VAHTS Universal DNA Library Prep Kit for MGI

NDM607

Vazyme

Instruction for Use

Version 23.1

Vazyme

Contents

01/Product Description

VAHTS Universal DNA Library Prep Kit for MGI is a library preparation kit optimized for MGI high-throughput sequencing platform. It can convert 100 pg - 4 μg Input DNA into a specialized MGI library. As an upgraded version, VAHTS Universal DNA Library Prep Kit for MGI makes overall improvements to the end repair module, ligation module and library amplification module. It greatly improves the library conversion rate and library output. This kit is widely applicable to PCR library preparation of multiple sample types. 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	NDM607-01 (24 rxns)	NDM607-02 (96 rxns)
End Prep Mix 4	360 μl	4 × 360 μl
Rapid Ligation Buffer 2	600 μl	4 × 600 μl
Rapid DNA Ligase	120 μl	480 μl
VAHTS HiFi Amplification Mix	600 μl	4 × 600 μl
PCR Primer Mix for MGI	120 μl	480 μl
Control DNA (264 bp, 50 ng/μl)	10 μl	10 μl

03/Storage

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

04/Applications

This kit is applicable for preparing a dedicated library for the MGI high-throughput sequencing platform. It is compatible with multiple sample types: genomic DNA, free DNA (cfDNA and ctDNA), FFPE DNA, chromatin immunoprecipitation DNA (ChIP DNA), amplicons, etc. For small genome, free DNA (cfDNA and ctDNA), ChIP DNA, amplicons and other samples with low complexity, the amount of Input DNA can be as low as 100 pg. Recommended for:

◇ Whole genome sequencing

◇ Amplicons sequencing

◇ ChIP sequencing

◇ Metagenome sequencing

◇ Methylation sequencing (collocation with EpiArt DNA Enzymatic Methylation Kit, Vazyme #EM301; EpiArt DNA Methylation Bisulfite Kit, Vazyme #EM103)

05/Self-prepared Materials

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

DNA quality control: Agilent Technologies 2100 Bioanalyzer or other equivalent instruments;

DNA Adapter:

VAHTS DNA Adapters Set 8 for MGI (Vazyme #NM108);

#NM108 contains 96 types of 10 bp single-indexed adapters.

or VAHTS Dual UMI UDB Adapters Set 1 - Set 8 for MGI (Vazyme #NM35101 - NM35108).

#NM35101 - NM35108 contains dual-indexed UMI short adapters and 192 types of 10bp dual-indexed adapter primers.

Other materials: absolute ethanol, ddH_2O, 0.1 × TE, eluent (10 mM Tris-HCl, pH 8.0 - 8.5), low adsorption EP tube, PCR tube, magnetic rack, PCR instrument, etc.

06/Notes

For research use only. Not for use in diagnostic procedures.

Due to a number of factors such as the sample type, plan, equipment and operations, it may be necessary to adjust the parameters of library preparation according to the actual situation. In order to obtain a high-quality library, it is important to read the following precautions carefully. In case of any queries during the process, please contact Vazyme at: info.biotech@vazyme.com.

06-1/Input DNA & Fragmentation

◇ Starting material: 100 pg - 4 μg input DNA. High-quality input DNA (A260/A280 = 1.8 - 2.0) should be used. Table 1 lists the recommended input DNA amounts for conventional applications.

Table 1. Recommended input DNA amounts for conventional applications

Application	Sample type	Recommended Input DNA amount
Whole genome sequencing	Complex genome	50 ng - 4 μg
Target capture sequencing	Complex genome	10 ng - 4 μg
Whole genome/target capture sequencing	FFPE DNA	≥50 ng
Whole genome/target capture sequencing	cfDNA/ctDNA	≥100 pg
Whole genome sequencing	Microbial genome	1 ng - 1 μg
ChIP sequencing	ChIP DNA	≥100 pg
Target capture sequencing	Amplicons	≥100 pg

▲The above table shows the recommended amount of input DNA when using high-quality DNA. The amount should be increased as appropriate if the quality of Input DNA is low.

◇ Input DNA refers specifically to the DNA used in End Preparation step. If size selection or purification is performed after fragmentation, the concentration needs to be re-determined. The amount of DNA prior to fragmentation cannot be directly used as the amount of Input DNA. Otherwise, the library yield may be low due to insufficient amplification cycles.

◇ If high concentrations of metal ion chelating agents or other salts are introduced during the preparation process of input DNA, the efficiency of the End Preparation step may

be affected. When using the mechanical method for fragmentation, if the product is directly input for library preparation without purification or size selection, please dilute the DNA in 0.1 × TE for fragmentation, do not perform in ddH_2O. When using the restriction enzyme digestion method for fragmentation, if the product is directly input for library preparation without purification or size selection, please confirm that the Stop Buffer does not contain excessive metal ion chelating agents. If the above conditions are not met, fragmentation products can be preformed purification or size selection first and then dissolved in 0.1 × TE or ddH_2O (≤50 μl) before library preparation.

