VAHTS Universal Plus DNA Library Prep Kit V4

ND801

Instruction for Use

Version 25.1

Vazyme

Contents

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

01/Product Description

VAHTS Universal Plus DNA Library Prep Kit V4 is an enzyme-based fragmented DNA library preparation kit specifically developed for next-generation sequencing (NGS) platforms. The kit is optimized to improve library uniformity and enhance tolerance to EDTA in the DNA sample buffer. It integrates DNA fragmentation, end repair, and dA-tailing into a single step, with no purification required prior to adapter ligation. Adapter ligation, library amplification, and size selection can then be performed directly, converting 1 ng - 1 μg of input DNA into libraries compatible with Illumina or MGI sequencing platforms. All the reagents provided in the kit have undergone rigorous quality control and functional testing to ensure the stability and reproducibility of library preparation.

02/Components

	Components	ND801-01 (24 rxns)	ND801-02 (96 rxns)
	FEA Buffer V4	120 μl	480 μl
	FEA Enzyme Mix V4	240 μl	960 μl
	Rapid Ligation Buffer V4	600 μl	4 × 600 μl
	Rapid DNA Ligase V4	120 μl	480 μl
	VAHTS Ultra Amplification Mix	600 μl	4 × 600 μl
	PCR Primer Mix for Illumina	120 μl	480 μl
	PCR Primer Mix for MGI	120 μl	480 μl

03/Storage

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

04/Applications

The kit is compatible with dsDNA inputs ranging from 1 ng to 1 μg, including genomic DNA from a wide variety of sources, such as animals, plants, and microorganisms. This kit is recommended for:

◇ Whole-genome sequencing (WGS)

◇ Whole-exome sequencing (WES) or other targeted capture sequencing

◇ Metagenomic sequencing

05/Self-prepared Materials

Magnetic Beads: VAHTS DNA Clean Beads (Vazyme #N411);

Quality Control: Agilent Technologies 2100 Bioanalyzer or other equivalent products;
Equalbit 1 × dsDNA HS Assay Kit (Vazyme #EQ121);

DNA Adapter for Illumina:

VAHTS Maxi Unique Dual Index Primers Set 5 - Set 8 for Illumina (Vazyme #N34405/N34406/N34407/N34408);

#N34405/N34406/N34407/N3448 are stubby adapters with 10 bp Unique Indexes at two ends, providing 384 different indexes across 4 sets.

DNA Adapter for MGI:

VAHTS Maxi Unique Dual Barcode Primers Set 1 - Set 4 for MGI (Vazyme #NM34401/NM34402/NM34403/NM34404);

#NM34401/NM34402/NM34403/NM34404 are stubby adapters with 10 bp Unique Indexes at two ends, providing 384 different indexes across 4 sets.

▲ For other CDI, UMI, or single index adapters, please contact support@vazyme.com for technical support.

Other Materials: Absolute ethanol, ddH_2O, low adsorption EP tube, PCR tube, magnetic rack, PCR instrument, etc.

06/Notes

The parameters in library preparation may require adjustment depending on factors such as sample type, protocol, instrument, and operational conditions. To ensure high-quality results, please carefully read the following notes. Contact support@vazyme.com for assistance in case of any issues during use.

06-1/Input DNA and Fragmentation

1. Starting material: 1 ng - 1 μg of high-quality DNA (A260/A280 = 1.8 - 2.0). Recommended input amounts for common applications are listed in Table 1.

Table 1. Recommended input amounts for common applications

Application	Sample type	Recommended amount of Input DNA
Whole Genome Sequencing	Complex gDNA	50 ng - 1 μg
Targeted Capture Sequencing	Complex gDNA	10 ng - 1 μg
Whole Genome/Targeted Capture Sequencing	FFPE DNA	≥50 ng
Whole Genome Sequencing	Microbial genome	1 ng - 1 μg
Whole Genome Sequencing (PCR-free)	Complex/Simple genome	≥50 ng (without size selection) ≥200 ng (with size selection)

▲ Table 1 lists the recommended amounts of high-quality Input DNA. When the Input DNA is of poor quality, the usage amount should be increased appropriately.

2. Recommended solvents for DNA samples are ddH_2O, 10 mM Tris-HCl (pH 7.5 - 8.0), or Low TE (10 mM Tris, 0.1 mM EDTA, pH 8.0). FEA Enzyme Mix V4 is sensitive to high EDTA concentrations. Please confirm the EDTA concentration in DNA samples and ensure that the final EDTA concentration in the fragmentation reaction does not exceed 0.3 mM. If the final concentration is ≥0.3 mM, the fragmentation time may be extended to achieve the desired DNA fragment size.

   Final EDTA concentration (mM) = (EDTA concentration in input DNA (mM) × volume of input DNA (μl)) ÷ total reaction volume (50 μl).

3. After incubating the PCR tube at 65°C for 30 min in the PCR instrument (step 4 of **08-1/Fragmentation, End Preparation & dA-tailing**), a decrease in transparency of the reaction mixture is normal and does not affect subsequent ligation reaction. Proceed with the following steps as described in the manual.

