VAHTS Universal Plus Fragmentation, End Preparation & dA-Tailing Module for Illumina V2

N219

Version 22.1

Product Description

VAHTS Universal Plus Fragmentation, End Preparation & dA-Tailing Module for Illumina V2 is developed specifically for Illumina high-throughput sequencing platform, supports fragmentation, end-repair and dA-tailing in a single reaction step. This module offers improved fragmentation performance, which significantly reduces the proportion of Artificial Invert Chimera Reads in the FFPE sample DNA library and improves the reliability of SNV detection. The module has been optimized to convert 100 pg - 1 μg of DNA to fragmented, end-repaired DNA having 5' phosphorylated, 3' dA-tailed ends and qualified with automation methods, greatly reducing the total operation time. 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.

Components

	Components	N219-01 (24 rxns)	N219-02 (96 rxns)
■	FEA Buffer V2	120 μl	480 μl
■	FEA Enzyme Mix V2	240 μl	960 μl
□	Neutralization Buffer	120 μl	480 μl

Storage

Store at -30 ~ -15℃ and transport at ≤0℃.

Self-prepared materials

VAHTS Universal Adapter Ligation Module for Illumina (Vazyme #N204)
VAHTS DNA Clean Beads (Vazyme #N411)
VAHTS HiFi Universal Amplification Mix for Illumina (Vazyme #N618)

Applications

It supports a broad DNA input range (100 pg - 1 μg) and multiple DNA input types such as animals, plants, microorganisms and other different sources of genomic DNA and paraffin-embedded DNA (FFPE DNA), etc.

Notes

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

1. Since the kit is shipped on dry ice, the buffer and enzymes will be frozen. Ensure that the product has been fully thawed and mixed before use.
2. Please tighten the caps and store the tubes at the appropriate temperature after using the reagents.

Experiment Process

Experiment material: 100 pg - 1 μg Input DNA

1. Before starting the experiment, please confirm which solvent the template DNA is dissolved in and whether the solvent contains EDTA (recommand use ddH_2O). Pretreat the DNA sample according to Step 2 if the solvent contains EDTA, proceed directly to Step 3 if not.
2. If the solvent contains EDTA, purify the template DNA with 2.2 × beads and elute with ddH_2O. Alternatively, add the corresponding volume of Neutralization Buffer according to the final concentration of EDTA in the fragmentation solution to neutralize the EDTA.

   ▲ EDTA final concentration of fragmentation solution = EDTA concentration in DNA solution × volume of DNA used/50 μl. For example, if the DNA is dissolved in TE containing 1 mM of EDTA and 10 μl is used to construct one library, the EDTA final concentration will be 1 mM × 10 μl/50 μl = 0.2 mM.

The volume of Neutralization Buffer to be added is shown in the table below:

EDTA final concentration of fragmentation solution	Volume of Neutralization Buffer
1 mM	5 µl
0.8 mM	4 µl
0.6 mM	3 µl
0.5 mM	2.5 µl
0.4 mM	2 µl
0.2 mM	1 µl
0.1 mM	0.5 µl
<0.1 mM	0 µl

3. Thaw the FEA Buffer V2 and FEA Enzyme Mix V2, mix gently and centrifuge briefly and put them on ice before use. All of the following steps are performed on ice.

4. Prepare the reaction solution in a sterile PCR tube as follows:

Components	Volume	
Input DNA	x µl	
Neutralization Buffer	y µl	☐
FEA Buffer V2	5 µl	■
ddH_2O	To 40 µl	

5. Add 10 µl FEA Enzyme Mix V2 to each sample. Mix well by pipetting up and down or by vortexing , and centrifuge briefly to collect the reaction solution to the bottom of the tube, **immediately place it into PCR instrument to perform the reaction!**

▲ Fragmentation reaction is a time-dependent enzyme-based reaction, and the sizes of the fragmentation products depend on the reaction time. It is therefore recommended to add the FEA Enzyme Mix V2 to the reaction solution separately at the end, then immediately mix well and carry out the following reaction.

▲ Fragmentation reaction solution is sensitive to oxidation, so the tube caps of the FEA Buffer V2 and the FEA Enzyme Mix V2 should be tightly screwed on and stored at -20℃ as soon as possible after the reaction solution has been prepared .

6. Place the PCR tube into the PCR instrument and perform the following reaction:

Temperature	Time
Heating lid at 105°C	On
37℃	refer to the table below*
65℃	30 min
4℃	Hold

* Fragmentation time depends on Input DNA quality and target fragment size:

▲ If poly-A tail is not required to be added to the reaction product, the reaction can be completed at 37℃, performing the reaction at 65℃ is not required.

Expected insert size	Fragmentation time
150 bp	20 - 30 min
250 bp	15 - 20 min
350 bp	10 - 15 min
550 bp	6 - 10 min

▲ The recommended times given above are based on using high-quality human placenta gDNA for template testing. When using high-quality human placenta gDNA for library construction, different inputs within the recommended input range (100 pg - 1 µg) have the same reaction time, with little variation in the distribution of the fragmentation product (the distribution range is essentially consistent, but the main peak position may vary slightly). If the Input DNA quality is poor or the fragment size is not within the expected range, it is recommended to increase or decrease the fragmentation time by 2 - 5 min. FFPE DNA will reduce the fragmentation time depending on the integrity of the DNA. After the fragmentation reaction, the follow-up reaction should be carried out immediately.

7. Immediately use VAHTS Universal Adapter Ligation Module for Illumina (Vazyme #N204) to carry out the follow-up adapter ligation reaction, or use 2 × beads for purification for other purposes.