06-2/Adapter

◇ Vazyme offers two sets of indexes adapters for the MGI sequencing platform:
VAHTS DNA Adapters Set 8 for MGI (Vazyme #NM108);
VAHTS Dual UMI UDB Adapters Set 1 - Set 8 for MGI (Vazyme #NM35101 - NM35108).

◇ The quality and amount of adapters directly affect the preparation efficiency and library quality. The recommended molar ratio of Adapter : Input DNA is between 10:1 - 200:1. If the adapter input is too high, this may lead to residual adapter or adapter dimer. If the adapter input is too low, this may affect ligation efficiency and reduce library yield. Table 2 lists recommended adapter concentration for different input DNA amounts.

Table 2. Recommended adapter concentration for 100 pg - 4 μg input DNA

Input DNA	Adapter: Input DNA molar ratio	Concentration of adapter from other source	Vazyme adapter Pre-dilution factor
500 ng - 4 μg	10:1 - 20:1	10 μM	Undiluted
100 - 500 ng	20:1 - 100:1	10 μM	Undiluted
25 - 100 ng	40:1 - 200:1	5 μM	1:2
5 - 25 ng	50:1 - 200:1	1 μM	1:10
100 pg - 5 ng	60:1 - 3,000:1	0.2 μM	1:30 - 1:100

▲ The mole number of input DNA can be roughly calculated according to the following formula:
input DNA mole number (pmol) ≈ input DNA mass (ng)/[0.66 × average length of input DNA (bp)]

▲ It is recommended to use 0.1 × TE to dilute adapters according to the above table. This ensures that the adapters are used with a fixed volume (5 μl) during the library preparation process, avoiding incorrect loading volume.

▲ The quality of the adapters directly affects the molar ratio of the adapter and the input DNA, which in turn affects ligation efficiency and the library yield. High-quality adapters should be used. Use 0.1 × TE to dilute and store adapters. Avoid repeated freezing and thawing.

▲ Increasing the input amount of adapters can improve the library yield to some extent, especially when the input DNA is ≤25 ng. When it is necessary to optimize the efficiency of library preparation, you can try a higher input amount of adapters under the conditions recommended in the table above (recommended range of 2 - 10 folds). If the concentration of the adapter's original solution is limited and the input amount cannot be increased under a fixed input volume, you can try increasing the input volume of adapters. For example: the default input amount of adapters is 5 μl. When the input DNA is 500 ng - 4 μg, the input volume of adapters can be increased to 10 μl to improve the library yield by 5% - 15%.

06-3/Adapter Ligation Product Purification

◇ The Adapter Ligation product needs to remove residual adapters before subsequent library amplification (PCR library). The default purification condition of 0.6 × (100 μl of products, 60 μl of beads) is suitable for most cases. To obtain libraries with larger insert size, the amount of beads can be reduced to lower the content of small DNA fragments. However, this adjustment only changes the position of the main peak of the library. If you need to accurately control the library distribution, you can carry out size selection after this purification process.

◇ If library size selection is being performed later, the recommended elution volume is 105 μl. Otherwise, the recommended elution volume is 22.5 μl.

◇ If the data shows that the purification products are heavily contaminated with adapter or adapter dimer, it can be further purified with beads: the first purified product volume is made up to 50 μl with ddH_2O and the second purification is done with 50 μl beads (1 ×). This significantly reduces the residue level of the adapter or adapter dimer. Sometimes it may be necessary to reduce Adapter usage to completely eliminate Adapter or Adapter Dimer residues.

06-4/Magnetic Beads

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

◇ Precautions for magnetic beads operation:

▲ The amount of beads used is indicated by the usual multiplier "×", which indicates the proportion of the amount of beads used in relation to the original sample volume. For example, if the original volume of the sample is 100 μl, 1 × beads used for purification is 1 × 100 μl = 100 μl. 0.6 ×/0.2 × size selection means the beads amount is 0.6 × 100 μl = 60 μl in the first round and 0.2 × 100 μl = 20 μl in the second round.

▲ The amount of beads used directly affects the lower limit of DNA length that can be purified. The higher the multiplier is, the shorter the purified DNA length is, and vice versa. For example: 1 × magnetic beads can only be used to efficiently purify DNA longer than 250 bp, shorter DNA will be lost in large quantities during purification and 150 bp DNA can also be efficiently purified after the multiplier has been increased to 1.8 ×.

▲ Beads should be balanced to room temperature (30 min at room temperature) before use, otherwise it could result in poor yield or poor selection effect.

▲ Thoroughly vortex or mix the beads before use.

