTS Index 16 Primer for Illumina (25 μM)	20 μl
VAHTS Index 18 Primer for Illumina (25 μM)	20 μl
VAHTS Index 19 Primer for Illumina (25 μM)	20 μl

VAHTS Mate Pair Library Prep Kit-Box3

Component	ND104-01(48 rxn)
Bead Bind Buffer	20 ml
Bead Wash Buffer	80 ml
Resuspension Buffer	45 ml

Component	ND104-01(48 rxn)
VAHTS Super-Fidelity DNA Polymerse	48 μl
VAHTS Super-Fidelity PCR Buffer(2×)	1.2 ml

3. Storage

Box1, Box2 and Box4 should be stored at -20 ℃ and Box 3 can be stored at room temperature.

4. Other Required Material

70% of Ethanol

Ultrapure Sterile Water

Magnetic separation rack

Low adsorption EP tube (Eppendorf #022431021)

AMPure XP beads (Beckman Coulter, Inc. #A63880)

Dynabeads M-280 streptavidin magnetic beads (Invitrogen #112-05D)

Covaris AFATM Ultrasonicator (CovarisS2 or S220Ultrasonicator device)

CovarisT6 (6×32) glass tubes (Covaris #520031)

Covaris Snap-Cap-Teflon Silicone Septa 8mm (Covaris #520041)

Zymo Genomic DNA Clean & Concentrator Kit (ZYMO RESEARCH #D4010)

Zymoclean™ Large Fragment DNA Recovery Kit (ZYMO RESEARCH #D4045)

