VAHTS Universal Plus DNA Library Prep Kit for Illumina V2 (Plate)

NDB627

Vazyme

Instruction for Use

Version 24.2

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For Research Use Only. Not for use in diagnostic procedures.

01/Product Description

VAHTS Universal Plus DNA Library Prep Kit for Illumina V2 (Plate) is a 96-well plate library preparation kit based on enzymatic fragmentation for Illumina sequencers. It can convert 100 pg - 1 μg DNA into a specialized Illumina library. This kit can be compatible with various samples and different inputs, and simply adjust the fragmentation time according to the expected insert size to obtain expected library size. All the reagents provided in the kit have undergone rigorous quality control and functional testing to ensure the optimal stability and repeatability of library preparation.

02/Components

Components	NDB627-01
DNA Reagents (Prepackaged for NDB627)	96 rxns

Detailed component

	Components	NDB627-01 (96 rxns)
①	FEA Buffer V2	90 μl each
②	FEA Enzyme Mix V2	180 μl each
③	Rapid Ligation Buffer V2	350 μl each
④	Rapid DNA Ligase V2	80 μl each
⑤	VAHTS HiFi Amplification Mix	350 μl each
⑥	PCR Primer Mix 3 for Illumina	80 μl each

NDB627-01 Layout

Component

Unit/Well: μl

	①	②	③	④	⑤	⑥	7	8	9	10	11	12
	1	2	3	4	5	6						
A	90	180	350	80	350	80						
B	90	180	350	80	350	80						
C	90	180	350	80	350	80						
D	90	180	350	80	350	80						
E	90	180	350	80	350	80						
F	90	180	350	80	350	80						
G	90	180	350	80	350	80						
H	90	180	350	80	350	80						

Empty Well*

* Columns 7 to 12 are empty wells, which can be utilized as needed for experimental requirements to prepare reaction solution or to add reagents such as magnetic beads or Adapters.

03/Storage

Store at -30 ~ -15°C and ship at ≤0°C.

04/Applications

The kit is suitable for preparing a dedicated library for Illumina sequencers. It is suitable for various gDNA (Genomic DNA) samples from animals, plants and microorganisms, FFPE DNA, etc.

05/Self-prepared Materials

Clean up magnetic beads: VAHTS DNA Clean Beads (Vazyme #N411);

DNA Adapter:

VAHTS Maxi Unique Dual Index Primers Set 1 - Set 4 for Illumina (Plate) (Vazyme #NB34401 - NB34404);

Other materials: Freshly prepared 80% ethanol, Nuclease-free ddH_2O, 0.1 × TE, Elution buffer (10 mM Tris-HCl，pH 8.0 - 8.5), etc.

06/Notes

1. This kit is sensitive to oxidization, please do not open the aluminum foil bag until use.
2. Before the experiment, clean the instruments and environment thoroughly. First, wipe the surfaces with dust-free paper soaked in 75% ethanol, then followed by the dry dust-free paper. Perform UV to eliminate potential aerosol contamination and ensure the accuracy results.
3. Thaw the plate-based components at room temperature before use. After thawing, mix thoroughly by inverting 3 times and centrifuge briefly. Place on ice before use.
4. Due to the influence of various factors such as samples, protocols, instruments and operations, the parameters of the library preparation workflow need to be adjusted based on actual conditions. If you have any questions during use, please contact Vazyme Technical Support for assistance.

07/Workflow

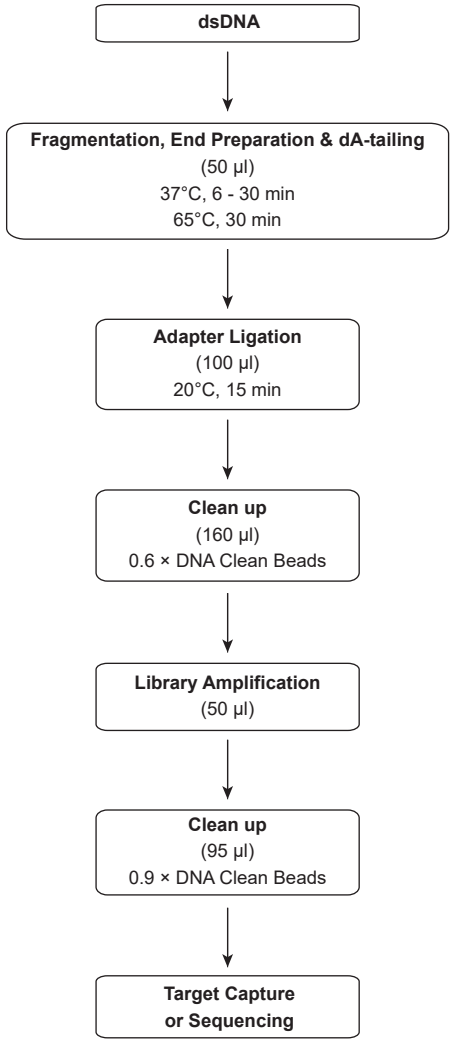

Fig 1. Workflow of VAHTS Universal Plus DNA Library Prep Kit for Illumina V2 (Plate)

08/Experiment Process

Thaw the plate-based components and put the plate on ice. Mix gently by pipetting 10 times before use.