▲ When a sample is fully mixed with the beads and placed on the magnetic rack to separate, the solution must be completely clear before the supernatant is aspirated. About 2 - 3 μl supernatant should be left. If the beads were accidentally removed, reduced yields or poor selection effect may be caused, and this may even affect subsequent enzyme reactions. To solve this problem, the beads can be fully mixed again and placed back on the magnetic rack for further separation. Due to the different performance of the magnetic rack, the default separation time may sometimes need to be extended to completely separate the beads and liquid.

▲ Rinse the beads by freshly prepared 80% ethanol. The EP tube should always be placed on the magnetic rack during the rinse process. Make sure not to disturb the beads.

▲ The beads should be allowed to dry at room temperature before elution. Insufficient drying can lead to absolute ethanol residue which affects the subsequent reaction. Excessive drying can cause the beads to crack, which reduces the purification rate. The beads will usually dry sufficiently when left at room temperature for 5 - 10 min. Do not heat to dry the beads (e.g. drying out at 37°C in an oven).

▲ Generally, it is recommended to use an eluent (10 mM Tris-HCl, pH 8.0 - 8.5) to perform product elution, as this is better for the stable storage of the product. If targeted capture of the library is required in the future, in order to facilitate the drying and concentration of the library before capture and to prevent any impact on the subsequent capture reaction, the product elution should be carried out with ddH_2O.

▲ The eluent can remain stable for one week at 4°C. Store at -20°C for long-term storage. Avoid repeated freezing and thawing.

06-5/Size Selection

◇ If the input DNA distribution range is wide, size selection is usually required to control the final library size distribution. It is recommended to use two rounds of beads selection, or selection can also be performed using gel extraction.

◇ Size selection can be carried out before End Preparation, after Adapter Ligation, or after Library Amplification. The size selection steps are not included in the standard experiment protocol. For more information, please refer to Appendix I: Two Rounds of Size Selection.

◇ The amount of DNA loss involved in size selection is about 60% - 95%. Sometimes it is necessary to choose between the library size distribution (with size selection) and the library complexity (no size selection). When the amount of input DNA is low, it must be guaranteed that the size selection stage occurs only once. Two or more size selections can lead to a significant reduction in library complexity and yield.

◇ Over-amplification typically results in trailing band or tail peak appeared at the high molecular weight position. The corresponding products are mostly non-complementary chain cross-annealing products (refer to 06-6/Library Amplification). The recommended solution is to adjust the number of amplification cycles to avoid over-amplification. It is not recommended to resolve trailing band or tail peak by means of size selection.

◇ The high concentration of PEG in Rapid Ligation Buffer 2 has a significant impact on two rounds of size selection and gel extraction. Therefore, if size selection is performed after Adapter Ligation, the Adapter Ligation product purification steps (08-2/Adapter Ligation/Step 6. Purify the reaction products using VAHTS DNA Clean Beads) must not be omitted and the purified products must be eluted in a suitable volume of eluent, followed by two rounds of size selection or gel extraction. If selection must be performed after Adapter Ligation, the selection condition should be explored separately.

◇ If size selection is performed after Library Amplification, the amplification products can be filled up to 100 μl and proceed directly to two rounds of size selection or gel extraction.

06-6/Library Amplification

◇ PCR Primer Mix for MGI is designed to be used for amplification of the MGI high-throughput sequencing platform library with full length adapter. Replace the amplification primers for stubby adapters or other platform libraries, and the recommended final amplification concentration for each primer is 0.25 - 1 μM.

◇ During the late stage of PCR, primers are usually depleted before dNTP. At this point, too many cycles can cause non-specific annealing after the amplification products unwinding, resulting in non-complementary chain cross-annealing products. These products migrate slower and are diffused in higher molecular weight region in electrophoresis-based analysis. They are made up of single-strand libraries that have the correct length, which can be cyclized and sequenced after denaturation. Therefore, its presence or absence has no significant effect on sequencing. However, the existence of these products has a decisive impact on the library's quantitative methods. Because the product is not a complete double stranded structure, when library quantification is performed by using fluorescent dyes (Equalbit 1 × dsDNA HS Assay Kit, Vazyme #EQ121) that recognize double-stranded DNA, the quantification results are lower than the actual values.

◇ The Library Amplification step requires strict control of the number of amplification cycles. An insufficient number of cycles will lead to an insufficient library yield, while an excessive number of cycles will lead to various adverse effects such as over-amplification, increased amplification bias, duplication rate, chimeric products, and amplification mutations. Table 3 specifies the recommended number of amplification cycles to obtain a 100 ng or 1 μg library when using 100 pg - 1 μg of high-quality input DNA.

Table 3. Recommended amplification cycles for 100 pg - 1 μg input DNA

Input DNA (Into End Preparation)	Number of cycles required to generate	
	100 ng	1 μg
100 pg	16 - 18	17 - 20
1 ng	10 - 12	13 - 15
5 ng	8 - 10	11 - 14
10 ng	7 - 9	9 - 12
50 ng	5 - 7	8 - 10
100 ng	3 - 5	6 - 8
250 ng	3*	4 - 7
500 ng	3*	3 - 5
1 μg	3*	3 - 5

* The library yield will be larger than 100 ng. However, due to the MGI platform adapter design, library amplification must be performed before cyclization when using NM108.