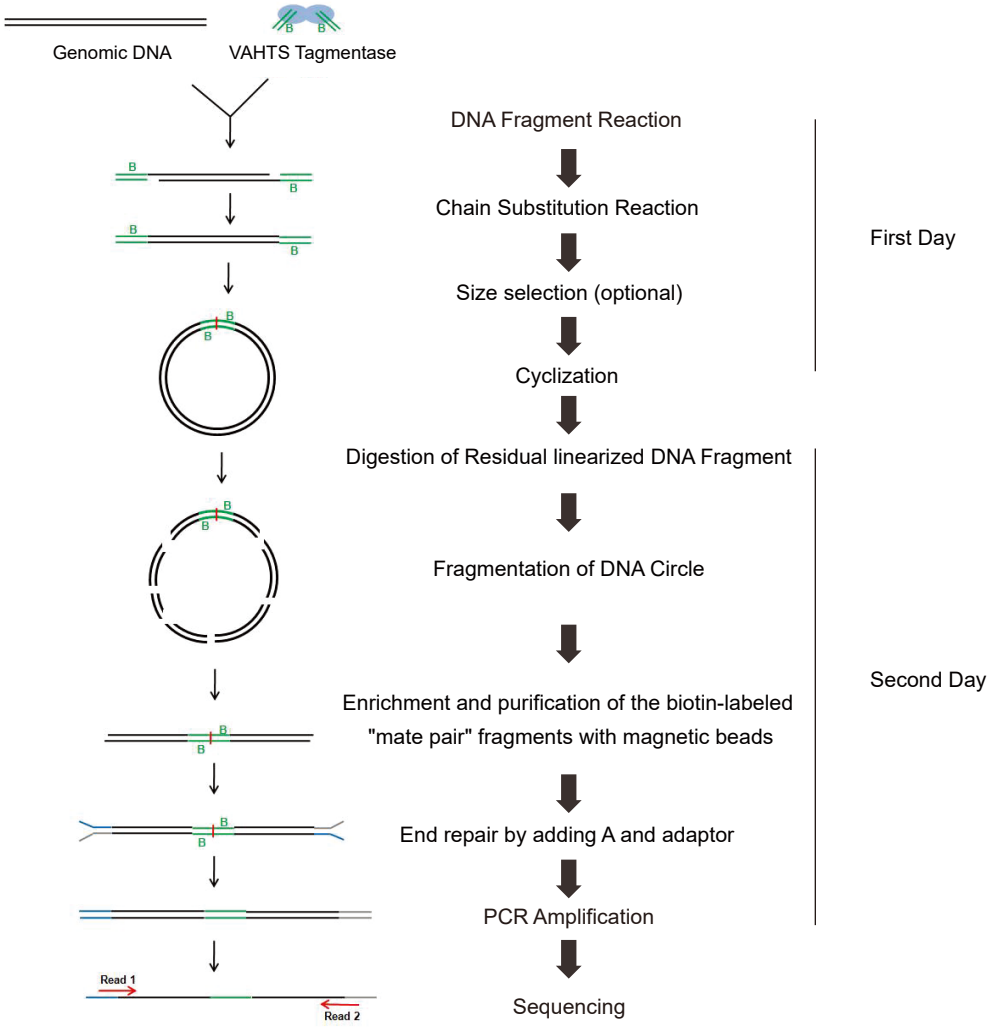
5. Applications

The kit can be used to prepare the specific library for high-throughput sequencing platform of Illumina with1 μg–4 μg of DNA as input.

6. Index Sequence

	Sequences
VAHTS Index 2 Primer for Illumina (25 μM)	CGATGT
VAHTS Index 4 Primer for Illumina (25 μM)	TGACCA
VAHTS Index 5 Primer for Illumina (25 μM)	ACAGTG
VAHTS Index 6 Primer for Illumina (25 μM)	GCCAAT
VAHTS Index 7 Primer for Illumina (25 μM)	CAGATC
VAHTS Index 12 Primer for Illumina (25 μM)	CTTGTA
VAHTS Index 13 Primer for Illumina (25 μM)	AGTCAA
VAHTS Index 14 Primer for Illumina (25 μM)	AGTTCC
VAHTS Index 15 Primer for Illumina (25 μM)	ATGTCA
VAHTS Index 16 Primer for Illumina (25 μM)	CCGTCC
VAHTS Index 18 Primer for Illumina (25 μM)	GTCCGC
VAHTS Index 19 Primer for Illumina (25 μM)	GTGAAA

7. Schematic process of library construction



8. Protocol

1. Fragmentation of DNA and addition of biotin-labeled adaptor at the two ends.

The kit provided two kinds of plan for library construction: None gel extraction and gel extraction. The plan of non-gel extraction, a simple way of library construction, can produce more kinds of insert fragments for library construction with less initial DNA. But the size of insert fragments distributed in a large range. Under normal circumstances, the size of inserts fragments distributed between 2 and 15 kb and mainly concentrated between 3-5 kb. The plan of gel extraction, a more challenging way of library construction, can obtain the insert fragment with longer size and more concentrated distribution range. In the plan of gel extraction, several libraries can be constructed with one sample through choosing 1-3 of different ranges of gel extraction if sufficient quantities of DNA can be recovered. The user can choose one of the plans for library construction according to their need.

1) Add the following components in order to the EP tube:

	None gel extraction	Gel extraction
Genomic DNA	x μl (1 μg)	x μl (4 μg)
ddH_2O	76-x μl	308-x μl
VAHTS Tagmentase Buffer	20 μl	80 μl
VAHTS Tagmentase	4 μl	12 μl
Total	100 μl	400 μl

2) Mix the reaction system 5 times by gentle pipetting. Collect the mixture to the bottom of the tube with transient centrifugation (1 to 2 seconds).

3) Incubate at 55 ℃ for 10 min.

4) Purify DNA fragment with Zymo Genomic DNA Clean & Concentrator Kit. Elute the DNA fragment with 30 μl of washing buffer and then the eluted DNA can be applied to the next step of chain substitution reaction.

2. Chain Substitution Reaction

One gap was left in DNA fragment reaction of the first step. In this step, the gap will be filled with DNA polymerase to guarantee that all the DNA fragments were blunt-ended to fit the reaction of loop.

1) Add the following components in order to a EP tube:

	None gel extraction	Gel extraction
Fragmented DNA	30 μl	30 μl
ddH_2O	8 μl	122 μl
VAHTS Strand Disp Buffer	10 μl	40 μl
dNTPs	1 μl	4 μl
VAHTS Strand Disp Polymerase	1 μl	4 μl
Total	50 μl	200 μl

2) Mix the reaction system 5 times with gentle pipetting. Collect the mixture to bottom of the tube with transient centrifugation (1 to 2 seconds).

3) Incubate at 72 ℃ for 30 min.

4) The products of DNA fragment chain substitution reaction can be purified with AMPure beads in the next step.