06-2/DNA Adapter

The quality and amount of adapters directly affect the preparation efficiency and library quality. Excess adapter input may lead to residual adapters or adapter dimers, while insufficient adapter input may affect ligation efficiency and reduce library yield. Recommended adapter concentrations for different input DNA amounts are listed in Table 2.

Table 2. Recommended adapter concentration for 1 ng - 1 μg Input DNA

Input DNA	Concentration of adapter from other sources	Vazyme Adapter: ddH_2O
1 ng	0.1 μM	1:99
10 ng	1 μM	1:9
50 ng	5 μM	1:1
100 ng - 1 μg	10 μM	Undiluted

▲ It is recommended to dilute the adapter according to the concentration provided in the table or based on the dilution ratio of the Vazyme Adapter, ensuring that a fixed volume of 5 μl is used during library preparation to avoid pipetting errors.

▲ Adapter quality directly affects the molar ratio of adapter to input DNA, which in turn impacts ligation efficiency and library yield. Use high-quality adapters and avoid repeated freeze-thaw cycles.

▲ Increasing the use of adapters can improve the library yield to some extent; however, it is important to note that elevated adapter concentrations may increase adapter residues in the library, resulting in wasted sequencing data.

▲ For complete removal of adapters, DR Flowsizer Buffer (Vazyme #N001) is recommended.

06-3/Adapter Ligation Product Purification

1. Excess adapters must be removed prior to library amplification (for PCR-amplified libraries) or sequencing (for PCR-free libraries). A purification ratio of 0.6 × (60 μl beads per 100 μl product) is recommended for most applications. Reducing the bead volume can increase the insert size by lowering the proportion of small DNA fragments; however, this adjustment primarily shifts the main library peak. For precise control of library size distribution, perform size selection after purification.

2. If library size selection is to be performed, the recommended elution volume of 105 μl is recommended; otherwise, the recommended elution volume is 25 μl.

3. If the data indicate that the purified products are heavily contaminated with adapters or adapter dimers, an additional bead purification step can be performed. Adjust the volume of the initial purified product to 50 μl with ddH_2O, then perform a second purification using 50 μl of beads (1 ×). This process significantly reduces residual adapter dimers, particularly during the preparation of PCR-free libraries. It may also be necessary to reduce the amount of adapter used to eliminate adapter dimer residues.

06-4/Magnetic Beads

1. It is recommended to use VAHTS DNA Clean Beads (Vazyme #N411) for purification.
   ▲ The purification conditions may need adjustment if beads from other sources are used.

2. General precautions for the use of magnetic beads:
   a. The amount of beads used is indicated by the multiplier "×", which represents the proportion of bead volume relative to the original sample volume. For example, if the original sample volume is 100 μl, using 1 × beads for purification means adding 1 × 100 μl = 100 μl of beads. A 0.6 ×/0.2 × size selection means 0.6 × 100 μl = 60 μl of beads are used in the first round, followed by 0.2 × 100 μl = 20 μl in the second round.
   b. The amount of magnetic beads used directly affects the minimum size of DNA fragments that can be efficiently purified. A higher bead-to-sample ratio allows for the recovery of shorter DNA fragments, whereas a lower ratio favors the purification of longer fragments. For example, at a 1 × bead ratio, only DNA fragments longer than 250 bp can be efficiently purified, with shorter fragments largely lost during purification. When the ratio is increased to 1.8 ×, fragments as short as 150 bp can be effectively recovered.
   c. Equilibrate the beads at room temperature for 30 min before use, or the yield and selection efficiency may be affected.
   d. Mix the beads well by vortexing or pipetting before use.
   e. Mix the sample well with the beads, and place the tube on the magnetic rack for separation. Leave about 2 - 3 μl of the supernatant to avoid disturbing the beads, otherwise the yield, selection efficiency, and the subsequent enzyme reaction may be affected. If disturbance occurs, remix the beads and place the tube on the magnetic rack for another separation. Due to the different performances of the magnetic rack, the default separation time may need to be extended for complete separation.
   f. Rinse the beads with freshly prepared 80% ethanol. Keep the EP tube on the magnetic rack during the rinse. Do not disturb the beads.

g. Air-dry the beads at room temperature before elution. Insufficient drying may leave residual absolute ethanol, which can interfere with the subsequent reactions, while over-drying may cause the beads to crack, reducing recovery efficiency. The beads are sufficiently air-dried when left at room temperature for 5 - 10 min. Do not heat the beads (e.g., drying at 37°C in an oven).

h. Eluent (10 mM Tris-HCl, pH 8.0 - 8.5) is generally recommended to ensure stable product storage. However, if the library will undergo targeted capture, ddH_2O is preferred. This facilitates drying and concentration of the library before capture and helps avoid interference with subsequent capture reactions.

