VAHTS Universal DNA Library Prep Kit for Illumina V4 (Plate)

NDB610

Vazyme

Instruction for Use

Version 24.1

Contents

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

01/Product Description

VAHTS Universal DNA Library Prep Kit for Illumina V4 (Plate) is a 96-well plate library preparation kit for Illumina sequencers. It can convert 100 pg - 1 μg fragmented Input DNA into a specialized Illumina library. The kit is applicable to prepare the PCR or PCR-Free libraries from various samples and compatible with the targeted hybridization capture. 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	NDB610-01
DNA Reagents (Prepackaged for NDB610)	96 rxns

Detailed component

	Components	NDB610-01 (96 rxns)
①	End Prep Buffer 2	130 μl each
②	End Prep Enzyme 2	60 μl each
③	Rapid Ligation Buffer 5	420 μl each
④	Rapid DNA Ligase 2	150 μl each
⑤	VAHTS HiFi Amplification Mix 2	350 μl each
⑥	PCR Primer Mix 3 for Illumina	80 μl each

NDB610-01 Layout

Component

Unit/Well: μl

	1	2	3	4	5	6	7	8	9	10	11	12
	①	②	③	④	⑤	⑥						
A	130	60	420	150	350	80						
B	130	60	420	150	350	80						
C	130	60	420	150	350	80						
D	130	60	420	150	350	80						
E	130	60	420	150	350	80						
F	130	60	420	150	350	80						
G	130	60	420	150	350	80						
H	130	60	420	150	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 the Illumina sequencers. It is suitable for various DNA samples such as gDNA, cfDNA, ctDNA, FFPE DNA, ChIP DNA and Amplicons, etc. The amount of Input DNA can be as low as 100 pg.

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. 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.
2. 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.
3. 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

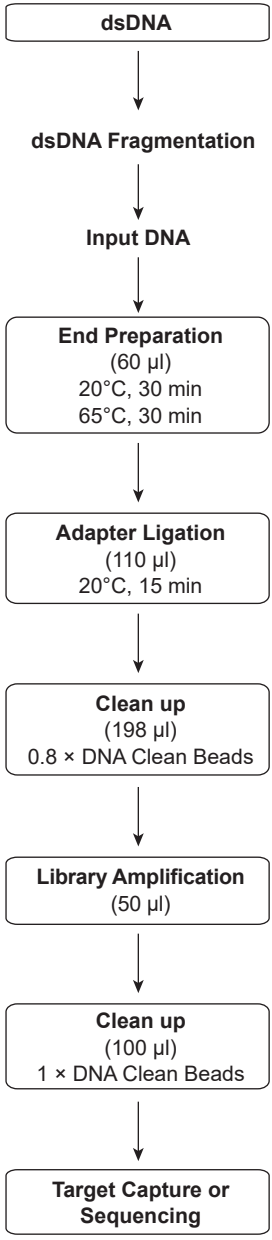

Fig 1. Workflow of VAHTS Universal DNA Library Prep Kit for Illumina V4 (Plate)

08/Experiment Process

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

08-1/End Preparation

1. Prepare the following reaction on ice.

Components	Volume
Input DNA	x μl
End Prep Buffer 2	7 μl
End Prep Enzyme 2	3 μl
ddH_2O	To 60 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
20°C	30 min
65°C	30 min
4°C	Hold

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 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	3 - 5
100 ng	5 - 10 μM	1 : 2 or Undiluted	6 - 8
10 ng	2 μM	1 : 5	10 - 13
1 ng	0.5 μM	1 : 30	13 - 15
100 pg	0.1 μM	1 : 200	17 - 19

▲ 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 and put them on ice before use. Prepare the reaction solution as follows.

Components	Volume
Products from previous step	60 μl
Rapid Ligation Buffer 5	30 μl
Rapid DNA Ligase 2	10 μl
DNA Adapter X	5 μl
ddH_2O	5 μl
Total	110 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

▲When the amount of Input DNA is little, the ligation time may be doubled as appropriate. However, longer reaction time may increase Adapter dimers. The concentration of Adapter needs to be adjusted if necessary.

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 88 μl VAHTS DNA Clean Beads to 110 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 23 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 Size 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 2	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
98°C	45 sec	1
98°C 60°C 72°C	15 sec 30 sec 30 sec	For number of cycles, refer to Table 1
72°C	1 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 50 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.

   h. Remove the PCR tube from the magnetic rack for elution. Add 23 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

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 the 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).

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 23 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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