08-1/Fragmentation, End Preparation & dA-tailing

1. Prepare the following reaction (on ice).

Components	Volume
Input DNA	x μl
FEA Buffer V2	5 μl
FEA Enzyme Mix V2	10 μl
ddH_2O	To 50 μl

2. Mix gently by pipetting 10 times. Place the PCR plate in the PCR instrument and perform the following reaction.

Temperature	Time
105°C (Preheated lid)	On
4°C	1 min
37°C	Refer to the table below*
65°C	30 min
4°C	Hold

* Fragmentation time depends on the quality of Input DNA and expected insert size:

Expected insert size	Fragmentation time
150 bp	20 - 30 min
250 bp	8 - 12 min
350 bp	5 - 10 min
550 bp	≤5 min

▲ The recommended time given above was tested by using high-quality human gDNA as a template. When using high-quality human gDNA for library preparation, different inputs within the recommended input range (100 pg - 1 μg) with the same reaction time resulted in little variation in the distribution of fragmented product (the distribution range is essentially consistent, but the main peak size may vary slightly different). If the quality of Input DNA is poor or the fragmented size is not within the expected range, it is recommended to increase or decrease the fragmentation time by 2 - 5 min. For FFPE DNA samples, there is no need to adjust the fragmentation time according to its integrity if there is no strict requirement for the insert size.

08-2/Adapter Ligation

This step will ligate the Adapter to the end of the product in the previous step.

1. Dilute the Adapter to an appropriate concentration according to the amount of Input DNA, refer to the table below.

Table 1. Recommended the Adapter concentration and amplification cycles for 100 pg - 1μg Input DNA

Input DNA	Concentration of Adapter from other brand	Vazyme Adapter dilution ratio	Number of cycles required to generate 1 μg
1 μg	10 μM	Undiluted	0 - 2
100 ng	10 μM	Undiluted	4 - 6
50 ng	5 μM	1 : 2	5 - 7
10 ng	1 μM	1 : 10	7 - 9
1 ng	0.1 μM	1 : 100	11 - 13
100 pg	0.05 μM	1 : 200	14 - 16

▲ When DNA quality is poor and library size is longer, the number of cycles must be adjusted to obtain sufficient library yields.

2. Mix gently by pipetting 10 times. Prepare the reaction solution as follows.

Components	Volume
Products from previous step	50 μl
Rapid Ligation Buffer V2	25 μl
Rapid DNA Ligase V2	5 μl
DNA Adapter X	5 μl
ddH_2O	15 μl
Total	100 μl

3. Mix gently by pipetting 10 times. Place the PCR plate in the PCR instrument and perform the following reaction.

Temperature	Time
105°C (Preheated lid)	On
20°C	15 min
4°C	Hold

4. Clean up the reaction product using VAHTS DNA Clean Beads as follows:

a. Keep the VAHTS DNA Clean Beads at room temperature for 30 min. Resuspend the beads by vortexing.

b. Add 60 μl VAHTS DNA Clean Beads to 100 μl product after Adapter Ligation and mix by pipetting 10 times to resuspend.

c. Incubate at room temperature for 5 min.

d. Briefly centrifuge the PCR tube and place it on a magnetic rack until the supernatant is clear (~ 5 min), carefully **discard the supernatant**.

e. Keep the tube on the magnetic rack and wash the beads with 200 μl of freshly prepared 80% ethanol without disturbing the beads. Incubate at room temperature for 30 sec, carefully **discard the supernatant**.

f. Repeat step e, wash twice in total.

g. Keep the tube always on the magnetic rack and air-dry the beads for 3 - 5 min.

▲ Do not over dry the magnetic beads, which may reduce the efficiency of DNA elution and affect the yield.

h. Remove the PCR tube from the magnetic rack for elution:

▲ If the purification products do not perform Two Rounds Size Selection: Add 22.5 μl of Elution buffer (10 mM Tris-HCl, pH 8.0 - 8.5) or ddH_2O for elution, pipette gently to resuspend beads and incubate at room temperature for 5 min. Briefly centrifuge the PCR tube and place it on the magnetic rack until the supernatant is clear (~ 5 min), carefully transfer 20 μl of supernatant to a new EP tube. Do not disturb the magnetic beads.

▲ If the purification products perform Two Rounds Szie Selection: Add 105 μl of Elution buffer (10 mM Tris-HCl, pH 8.0 - 8.5) or ddH_2O for elution, vortex or pipette gently to resuspend beads and incubate at room temperature for 5 min. Briefly centrifuge the PCR tube and place it on the magnetic rack until the supernatant is clear (~ 5 min), carefully transfer 100 μl of supernatant to a new EP tube. Do not disturb the magnetic beads.Then perform the size selection, refer to Appendix I: Protocol for Tow Rounds Size Selection.