06-5/Size Selection

1. If the Input DNA distribution range is wide, size selection is usually required to control the final library size distribution. It is recommended to perform Two Rounds Size Selection or gel extraction.
2. Size selection can be carried out after Adapter Ligation or Library Amplification. The size selection steps are not included in the standard experimental protocol. Refer to **Appendix: Two Rounds Size Selection** for more information.
3. Size selection often results in substantial DNA loss. Sometimes, a trade-off must be made between achieving a defined library size distribution (with size selection) and preserving library complexity (without size selection). When the Input DNA amount is low, size selection should be performed only once, as two rounds or above can significantly reduce library complexity and yield.
4. Over-amplification typically results in a trailing band or a tailing peak in the high-molecular-weight region. These products are primarily caused by non-complementary strand cross-annealing (see **06-6/About Library Amplification**). It is recommended to adjust the number of amplification cycles to avoid over-amplification, while size selection is not recommended for eliminating the trailing band or tailing peak.
5. The high concentration of PEG in Rapid Ligation Buffer V4 significantly impacts Two Rounds Size Selection and gel extraction. If size selection is performed after adapter ligation, it is recommended to purify the ligation products first, followed by size selection of the purified products using the bead volumes specified in **Table 7**. If size selection is performed directly without prior purification, refer to the recommended bead volumes in **Table 9**. Please note that size selection efficiency is highly influenced by factors such as the total reaction volume and the volume of beads added. If the resulting fragment size distribution is not optimal, minor adjustments may be made based on the observed results. If size selection is performed after library amplification, the purification step may be replaced with Two Rounds Size Selection, using bead volumes as recommended in **Table 7**.

06-6/Library Amplification

1. In the later cycles of PCR, primers are typically depleted before dNTPs. At this stage, excessive cycling can lead to nonspecific annealing following denaturation of the amplification products, resulting in cross-annealing between non-complementary strands. These products migrate slowly during electrophoresis-based detection and appear as diffuse bands in the high molecular weight region. They are composed of single-stranded libraries of correct length and, after denaturation, can hybridize normally to the flow cell and be sequenced. Therefore, their presence has no significant impact on sequencing performance. However, the presence of such products has a critical impact on the choice of library quantification method. Since these products are not fully double-stranded, quantification using fluorescence dyes that specifically bind to double-stranded DNA (such as Equalbit 1 × dsDNA HS Assay Kit, Vazyme #EQ121) may underestimate the actual library concentration. In contrast, qPCR-based quantification methods (such as VAHTS Library Quantification Kit for Illumina, Vazyme #NQ105 - NQ107), which include a denaturation step during quantification, can still accurately measure the concentration of such over-amplified libraries.

2. Library Amplification requires strict control of the number of amplification cycles. Insufficient cycles may lead to an insufficient library yield, while excessive cycles will lead to various adverse effects such as over-amplification, increased amplification bias, PCR duplicates and chimeric products, and amplification mutations. Table 3 provides the recommended number of amplification cycles to obtain a library of 100 ng or 1 μg when 1 ng - 1 μg of high-quality Input DNA is used.

Table 3. Recommended amplification cycles for 1 ng - 1 μg Input DNA

	Number of cycles required to generate	
Input DNA	100 ng	1 μg
1 ng	11 -13	15 - 17
10 ng	6 - 8	9 - 11
50 ng	2 - 4	6 - 8
100 ng	0 - 2	5 - 7
200 ng	0 - 2	4 - 6
500 ng	/	3 - 4
1 μg	/	2 - 3

▲ Table 3 shows the number of cycles used for library preparation with high-quality human 293 gDNA fragmented for 10 min at 37°C. When DNA quality is poor, the number of cycles must be adjusted to obtain sufficient library yield.

▲ If size selection is performed after adapter ligation, a higher number of amplification cycles should be used; otherwise, a lower number of cycles is sufficient.

3. When performing adapter ligation with full-length adapters (VAHTS Maxi Unique Dual Index DNA Adapters Set 1 - Set 4 for Illumina,Vazyme #N34201 - N34204), and the library yield is sufficient, the library amplification step can be omitted to obtain a PCR-free library.
4. When performing adapter ligation with stubby adapters, at least 2 additional PCR cycles are required to complete the adapter sequences at the ends of the library.

06-7/Library Quality Control

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

1. Library size distribution analysis:
   a. Library size distribution can be assessed using electrophoresis-based instruments, such as the LabChip series, Agilent Technologies 2100 Bioanalyzer, and Qsep.
   b. Adapters designed for both Illumina and MGI platforms contain single-stranded forked regions at their ends. Consequently, PCR-free libraries without amplification retain these single-stranded forked ends after adapter ligation. During library size distribution analysis, these single-stranded structures can slow down library migration, resulting in apparently larger fragment sizes, broader size distributions, and abnormal peak shapes. To accurately assess library size distribution, a small portion of the PCR-free library can be subjected to limited PCR amplification. The amplified products should then be purified and analyzed to reflect the true size distribution of the original PCR-free library.
2. Library concentration analysis:

   Two methods are generally used for library quantification: (1) methods based on double-stranded DNA fluorescent dyes, such as the Equalbit 1 × dsDNA HS Assay Kit (Vazyme #EQ121), Qubit, or PicoGreen; and (2) absolute quantification by qPCR, such as the VAHTS Library Quantification Kit for Illumina (Vazyme #NQ105 - NQ107). Although the first method is simpler to perform, the second method is recommended for library quantification for the reasons described below:

   a. When full-length adapters are used and adapter ligation is complete, the products from any subsequent step can be quantified using the qPCR-based absolute quantification. This allows monitoring of the efficiency of adapter ligation, magnetic bead purification/size selection, and library amplification, supporting workflow optimization and analysis of library preparation issues.
   b. PCR-free libraries are prepared without library amplification, resulting in a mixture of molecules, including fragments with adapters ligated to both ends, fragments with a single adapter ligated, and fragments lacking adapters altogether. Fluorescent dye-based quantification can not distinguish these molecules effectively. In contrast, qPCR-based absolute quantification specifically measures only molecules with adapters on both ends (i.e., sequenceable libraries), eliminating interference from non-sequenceable products with single-ended or no adapter ligation. Therefore, PCR-free libraries must be quantified using qPCR-based absolute quantification methods.

c. Over-amplified libraries contain a large proportion of incomplete double-stranded DNA structures and therefore can not be accurately quantified using dsDNA fluorescent dye-based methods such as Qubit and PicoGreen, while qPCR-based absolute quantification remains unaffected.

06-8/Other Notes

1. The size and distribution range of DNA fragments are determined by a time-dependent enzymatic reaction, thus the fragmentation reaction should be carried out on ice.
2. During transportation on dry ice, both buffer and enzyme remain frozen. Thaw all the components at room temperature before use. Then mix well and centrifuge briefly before putting them on ice.
3. To avoid cross-contamination of samples, it is recommended to use tips with a filter and replace them between samples.
4. It is recommended to use a PCR instrument with a heated lid for the reaction at each step. Preheat the PCR instrument to the appropriate reaction temperature before use.
5. PCR products are highly susceptible to aerosol contamination caused by improper handling, which can affect the accuracy of the experiment results. Therefore, PCR setup should be performed in an area physically separated from PCR product purification and analysis. It is also recommended to use dedicated pipettes, and regularly clean each laboratory area using RNase, RNA and DNA remover (Vazyme #R504) to help maintain a contamination-free environment.

07/Workflow

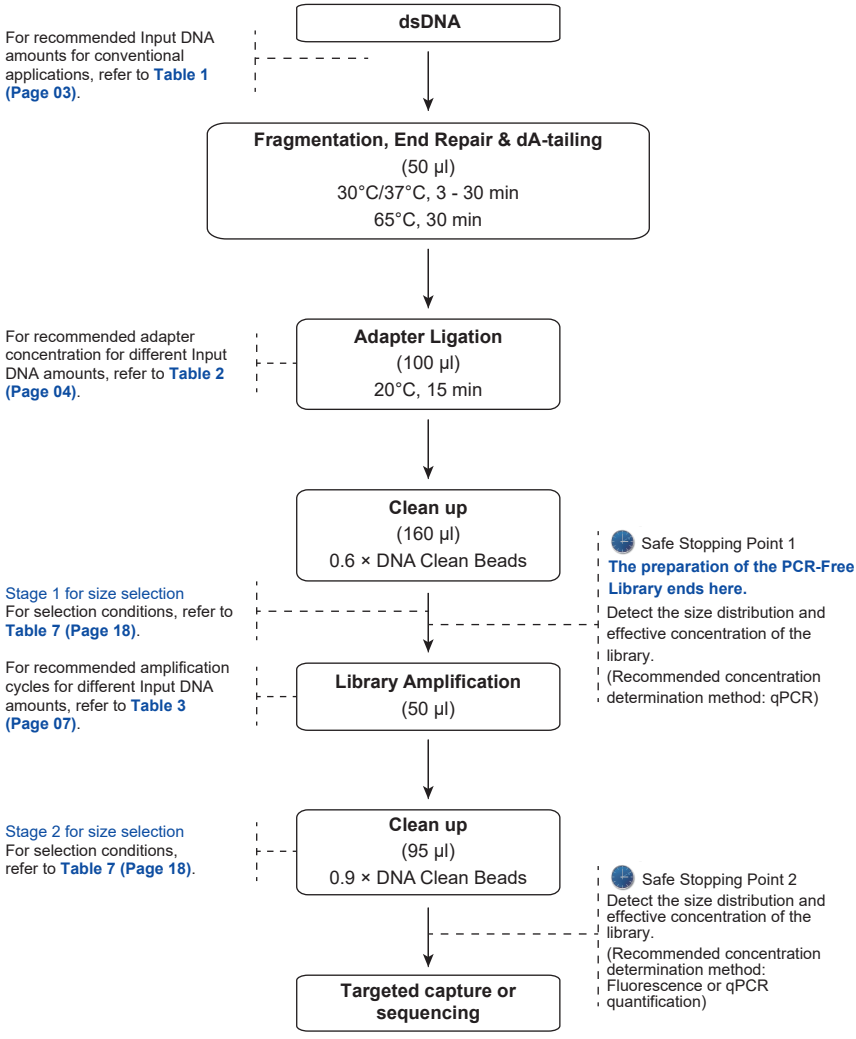


Fig 1. Workflow of VAHTS Universal Plus DNA Library Prep Kit V4

08/Experiment Process

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

This step is performed to fragment the Input DNA while simultaneously repairing the fragmented ends, phosphorylating the 5' ends, and adding a dA tail to the 3' ends.