▲ The above table shows the number of cycles measured for high-quality input DNA of approximately 200 bp. When DNA quality is poor or the library length is long, the number of cycles must be appropriately increased to obtain sufficient library.

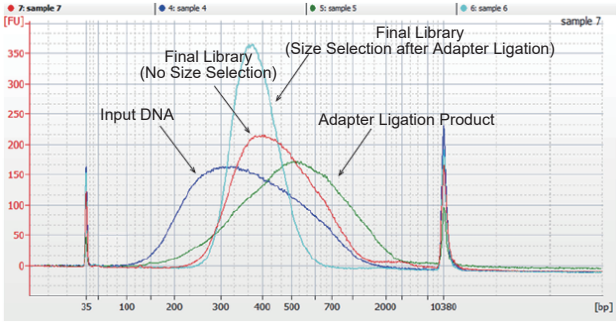
▲ If the size selection is performed during library preparation, Library Amplification should be carried out with higher number of cycles; otherwise, the lower number of cycles is sufficient.

06-7/Library Quality Control

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

◇ Library size distribution analysis:

Library size distribution analysis can be performed using equipment based on electrophoretic separation, such as LabChip GX, GXII, GX Touch (PerkinElmer); Bioanalyzer, Tapestation (Agilent Technologies); Fragment Analyzer (Advanced Analytical) etc. When using VAHTS Universal DNA Library Prep Kit for MGI to prepare the library, the size distribution analysis of each step product is shown in the figure below.



VAHTS Universal DNA Library Prep Kit for MGI
The size distribution analysis of each step products during library preparation

◇ Library concentration analysis:

Common library concentration analysis methods are based on double-strand DNA fluorescent dyes, such as the Equalbit 1 × dsDNA HS Assay Kit (Vazyme #EQ121).

06-8/Other Notes

◇ Thaw all the components at room temperature before use. After thawing, mix thoroughly and centrifuge briefly before putting them on ice.

◇ It is recommended to pipette up and down to mix the reaction liquid. Vigorous oscillation may cause a reduction in the library yield.

◇ To avoid cross-contamination of samples, it is recommended to use tips with a filter and to replace the tips between samples.

◇ It is recommended to use a PCR instrument with heat lid function when carrying out the reaction in each step. Preheat the PCR instrument close to the reaction temperature before use.

◇ PCR products are highly susceptible to aerosol contamination caused by improper handling, which can affect the accuracy of the experiment results. Therefore, we recommend physically isolating the PCR preparation area and the PCR product purification testing area, using equipment such as specialist pipettes, and periodically cleaning each laboratory area (wipe down with 0.5% sodium hypochlorite or 10% bleach) to ensure proper cleanliness of the laboratory environment.