3. Purify chain substitution products with AMPure beads

In this step, large DNA fragments will be purified with AMPure beads by binding to the beads and the small DNA fragments which are not needed will be removed.

Note: Incubate the AMPure beads at room temperature for at least 30 min before used and mix well by vortex.

1) Add the following components in order to a EP tube:

	None gel extraction	Gel extraction
Strand Displaced DNA	50 μl	200 μl
ddH_2O	50 μl	0 μl
AMPure XP Beads	40 μl	100 μl
Total	140 μl	300 μl

Note: The volume of all the components in this step is very important. Please precisely quantify the volume of each component.

2) Flick the tube for 5 times to mix the beads and the solution, and collect with transient centrifugation.

3) Incubate the mixture of beads and DNA at room temperature for 15 min and flick the tube every 2 minutes to re-suspend the beads.

4) Collect the mixture to bottom of the tube with transient centrifugation (1 to 2 seconds).

5) Put the EP tube on the magnetic separation rack for 5 min and discard the liquid. Please do not stir the beads.

6) Keep the tube on the magnetic separation rack, add 400 μl of 70% ethanol and discard the liquid. Please do not stir the beads.

7) Repeat step 6 for one time.

8) Keep the tube on the magnetic separation rack and open the lid to dry for 10-15 min.

9) Take the EP tube from the magnetic separation rack, add 30 μl of Resupension Buffer to elute the DNA from the beads and mix well by flicking the tube.

10) Collect the beads and Resupension Buffer to the bottom of tube with transient centrifugation and incubate at room temperature for 5 min.

11) Put the EP tube on the magnetic separation rack for 5 min and transfer the supernatant to a new EP tube.

12) The purified DNA can be cyclized directly or after Fragment size selection in the next step.

Option: Fragment size selection (Gel extraction)

This scheme provides a strict control of fragment size to construct the library with larger and more concentrated size.

1) Prepare 0.6% of agarose gel and TAE buffer.

2) Do electrophoresis with 30 μl of chain substitution DNA sample purified with AMPure beads with loading buffer.

3) Run the electrophoresis at 100 V for 120 min.

4) Cut the gel containing the target DNA fragment carefully.

Note: Please extend the width of the target region by several kbs up and down (e.g., 4 - 6 kb, 7 - 10 kb, 9 - 12 kb). Extension of the width of the target region can increase the recovered amount of DNA and increase the diversity of the library.

5) Recover DNA fragments in the agarose gel with Zymoclean™ Large Fragment DNA Recovery Kit. The protocol is referenced to the instruction manual of Zymoclean™ Large Fragment DNA Recovery Kit. Elute the DNA with 30 μl of Respension Buffer.

6) The eluted DNA can be cyclized in the next step.

4. Cyclization

In this step, molecular internal blunt-end ligation will be performed with the obtaiined fragmented DNA. Please determine the concentration of the purified DNA before the cyclization reaction. Qubit quantify is recommended for the concentration determination. With more fragment DNA to carry out the cyclization reaction can increase the output and diversity of the final Library. But it also increases the probability of the formation of chimera ligations. Considering the two factors, 200 - 600 ng of DNA fragment was recommended to carry out the cyclization reaction.

1) Add the following components in order to a EP tube:

	Volume
Purified DNA fragment (200-600 ng)	x μl
ddH_2O	263-x μl
VAHTS Circulation Buffer	30 μl
VAHTS Circulation Ligase	7 μl
Total	300 μl

2) Mix the reaction system for 5 times by gentle pipetting. Collect the mixture to bottom of the tube with transient centrifugation (1 to 2 seconds).

3) Incubate at 22 ℃ overnight (about 12-16 hours).

4) Incubate at 65 ℃ for 10 min to inactive VAHTS Circulation Ligase.

5) Perform the next step of Digest Linear DNA after cooling the reaction system to room temperature.

5. Digest Linear DNA

In this step, digest the linear DNA with exonuclease and inactive the exonuclease after the reaction.

1) Add 6 μl of VAHTS Exonuclease to the overnight incubated cyclization reaction system, mix by gently pipette for 5 times and collect to the bottom of the tube by transient centrifugation.

2) Incubate at 37 ℃ for 30 min.

3) Incubate at 70 ℃ for 30 min to inactive VAHTS Exonuclease.