1. Thaw the FEA Buffer V4 and FEA Enzyme Mix V4. Mix well, centrifuge briefly to collect the solution at the bottom of the tube, and put them on ice before use. All of the following steps should be performed on ice.
2. Prepare the reaction solution in a sterile PCR tube as follows:

Components	Volume
Input DNA	X μl
FEA Buffer V4	5 μl
ddH_2O	To 40 μl

3. Add 10 μl of FEA Enzyme Mix V4 to each sample and mix well by pipetting or vortexing. Centrifuge to collect the reaction solution to the tube bottom and **place it in the PCR instrument immediately for reaction**.

▲ Fragmentation is an enzyme-based reaction, and the resulting fragment size depends on the reaction time. It is recommended to add FEA Enzyme Mix V4 to the reaction solution last. Mix well immediately, and proceed with the subsequent step.

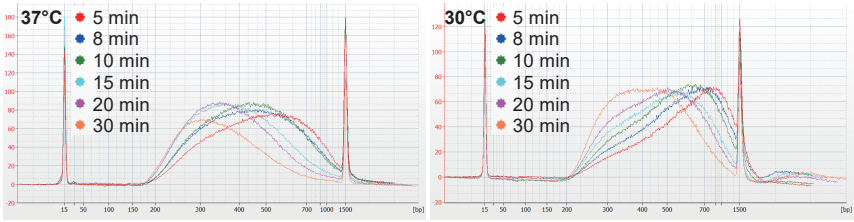
4. Place the PCR tube into the PCR instrument and perform the following program:

Temperature	Time
Heated lid 105°C	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 target fragment size:

Expected insert size	Fragmentation temperature/time	Fragmentation temperature/time
100 - 200 bp	37°C 20 - 30 min	30°C ≥30 min
200 - 300 bp	37°C 10 - 20 min	30°C 20 - 30 min
300 - 400 bp	37°C 5 - 10 min	30°C 10 - 20 min
>500 bp	37°C 3 - 5 min	30°C 5 - 10 min

▲ The recommended times above are for reference only, as fragment size is determined by the enzyme amount and actual reaction time. The following data were generated using 100 ng of high-quality human gDNA dissolved in ddH_2O as input, fragmented at 30°C or 37°C for 5 - 30 min, with #N342 UDI adapters and 5 cycles of PCR amplification.



08-2/Adapter Ligation

Adapter is added to the products from the previous step.

1. Dilute the adapters to the appropriate concentration according to the table below.

Table 4. Recommended adapter concentration for 1 ng - 1 μg Input DNA

Input DNA	Concentration of Adapter from other sources	Vazyme Adapter: ddH_2O
1 ng	0.1 μM	1:99
10 ng	1.0 μM	1:9
50 ng	5 μM	1:1
100 ng - 1 μg	10 μM	Undiluted

2. Thaw the Rapid Ligation Buffer V4 and Rapid DNA Ligase V4. Mix well, centrifuge briefly to collect the solution at the bottom of the tube, and put them on ice before use.

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

Components	Volume
Product from the previous step	50 μl
Rapid Ligation Buffer V4	25 μl
Rapid DNA Ligase V4	5 μl
ddH_2O	15 μl
DNA Adapter (pre-diluted as indicated above)*	5 μl

DNA adapter usage varies depending on the adapter type. With full-length adapters, different samples are distinguished during the ligation step using different DNA adapters. With stubby adapters, all samples are ligated with universal adapters, and sample identification is achieved during the final library amplification using different index primers. The corresponding DNA adapter components in the adapter kits are listed in the table below.

Sequencer	Product Form	Adapter Cat. No.	Adapter Type	DNA Adapter Components
Illumina	Tube-based	N805 - N808	Full-length	DNA Adapter for Ilumina 96-XX
		N34201 - N34208		VAHTS Maxi Unique Dual Index DNA Adapter for Illumina UDIAXXX
		N321 - N322	Stubby	VAHTS Adapter-S for Illumina
		N34405 - N34408		VAHTS Universal Adapter for Illumina
		N351 - N354		VAHTS Dual UMI Adapters for Illumina
	Plate-based	N805-PB2	Full-length	DNA Adapter 96-XX
		N34201-P2 - N34204-P2 NB34205 - NB34208		VAHTS Maxi Unique Dual Index DNA Adapter for Illumina UDIAXXX
		NB34405 - NB34412	Stubby	VAHTS Universal Adapter for Illumina
MGI	Tube-based	NM108 NM10901 - NM10904	Full-length	DNA Adapter for MGI 96-XX
		NM35101 - NM35108	Stubby	VAHTS Dual UMI Adapters for MGI UDB
		NM34401 - NM34404		VAHTS Universal Adapter for MGI
	Plate-based	NMB108 NMB108-C2 NMB109-PB2 NMB109-CB1	Full-length	DNA Adapter 96-XX
		NMB34401 - NMB34408	Stubby	VAHTS Universal Adapter for MGI