07/Workflow

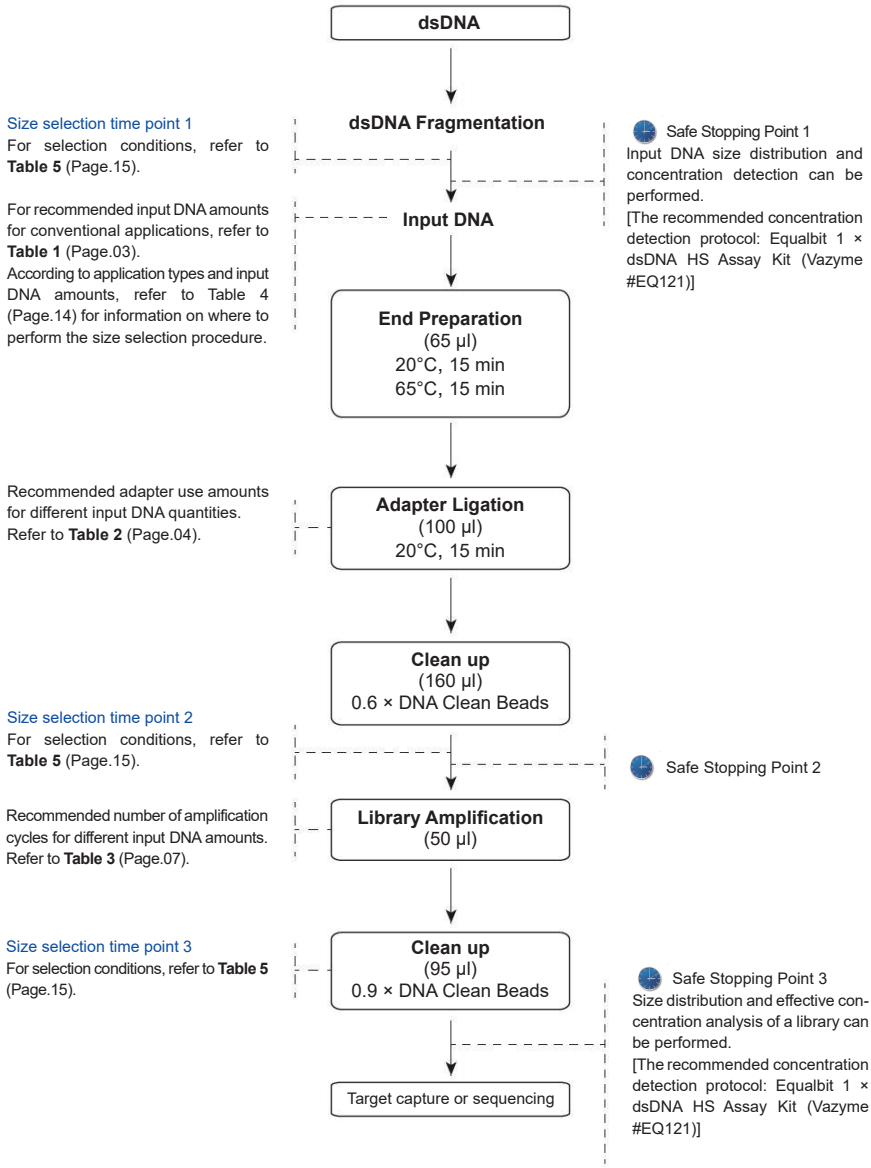


Fig 1. Workflow of VAHTS Universal DNA Library Prep Kit for MGI

08/Experiment Process

08-1/End Preparation

This step flattens the Input DNA end, the 5' end phosphorylation and the 3' end dA tailing.

1. Thaw the End Prep Mix 4 and mix it thoroughly, and prepare the reaction solution in a sterile PCR tube as follows:

Components	Volume
Input DNA	x µl
End Prep Mix 4	15 µl
ddH_2O	To 65 µl

2. Mix well by pipetting up and down (Do not vortex), then centrifuge briefly to the bottom of the tube.
3. Place the PCR tube into the PCR instrument and perform the below reaction:

Temperature	Time
105°C (Heated lid)	On
20°C	15 min
65°C	15 min
4°C	Hold

08-2/Adapter Ligation

This step ligates adapters to the end of the End Preparation products.

1. Dilute the adapters to the appropriate concentration according to **Table 2** (Page.04).
2. Thaw the Rapid Ligation Buffer 2 and mix it upside-down, then place it on ice.
3. Prepare the reaction solution in the sterile PCR tube of the End Preparation step as follows:

Components	Volume
End Preparation Products	65 µl
Rapid Ligation Buffer 2	25 µl
Rapid DNA Ligase	5 µl
DNA Adapter X	5 µl
Total	100 µl

▲ If VAHTS Dual UMI UDB Adapters Set 1 - Set 8 for MGI (Vazyme #NM35101-NM35108) are used, then paired Dual UMI Adapters for MGI must be used, and the amount used is still 5 µl.

4. Mix well by pipetting up and down (Do not vortex), then centrifuge briefly to the bottom of the tube.

5. Place the PCR tube into the PCR instrument and perform the below reaction:

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

▲ If the input DNA amount is low, consider doubling the ligation time. However, longer reaction time may lead to increased adapter dimers. If necessary, the adapter concentration may also need to be optimized.

6. Use VAHTS DNA Clean Beads to purify the reaction product:

1/ When the beads are balanced to room temperature, vortex to mix the VAHTS DNA Clean Beads.

2/ Add 60 μl of VAHTS DNA Clean Beads to 100 μl of Adapter Ligation products. Mix thoroughly by vortexing or pipetting.

3/ Incubate for 5 min at room temperature.

4/ Briefly centrifuge the PCR tube and place it on the magnetic rack to separate the beads and liquid. Once the solution is clear (approximately 5 min), carefully **remove and discard the supernatant**.

5/ Always keep the PCR tube on the magnetic rack, add 200 μl of freshly prepared 80% ethanol to rinse the beads. Incubate for 30 sec at room temperature, then carefully **remove and discard the supernatant**.

6/ Repeat step 5/, rinse twice in total.

7/ Always keep the PCR tube on the magnetic rack. Open the lid to air dry the beads for 5 - 10 min until there is no ethanol residue.

8/ Remove the PCR tube from the magnetic rack for elution:

▲ If the purification products do not perform two rounds of size selection: Add 22.5 μl of eluent (10 mM Tris-HCl, pH 8.0 - 8.5) for elution, vortex or gently pipette up and down to mix thoroughly and place it at room temperature for 2 min. Briefly centrifuge the PCR tube and place it on the magnetic rack. Once the solution is clear (approximately 5 min), carefully transfer 20 μl of supernatant to a new EP tube. Do not touch the magnetic beads.