4) Perform the next step of fragment the cyclized DNA.

6. Fragment cyclized DNA

In this step, large fragment cyclic DNA will be cut into small DNA fragments (about 3000 - 1000 bp) by physical methods. Covaris S2 or S220 were recommended to fragment the DNA.

1) Transfer the sample into Covaris T6 glass tube.

2) Make sure that the entire tube is filled with the sample. Add water to fill if needed. Cover the lid and check the tube to ensure that no air bubbles were in the tube

3) Set parameter for fragmentation of the DNA according to the used instrument.

Parameter	S2	S220
Peak Power Intensity	N.A	240
Intensity	8	N.A
Duty Cycle/Duty Factor	20%	20%
Cycles Per Burst	200	200
Time	40 s	40 s
Temperature	6℃	6℃

4) Transfer the DNA sample into a new tube after the fragmentation. Perform the next step of capturing the target DNA fragment with Streptavidin beads.

7. Capture the target DNA fragment with Streptavidin beads

1) Mix Streptavidin M280 Dynabeads by shaking before use.

2) Take 20 μl of M280 Dynabeads and put into a new tube.

3) Put the EP tube filled with M280 Dynabeadson on magnetic separation rack for 1 min and discard the supernatant.

4) Add 50 μl of Bead Bind Buffer to resuspend the beads.

5) Put the EP tube on the magnetic separation rack for 1 min and discard the supernatant

6) Repeat step 4 and 5 for one time.

7) Take the EP tube off from the magnetic frame and add 300 μl of Bead Bind Buffer to resuspend the beads.

8) Add 300 μl of the resuspended beads to 300 μl of fragmented DNA sample, and mix well.

9) Incubate at 20 ℃ for 15 min. Re-suspend the beads by shaking upside down every 2 min.

10) Put the EP tube on the magnetic separation rack for 1 min.

11) Discard the supernatant after transient centrifugation (1-2 seconds).

12) Take the EP tube off from the magnetic separation rack, flick the tube to resuspend the beads and put the EP tube on the magnetic separation rack after transient centrifugation (1-2 seconds).

13) Repeat step 11 and 12 for three times.

14) Discard the supernatant and add 200 μl of Resuspension Buffer.

15) Take the EP tube off from the magnetic separation rack, flick the tube to re-suspend the beads and put the EP tube on the magnetic separation rack after transient centrifugation (1-2 seconds).

16) Repeat step 14 and 15 for one time.

17) Perform the next step of end repair, phosphorylation and dA-Tailing. Leave the beads in the Resuspension Buffer of the last step and keep them on the magnetic separation rack before preparing the mixture of end repair, phosphorylation and dA-Tailing.

8. End repair, Phosphorylation and dA-Tailing

In this step, DNA fragment will be end repaired, phosphorylated and dA-Tailing to be used for adaptor ligation.

1) Prepare the reaction mixture in a sterile PCR tube as follows:

	Volume
VAHTS Turbo End Prep Enzyme Mix	3 μl
VAHTS Turbo End Prep Reaction Buffer (10 ×)	6.5 μl
ddH_2O	55.5 μl
Total	65 μl

2) Mix by gentle pipetting and collect the reaction liquid to bottom of tube by transient centrifugal (1-2 sec)

3) Discard the Resuspension Buffer of the last step. Collect the residual solution on the wall of tube by transient centrifugation (1-2 seconds). Put the EP tube on the magnetic separation rack and carefully remove the left solution.

4) Take the EP tube off from the magnetic separation rack and add 65 μl of the reaction mixture above.

5) Flick the EP tube to resuspend the beads and collect the beads to the bottom of the tube with transient centrifugation (1-2 seconds). Please do not centrifuge for long time to avoid the accumulation of the beads.

6) Incubate at 20 ℃ for 30 min followed by incubation at 65 ℃ for 30 min.

7) Perform the next step of adaptor ligation.

9. Adaptor Ligation

In this step, DNA fragments will be ligated with adaptor to facilitate the PCR reaction.

1) Add the following components to the above 65 μl reaction system:

	Volume
VAHTS Turbo T4 DNA Ligase	2 μl
VAHTS Turbo Ligation Enhancer	30.5 μl
VAHTS Adapter for Illumina	2.5 μl
Total	100 μl

2) Mix by gentle pipetting and collect the reaction liquid to bottom of tube by transient centrifugation (1-2 seconds)

3) Incubate at 20 ℃ for 15 min.