4. Mix well and centrifuge briefly.

5. Place the PCR tube into the PCR instrument and perform the following program:

Temperature	Time
Heated lid 105°C	On
20°C	15 min
4°C	Hold

6. Purify the product using VAHTS DNA Clean Beads as follows:
   a. Equilibrate VAHTS DNA Clean Beads to room temperature, and mix well by vortexing.
   b. Add 60 µl of VAHTS DNA Clean Beads to 100 µl of Adapter Ligation product. Mix well by pipetting or vortexing.
   c. Incubate for 5 min at room temperature.
   d. Briefly centrifuge the PCR tube and place it on the magnetic rack. Carefully discard the supernatant after the solution is clear (~ 5 min). It is acceptable to leave 2 - 3 µl of the supernatant to avoid aspirating the beads.
   e. Keep the PCR tube on the magnetic rack, and add 200 µl of freshly prepared 80% ethanol to rinse the beads. Incubate for 30 sec at room temperature, then carefully discard the supernatant.

f. Repeat step e (wash twice in total).

g. Keep the PCR tube on the magnetic rack, and air-dry the beads for 2 - 5 min.

▲ Elute the beads when the surface changes from shiny brown to matte brown. Avoid over-drying as it can reduce the DNA recovery efficiency.

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

▲ If the purified products are not subjected to Two Rounds Size Selection, elute with 25 µl of 10 mM Tris-HCl (pH 8.0 - 8.5) or ddH_2O. Mix well by pipetting or vortexing and incubate at room temperature for 2 min. Briefly centrifuge the PCR tube and place it on the magnetic rack. Carefully transfer 20 µl of supernatant to a new EP tube after the solution is clear (~ 5 min). Do not disturb the magnetic beads.

▲ If the purified products are subjected to Two Rounds Size Selection: add 105 µl of eluent (10 mM Tris-HCl, pH 8.0 - 8.5) or ddH_2O. Mix well by pipetting or vortexing and incubate at room temperature for 5 min. Briefly centrifuge the PCR tube and place it on the magnetic rack. Carefully transfer 100 µl of supernatant to a new EP tube after the solution is clear (~ 5 min). Do not disturb the magnetic beads. Refer to Table 7 for size selection conditions.

Store the samples at -20°C and avoid repeated freeze-thaw cycles.

08-3/Library Amplification

This step involves PCR amplification of the Adapter Ligation products following purification or size selection. This step is optional and should be performed based on the amount of input DNA, the completeness of the adapters, and the downstream application. PCR amplification is required for libraries with stubby adapters. For libraries with full-length adapters, amplification is recommended when the input DNA is less than 50 ng.

1. Thaw the PCR Primer Mix for Illumina and VAHTS Ultra Amplification Mix. Mix well, and centrifuge briefly. Prepare the reaction solution in a sterile PCR tube as follows:

Illumina-compatible libraries:

Components	Volume	
Purified or selected Adapter Ligation products	20 µl	
PCR Primer Mix for Illumina	5 µl	■
VAHTS Ultra Amplification Mix	25 µl	■
Total	50 µl	

▲ The amplification primers and their volumes should be selected based on the adapter type. Please refer to the table below.

Product Form	Adapter Cat. No.	Adapter Type	PCR Adapter Primer Components	Volume	Source Kit
Tube-based	N805 - N808	Full-length	PCR Primer Mix for Illumina	5 μl	Library prep kit
	N34201 - N34208		PCR Primer Mix for Illumina	5 μl	
	N321 - N322	Stubby	VAHTS i5 PCR Primers (DM5XX)	2.5 μl	Adapter kit
			VAHTS i7 PCR Primers (DM7XX)	2.5 μl	
	N34405 - N34408		VAHTS Unique Dual Index Primer for Illumina (UDI XXX)	5 μl	
	N351 - N354		VAHTS UDI Primer for Illumina (UDI XXX)	5 μl	
Plate-based	N805-PB2	Full-length	PCR Primer Mix for Illumina	5 μl	Library prep kit
	N34201-P2 - N34204-P2		PCR Primer Mix for Illumina	5 μl	
	NB34205 - NB34208		PCR Primer Mix for Illumina	5 μl	
	NB34405 - NB344012	Stubby	VAHTS Unique Dual Index Primer for Illumina (UDI XXX)	5 μl	Adapter kit

▲ If stubby adapters or adapters from other platforms are used, amplification primers must be replaced accordingly.

MGI-compatible libraries:

Components	Volume
Purified or selected Adapter Ligation products	20 μl
PCR Primer Mix for MGI	5 μl
VAHTS Ultra Amplification Mix	25 μl
Total	50 μl

▲ The amplification primers and their volumes should be selected based on the adapter type. Please follow the instructions listed in the table below.