▲ If the purification products undergo two rounds of size selection: Add 105 μl of eluent (10 mM Tris-HCl, pH 8.0 - 8.5) for elution, vortex or gently pipette up and down to mix thoroughly and place it at room temperature for 2 min. Briefly centrifuge the PCR tube and place it on the magnetic rack. Once the solution is clear (approximately 5 min), carefully transfer 100 μl of supernatant to a new EP tube. Do not touch the magnetic beads. Perform size selection according to the two rounds of size selection criteria in **Table 5** (Page.15).

Samples can remain stable for one week at 4°C. Store at -20°C for long-term storage. Avoid repeated freezing and thawing.

08-3/Library Amplification

This step is for PCR amplification of the Adapter Ligation products after purification or size selection.

1. Thaw the PCR Primer Mix for MGI and the VAHTS HiFi Amplification Mix. Once thawed, mix thoroughly and prepare the reaction solution in the sterile PCR tube as follows:

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

▲If Vazyme #NM35101-NM35108 combination of adapters and primers are used, then paired Barcode Primer for MGI must be used, and the recommended amount is 5 μl.

2. Mix well by pipetting up and down (Do not vortex), then centrifuge briefly to the bottom of the tube.

3. Place the PCR tube into the PCR instrument and perform the below 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 3** (Page.07)
72°C	5 min	1
4°C	Hold	

4. For size selection, refer to Appendix I: Two Rounds of Size Selection. Use VAHTS DNA Clean Beads to purify the reaction products if size selection is not required:

   1/ When the beads are balanced to room temperature, vortex and mix the VAHTS DNA Clean Beads.

   2/ Add 45 μl of VAHTS DNA Clean Beads to 50 μl of Library Amplification solution. Mix thoroughly by vortexing or pipetting.

   3/ Incubate for 5 min at room temperature.

   4/ Briefly centrifuge the PCR tube and place it on the magnetic rack to separate the beads and liquid. Once the solution is clear (approximately 5 min), carefully **remove and discard the supernatant**.

   5/ Always keep the PCR tube on the magnetic rack, add 200 μl of freshly prepared 80% ethanol to rinse the beads. Incubate for 30 sec at room temperature, then carefully **remove and discard the supernatant**.

   6/ Repeat step 5/, rinse twice in total.

   7/ Always keep the PCR tube on the magnetic rack. Open the lid to air dry the beads for 5 - 10 min until there is no ethanol residue.

   8/ Remove the PCR tube from the magnetic rack for elution:

▲ If no subsequent target capture is being performed: add 22.5 μl of eluent (10 mM Tris-HCl, pH 8.0 - 8.5) or ddH_2O for elution, vortex or gently pipette up and down to mix thoroughly and place it at room temperature for 2 min. Briefly centrifuge the PCR tube and place it on the magnetic rack. Once the solution is clear (approximately 5 min), carefully transfer 20 μl of supernatant to a new EP tube. Do not touch the magnetic beads.

▲ If subsequent target capture is being performed: add 22.5 μl of ddH_2O to elute. Vortex or gently pipette up and down to mix thoroughly and place it at room temperature for 2 min. Briefly centrifuge the PCR tube and place it on the magnetic rack. Once the solution is clear (approximately 5 min), carefully transfer 20 μl of supernatant to a new EP tube. Do not touch the magnetic beads.

Samples can remain stable for one week at 4°C. Store at -20°C for long-term storage. Avoid repeated freezing and thawing.

08-4/Library Quality Control

Refer to 06-7/Library Quality Control.

Appendix I: Two Rounds of Size Selection

◇ To meet the needs of different applications, two rounds of size selection are often required during library preparation to control the distribution range of the library insert size. **It must be guaranteed that the size selection process is performed only once. Two or more selections can lead to a significant reduction in library complexity and yield!** Refer to Table 4 for information on size selection positions and the advantages and disadvantages of the different positions.

Table 4. Choosing the stage of the size selection

Stage of size selection	Applicable conditions	Advantages	Disadvantages	Examples of applicable samples
Before end preparation	The input DNA is sufficient, but the distribution range is wide or the main peak is not consistent with the expected insert size of the library; the input DNA purity is poor	Concentrated size distribution of selection products; accurate control of the amount of input DNA; further increase the purity of input DNA and improve the success rate of library preparation	Large quantity of DNA loss	Insufficient or excessive fragmentation of genomic DNA
After adapter ligation	Input DNA distribution is adequate and the amount is sufficient[a]	Reduce the loss of short input DNA; Suitable for most cases	Library size distribution range is slightly broad[b]	Proper fragmentation of genomic DNA or FFPE DNA with wider distribution range
After library amplification	Low input DNA amount[a]	Reduce the loss of input DNA during library preparation and increase the complexity of the library	Library size distribution range is broad[c]	cfDNA
No size selection during library preparation	Input DNA distribution range meets the library preparation requirements; input DNA amount is low	Reduce the loss of input DNA during library preparation and increase the complexity of the library	Library insert size cannot be controlled	Multiple PCR products, highly fragmented FFPE DNA

a. If the input DNA amount is ≥50 ng, it is recommended to perform the size selection after Adapter Ligation. If the input DNA amount is <50 ng or the sample copy number is limited, perform size selection after Library Amplification.

b. The effect of size selection is affected by the state of the DNA end. The single-stranded part of the input DNA end and the non-complementary region of adapter will lead to a wider distribution of selected product length.

c. It is relative to size selection in other stages. After products selected in other stages are subjected to Library Amplification, the library distribution will be more concentrated.