08-3/Library Amplification

This step is for PCR amplification of the Adapter Ligation products after clean up.

1. Prepare the following solution in the PCR tube:

Components	Volume
Products from previous step	20 μl
PCR Primer Mix 3 for Illumina	5 μl
VAHTS HiFi Amplification Mix	25 μl
Total	50 μl

2. Mix gently by pipetting 10 times. Place the PCR tube in the PCR instrument and perform the following reaction.

Temperature	Time	Cycles
95°C	3 min	1
98°C 60°C 72°C	20 sec 15 sec 30 sec	For number of cycles, refer to Table 1
72°C	5 min	1
4°C	Hold	

3. For size selection, refer to Appendix I: Protocol for Tow Rounds Size Selection. If size selection is not required, using VAHTS DNA Clean Beads to purify the reaction products as follows:
   a. Keep the VAHTS DNA Clean Beads at room temperature for 30 min. Resuspend the beads by vortexing.
   b. Add 45 μl VAHTS DNA Clean Beads to 50 μl product after Library Amplification and mix by pipetting 10 times to resuspend.

c. Incubate at room temperature for 5 min.

d. Briefly centrifuge the PCR tube and place it on a magnetic rack until the supernatant is clear (~ 5 min), carefully **discard the supernatant**.

e. Keep the tube on the magnetic rack and wash the beads with 200 µl of freshly prepared 80% ethanol without disturbing the beads. Incubate at room temperature for 30 sec, carefully **discard the supernatant**.

f. Repeat step e, wash twice in total.

g. Keep the tube always on the magnetic rack and air-dry the beads for 3 - 5 min.

▲ Do not over dry the magnetic beads, which may reduce the efficiency of DNA elution and affect the yield.

h. Remove the PCR tube from the magnetic rack for elution. Add 22.5 µl of Elution buffer (10 mM Tris-HCl, pH 8.0 - 8.5) or ddH_2O for elution, pipette gently to resuspend beads and incubate at room temperature for 5 min. Briefly centrifuge the PCR tube and place it on the magnetic rack until the supernatant is clear (~ 5 min), carefully transfer 20 µl of supernatant to a new EP tube. Do not disturb the magnetic beads.

08-4/Library Quality Control

Generally, a prepared library can be evaluated through size distribution and concentration analysis.

1. Library size distribution analysis

   a. Library size distribution analysis can be performed using equipment based on electrophoretic separation, such as Bioanalyzer, Tapestation (Agilent Technologies), etc.

   b. Take a little amount of PCR-Free libraries to perform PCR amplification, and the amplification products can be detected by VAHTS Library Quantification Kit for Illumina 2.0 (Vazyme #NQ107) to reflect the size distribution of the PCR-Free library.

2. Library concentration analysis

   a. Based on dsDNA fluorescent dyes, such as Equalbit 1 × dsDNA HS Assay Kit (Vazyme #EQ121) and PicoGreen, etc.

   b. Based on qPCR-based quantification, such as VAHTS Library Quantification Kit for Illumina 2.0 (Vazyme #NQ107).

   ▲ Over-amplified libraries contain non-complete fragment and can not be measured by Qubit or PicoGreen. Quantify with qPCR-based method is not affected by over-amplified libraries.

Appendix I: Protocol for Tow Rounds Size Selection

1. Keep the VAHTS DNA Clean Beads at room temperature for 30 min. Resuspend the beads by vortexing.

2. Add **X** μl of VAHTS DNA Clean Beads to the above 100 μl solution. Vortexing or pipetting to resuspend.
   ▲ If the solution is less than 100 μl. Fill up to 100 μl with ddH_2O.
3. Incubate for 5 min at room temperature.
4. Briefly centrifuge the tube and place it on a magnetic rack until the supernatant is clear (~ 5 min), carefully transfer the supernatant to a new PCR tube and **discard the beads.**
5. Add **Y** μl of VAHTS DNA Clean Beads to the supernatant. Vortexing or pipetting to resuspend.
6. Incubate for 5 min at room temperature.
7. Briefly centrifuge the tube and place it on a magnetic rack until the supernatant is clear (~ 5 min), carefully **discard the supernatant**.
8. Keep the tube on the magnetic rack and wash the beads with 200 μl of freshly prepared 80% ethanol without disturbing the beads. Incubate at room temperature for 30 sec, carefully **discard the supernatant**.
9. Repeat step 8, wash twice in total.
10. Keep the tube always on the magnetic rack and air-dry the beads for 5 min.
11. Remove the PCR tube from the magnetic rack and add 22.5 μl of Elution buffer (10 mM Tris-HCl, pH 8.0 - 8.5) or ddH_2O for elution, vortex or pipette gently to resuspend beads and incubate at room temperature for 2 min. Briefly centrifuge the PCR tube and place it on the magnetic rack until the supernatant is clear (~ 5 min), carefully transfer 20 μl supernatant to a new EP tube. Do not disturb the magnetic beads.

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