Product Form	Adapter Cat. No.	Adapter Type	PCR Adapter Primer Components	Source Kit
Tube-based	NM108	Full-length	PCR Primer Mix for MGI	Library prep kit
	NM10901 - NM10904		PCR Primer Mix for MGI	
	NM35101 - NM35108	Stubby	VAHTS Unique Dual Barcode Primer for MGI (UDB XXX)	Adapter kit
	NM34401 - NM34404		VAHTS Unique Dual Barcode Primer for MGI (UDB XXX)	
Plate-based	NMB108	Full-length	PCR Primer Mix for MGI	Library prep kit
	NMB108-C2		PCR Primer Mix for MGI	
	NMB109-PB2		PCR Primer Mix for MGI	
	NMB109-CB1			
	NMB34401 - NMB34408	Stubby	VAHTS Unique Dual Barcode Primer for MGI (UDB XXX)	Adapter kit

▲ If stubby adapters or adapters from other platforms are used, amplification primers must be replaced accordingly.

2. Mix well and centrifuge briefly.

3. Place the PCR tube into the PCR instrument and perform the following program:

Temperature	Time	Cycles
98°C	45 sec	1
98°C 60°C 72°C	15 sec 30 sec 30 sec	For the number of cycles, see Table 5
72°C	1 min	
4°C	Hold	1

Table 5. Recommended amplification cycles for 1 ng - 1 μg Input DNA

	Number of cycles required to generate	
Input DNA	100 ng	1 μg
1 ng	11 - 13	15 - 17
10 ng	6 - 8	9 - 11
50 ng	2 - 4	6 - 8
100 ng	0 - 2	5 - 7
200 ng	0 - 2	4 - 6
500 ng	/	3 - 4
1 μg	/	2 - 3

▲ The table above shows the number of cycles used for library preparation with high-quality human 293 gDNA fragmented for 10 min at 37°C. When DNA quality is poor, the number of cycles must be adjusted to obtain sufficient library yield.

▲ If size selection is performed during library preparation, a higher number of amplification cycles should be used; otherwise, a lower number of cycles is sufficient.

4. For size selection, refer to **Appendix: Two Rounds Size Selection**. Use VAHTS DNA Clean Beads to purify the reaction products if size selection is not required:
   a. Equilibrate VAHTS DNA Clean Beads to room temperature, and mix well by pipetting or vortexing.
   b. Add 45 μl of VAHTS DNA Clean Beads to 50 μl of Library Amplification solution. Mix well by pipetting or vortexing.
   c. Incubate for 5 min at room temperature.
   d. Briefly centrifuge the PCR tube and place it on the magnetic rack. Carefully discard the supernatant after the solution is clear (~ 5 min). It is acceptable to leave 2 - 3 μl of the supernatant to avoid aspirating the beads.
   e. Keep the PCR tube on the magnetic rack, and add 200 μl of freshly prepared 80% ethanol to rinse the beads. Incubate for 30 sec at room temperature, then carefully discard the supernatant.
   f. Repeat step e (wash twice in total).
   g. Keep the PCR tube on the magnetic rack, and air-dry the beads for 2 - 5 min.
   h. Remove the PCR tube from the magnetic rack for elution.

▲ Add 25 μl of eluent (10 mM Tris-HCl, pH 8.0 - 8.5) or ddH_2O for elution. Please note that if targeted capture is to be performed in subsequent steps, ddH_2O must be used for elution. Mix well by pipetting or vortexing and incubate at room temperature for 5 min. Briefly centrifuge the PCR tube and place it on the magnetic rack. Carefully transfer 20 μl of supernatant to a new EP tube after the solution is clear (~ 5 min). Do not disturb the magnetic beads.

Store the samples at -20°C and avoid repeated freeze-thaw cycles.

08-4/Library Quality Control

Refer to 06-7/Library Quality Control.

Appendix: Two Rounds Size Selection

1. To meet the needs of different applications, Two Rounds Size Selection is often required during library preparation to control the distribution range of the library insert size. See **Table 6** for information on choosing when to perform selection, as well as the advantages and disadvantages of performing size selection at different stages. **It must be guaranteed that the selection process is performed only once. Two or more selections can lead to a significant reduction in library complexity and yield!**

Table 6. Stage options for size selection

Size Selection Stage	Applications	Advantages	Disadvantages	Examples of applicable samples
After adapter ligation	Input DNA distribution is suitable and adequate[a]	The final library exhibits a relatively sharp peak profile	Library distribution cannot be accurately evaluated	Proper fragmentation of genomic DNA or FFPE DNA with wider distribution range
After library amplification	Low Input DNA amount[b]	Reducing the loss of Input DNA during library preparation and increasing the library complexity	Library size distribution range is slightly broad	/
No size selection during library preparation	Input DNA distribution range meets the library preparation requirements; low Input DNA amount	Reducing the loss of Input DNA during library preparation and increasing the library complexity	Library insert size cannot be controlled	Proper fragmentation of genomic DNA

a. The effectiveness of Two Rounds Size Selection is influenced by the DNA ends. Single-stranded overhangs at the Input DNA ends and non-complementary regions of the adapters may result in a broader size distribution of the selected products.

b. If the Input DNA amount is ≥100 ng, it is recommended to perform the size selection after Adapter Ligation. If the amount is <100 ng or the sample copy number is limited, perform the size selection after Library Amplification.