◇ Two rounds of size selection is made by controlling the amount of beads used to perform DNA size selection. The basic principle is: the first round of beads bind to DNA with a larger molecular weight and this kind of DNA is removed when the beads are discarded; while the second round of beads binds to DNA with a larger molecular weight in the remainder of the products and the smaller-sized DNA is removed by discarding the supernatant. Many components in the initial sample interfere with the two rounds of size selection. Therefore, when the stage of the size selection is different, the amount of beads used for two rounds selection would be different. Select the most appropriate selection parameter according to Table 5 based on the expected library insert size and the stage of the size selection.

Table 5. Library size selection

Stage and conditions for performing size selection	Expected library insert size (bp)									
Purification rounds	150	200	250	300	350	400	450	500	550	700
Before end preparation (fill sample volume up to 100 μl)	One round **X** (μl)	100	90	80	70	60	55	52	50	48	43
	Two round **Y** (μl)	30	20	20	20	20	20	15	15	15	12
After adapter ligation (sample volume 100 μl)	One round **X** (μl)	80	66	65	59	56	54	49	48	/	/
	Two round **Y** (μl)	20	20	15	15	12	12	10	10	/	/
After library amplification (fill sample volume up to 100 μl)	One round **X** (μl)	80	70	60	53	48	45	43	42	/	/
	Two round **Y** (μl)	20	20	20	20	20	20	20	15	/	/

◇ Sample pretreatment (important!)

▲ If size selection takes place before End Preparation, the sample volume should be 100 μl. If not, the sample should be filled up to 100 μl with ddH_2O.

▲ If size selection takes place after Adapter Ligation products purification, the sample volume should be 100 μl. If not, the sample should be filled up to 100 μl with ddH_2O.

▲ If size selection takes place 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 is not pretreated by volume, the beads amount can also be adjusted in proportion to the actual volume of the sample. However, if the sample volume is too small, this will increase pipetting errors, which in turn affect the accuracy of size selection. Therefore, direct size selection of sample volume <50 μl is not recommended.

◇ Protocol for selection (refer to Table 5 (Page.15) to confirm values of X and Y)

1. When the beads are balanced to room temperature, vortex to mix the VAHTS DNA Clean Beads.
2. Add X μl VAHTS DNA Clean Beads to the above 100 μl solution. Mix thoroughly by vortexing or gentlely pipetting up and down for 10 times.
3. Incubate for 5 min at room temperature.
4. Briefly centrifuge the PCR tube and place it on the magnetic rack to separate the beads and liquid. Once the solution is clear (approximately 5 min), carefully transfer the supernatant into a new PCR tube, **discarding the magnetic beads**.
5. Add Y μl of VAHTS DNA Clean Beads to the supernatant. Mix thoroughly by vortexing or gentlely pipetting up and down for 10 times.
6. Incubate for 5 min at room temperature.
7. Briefly centrifuge the PCR tube and place it on the magnetic rack to separate the beads and liquid. Once the solution is clear (approximately 5 min), carefully **remove and discard the supernatant.**
8. Always keep the PCR tube on the magnetic rack, add 200 μl of freshly prepared 80% ethanol to rinse the beads. Incubate at room temperature for 30 sec, then carefully **remove and discard the supernatant.**
9. Repeat step 8, rinse twice in total.
10. Always keep the PCR tube on the magnetic rack. Open the lid to air dry the beads for 5 - 10 min until there is no ethanol residue.
11. Remove the PCR tube from the magnetic rack for elution:

▲ If no subsequent target capture is being performed, add 22.5 μl of eluent (10 mM Tris-HCl, pH 8.0 - 8.5) or ddH_2O. Vortex or gently pipette up and down to mix thoroughly and place it at room temperature for 2 min. Briefly centrifuge the PCR tube and place it on the magnetic rack. Once the solution is clear (approximately 5 min), carefully transfer 20 μl of supernatant to a new EP tube. Do not touch the magnetic beads.

▲ If subsequent target capture is being performed: add 22.5 μl of ddH_2O to elute. Vortex or gently pipette up and down to mix thoroughly and place it at room temperature for 2 min. Briefly centrifuge the PCR tube and place it on the magnetic rack. Once the solution is clear (approximately 5 min), carefully transfer 20 μl of supernatant to a new EP tube. Do not touch the magnetic beads.