4) Collect the sample to the bottom of tube by transient centrifugation (1-2 seconds). Put the EP tube on the magnetic separation rack for 1 min.

5) Discard the supernatant and add 200 μl of Bead Wash Buffer.

6) Take the EP tube off from the magnetic separation rack, flick the EP tube to re-suspend the beads and put the EP tube on the magnetic separation rack for 30 sec after transient centrifugation (1-2 seconds).

7) Repeat step 5 and 6 for three times.

8) Discard the supernatant and add 200 μl of Resuspension Buffer.

9) Take the EP tube off from the magnetic separation rack, flick the EP tube to resuspend the beads and put the EP tube on the magnetic separation rack for 30 sec after transient centrifugation (1-2 seconds).

10) Repeat step 8 and 9 once.

11) Perform the next step of PCR amplification. Leave the beads in the Resuspension Buffer of the last step and keep them on the magnetic separation rack before preparing the mixture for PCR reaction.

10. PCR Amplification

In this step, DNA fragments with adaptors will be enriched.

1) Prepare reaction mixture in an EP tube as follows:

	Volume
VAHTS Super-Fidelity PCR Buffer(2 ×)	25 μl
VAHTS Universal PCR Primer for Illumina	1 μl
VAHTS Index Primer for Illumina	1 μl
VAHTS Super-Fidelity DNA Polymerase	1 μl
ddH_2O	22 μl
Total	50 μl

2) Discard the Resuspension Buffer of the last step. Collect the residual solution on the wall of tube by transient centrifugal (1-2 seconds). Put the EP tube on the magnetic frame and carefully remove the left solution.

3) Take the EP tube off from the magnetic separation rack and add the above 50 μl PCR mixture.

4) Mix the beads and PCR reaction well by gentle pipetting and then transfer into the PCR tube and perform PCR reaction as the follows.

	Temperature	Time	Cycle
Pre-denaturation	98℃	30 sec	1
Denaturation	98℃	10 sec	
Annealing	65℃	30 sec	15
Extension	72℃	30 sec	
Complete Extension	72℃	5 min	
Hold	4℃		

5) Perform the next step of PCR product purification

11. PCR Product Purification

In this step, PCR products will be purified and small fragments (<300 bp) of library will be excluded.

Note: Put the AMPure XP beads at the room temperature for at least 30 min and mix well by vortex.

1) Put the PCR tube on the magnetic separation rack for 1 min.

2) Transfer 45 μl of supernatant to a new EP tube.

3) Add 30 μl of AMPure XP beads to the EP tube, flick the tube to mix the beads and sample well. Collect the sample to the bottom of tube by transient centrifugation (1-2 sec).

4) Incubate at room temperature for 5 min.

5) Put the EP tube on the magnetic separation rack for 5 min and then discard the supernatant.

6) DO NOT take the EP tube off from the magnetic separation rack and add 200 μl of 70% ethanol into the tube. Incubate for 30 sec and then discard the ethanol carefully.

7) Repeat step 6 for one time.

8) Open the lid of the EP tube to dry in the air for 10-15 min.

9) Take the EP tube off from the magnetic separation rack, add 20 μl of Resuspension Buffer to elute the DNA. Flick the tube to resuspend the beads and incubate at room temperature for 5 min.

10) Put the EP tube on the magnetic separation rack again for 5 min and transfer the supernatant containing the library into a new tube.

Library Verification

The size of the enriched fragment from the library can be verified with agarose gel electrophoresis or Agilent Technologies 2100 Bio analyzer.

Verification by agarose gel electrophoresis:

1/10 of the total volume of the library was used in agarose gel and the library should be distributed at the range of 300-1000 bp.

Verification by Agilent Technologies 2100 Bioanalyzer:

For the constructed library by none gel extraction strategy, add 1 μl of the sample to 7500 or 12000 DNA Labchip. The expected size of the library is 300-1500 bp and the concentration is 5-50 nM.

For the constructed library by gel extraction strategy, add 1 μl of the sample to High Sensitivity DNA Labchip. The expected size of the library is 300-1500 bp and the concentration is 1.5-20 nM.