2. Two Rounds Size Selection is achieved by controlling the amount of beads used for DNA size selection. The basic principle is as follows: in the first round the beads bind to DNA with a larger molecular weight, and this kind of DNA is removed when the beads are discarded; in the second round the beads bind to DNA with a larger molecular weight in the remaining products, and the smaller-sized DNA is removed by discarding the supernatant. Many components in the initial sample interfere with the effectiveness of Two Rounds Size Selection. Therefore, when the stage for size selection is different, the amount of beads used for Two Rounds Size Selection will be different. Select the most appropriate selection parameter according to **Table 7 - 9** based on the expected library insert size and the stage for size selection.

▲ The efficiency of library size selection is directly influenced by the main peak size and the distribution concentration of DNA fragments after shearing. If the size selection result does not meet expectations, the selection conditions can be adjusted accordingly to achieve the desired library profile.

▲ Size selection performance may vary slightly across magnetic bead brands. Please optimize the selection conditions based on experimental results.

▲ The volume ratio between the sample and beads is important for size selection. Please ensure the accuracy of the initial sample volume and pipetting volume.

▲ Fragment size after size selection is influenced by selection conditions and fragmentation time. The insert size can be adjusted accordingly.

Table 7. Recommended size selection conditions for full-length adapter libraries

		Expected insert size (bp)							
Stage and conditions for size selection	Purification rounds	150	200	250	300	350	400	450	500
After adapter ligation (100 µl sample volume)	1st round X (µl)	78	68	65	59	56	53	51	50
	2nd round Y (µl)	20	20	15	15	12	12	10	10
After library amplification (100 µl sample volume)	1st round X (µl)	78	70	63	55	50	46	45	44
	2nd round Y (µl)	20	20	20	20	20	20	20	15

3. If stubby adapters are used, please refer to the following table to choose the volume of beads according to the expected insert size and the stage for size selection.

Table 8. Recommended size selection conditions for stubby adapter libraries

		Expected insert size (bp)							
Stage and conditions for size selection	Purification rounds	150	200	250	300	350	400	450	500
After adapter ligation (100 µl sample volume)	1st round X (µl)	100	90	75	65	60	55	53	50
	2nd round Y (µl)	20	20	20	20	20	20	20	18

4. If size selection is performed directly after adapter ligation without prior purification, refer to the table below for appropriate size-selection conditions.

Table 9. Recommended size selection conditions after adapter ligation

		Expected insert size (bp)		
Stage and conditions for size selection	Purification rounds	150	300	400
No Purification after adapter ligation (100 µl sample volume)	1st round X (µl)	30	20	10
	2nd round Y (µl)	10	10	10

5. Sample pretreatment (IMPORTANT!)
   - ▲ If size selection is performed after purification of the Adapter Ligation products, the sample volume should be 100 µl. If not, the sample should be filled up to 100 µl with ddH_2O.
   - ▲ If size selection is performed after Library Amplification, the sample volume should be 100 µl. If not, the sample should be filled up to 100 µl with ddH_2O.
   - ▲ If the sample volume is not adjusted in advance, the bead amount can be adjusted proportionally to the actual sample volume. However, smaller sample volumes increase pipetting errors, which may affect the accuracy of size selection. Therefore, direct size selection of samples with volumes <50 µl is not recommended.

6. Size selection protocol (refer to **Table 7 - 9** for values of **X** and **Y**)
   a. Equilibrate VAHTS DNA Clean Beads to room temperature, and mix well by vortexing.
   b. Add **X** µl of VAHTS DNA Clean Beads to 100 µl of the solution above. Mix well by vortexing or pipetting.
      - ▲ If the solution is <100 µl, fill up to 100 µl with ddH_2O.
   c. Incubate for 5 min at room temperature.
   d. Briefly centrifuge the PCR tube and place it on the magnetic rack. Carefully **transfer the supernatant to a new PCR tube** after the solution is clear (~ 5 min) and **discard the beads**.
   e. Add **Y** µl of VAHTS DNA Clean Beads to the supernatant. Mix well by vortexing or pipetting.
   f. Incubate for 5 min at room temperature.
   g. Briefly centrifuge the PCR tube and place it on the magnetic rack. Carefully **transfer the supernatant** after the solution is clear (~ 5 min).
   h. Keep the PCR tube on the magnetic rack and add 200 µl of freshly prepared 80% ethanol to rinse the beads. Incubate at room temperature for 30 sec, then carefully **discard the supernatant**.

i. Repeat step h (wash twice in total).

j. Keep the PCR tube on the magnetic rack. Air-dry the beads for 3 - 5 min until there is no ethanol residue.

k. Remove the PCR tube from the magnetic rack for elution.

▲ Add 25 µl of eluent (10 mM Tris-HCl, pH 8.0 - 8.5) or ddH_2O (use ddH_2O if downstream targeted capture is required). Mix well by vortexing or pipetting and incubate at room temperature for 5 min. Briefly centrifuge the tube and place it on the magnetic rack. Carefully transfer 20 µl of the supernatant to a new EP tube after the solution is clear (~ 5 min). Do not disturb the magnetic beads.

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