Appendix II: cfDNA Library Preparation

cfDNA is a blood-derived DNA with significant characteristics such as highly fragmented (about 180 bp) and low content. It is extremely valuable for detection in fields such as noninvasive prenatal diagnosis (NIPT) and liquid biopsy (ctDNA testing). VAHTS Universal DNA Library Prep Kit for MGI provides highly simplified and optimized library preparation protocols for these samples.

Notes

◇ Input DNA refers specifically to the DNA used in End Preparation step with a volume of ≤50 μl.

◇ cfDNA itself is highly fragmented and does not require fragmentation.

◇ It is recommended to carry out size selection (Agilent 2100 Bioanalyzer) and concentration detection (Qubit) of the cfDNA template to ensure library quality.

Library Preparation Process

Step 1: End Preparation (refer to 08-1/End Preparation)

Input DNA amount: 100 pg - 100 ng.

Step 2: Adapter Ligation (refer to 08-2/Adapter Ligation)

Adapter: Pre-dilute the adapters based on Table 2 (Page.04).

Clean up: Use 0.6 × beads for purification, 22.5 μl eluent for DNA elution, and remove 20 μl supernatant for the next step.

Step 3: Library Amplification (refer to 08-3/Library Amplification)

Number of cycles: 6 - 18 cycles are recommended. This may be independently adjusted depending on library yield requirements.

Clean up: Whether the cfDNA library size is selected depends on the sample situation and data analysis.

▲If two rounds of size selection is not performed: 0.9 × beads purification is used and the DNA is eluted with 22.5 μl eluent. 20 μl supernatant is transferred to a new EP tube, and is stored at -20°C.

▲If two rounds of size selection is performed: 0.78 ×/0.2 × two rounds of size selection is used and the DNA is eluted with 22.5 μl eluent. 20 μl supernatant is transferred to a new EP tube, and is stored at -20°C.

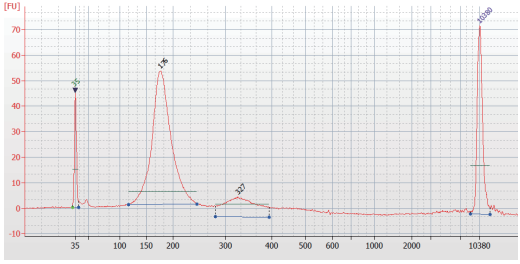
Step 4: Library Quality Control

Library concentration detection:

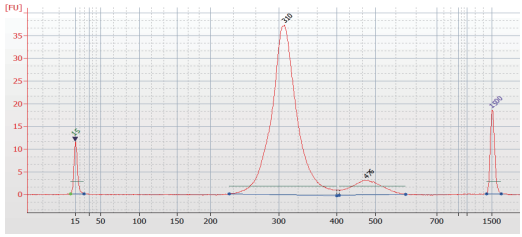
Equalbit 1 × dsDNA HS Assay Kit (Vazyme #EQ121) that is based on Fluorescent dye method is recommended to detect library concentration.

Library size distribution analysis:

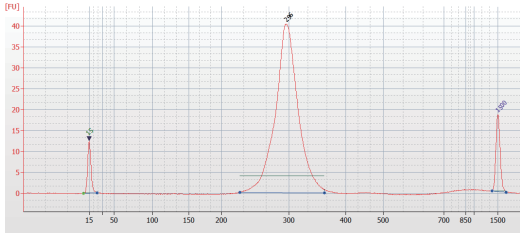
Size distribution analysis using Agilent 2100 Bioanalyzer.



cfDNA extracted with magnetic beads



cfDNA Library (no size selection)



cfDNA Library (size selection)

Appendix III: FFPE DNA Library Preparation

FFPE DNA is obtained from Formalin-Fixed and Parrffin-Embedded (FFPE) sections, which is characterized by difficult extraction (close cross-linking with histones) and low quality (serious degradation). FFPE samples are easy to preserve and widely sourced, which has high application value in the medical field. For these samples, VAHTS Universal DNA Library Prep Kit for MGI provides a highly simplified and optimized library construction solution.

Notes

- ◇ Input DNA refers specifically to DNA used in End Preparation step with a volume of ≤50 μl.
- ◇ The quality of extracted FFPE DNA is different due to tissue difference, embedding quality, storage time and other factors. When using low quality FFPE DNA library, the amount of input DNA or the number of amplification cycles should be appropriately increased.
- ◇ It is recommended to carry out size selection (Agilent 2100 Bioanalyzer) and concentration detection (Qubit) of the input DNA to ensure library quality.
- ◇ If the FFPE DNA Fragmentation degree is insufficient and the average molecular weight is large, fragmentation should be conducted before library construction.

Library Preparation Process

Step 1: End Preparation (refer to 08-1/End Preparation)

Input DNA amount: ≥50 ng.

Step 2: Adapter Ligation (refer to 08-2/Adapter Ligation)

Adapter: Pre-dilute the adapter based on Table 2 (Page.04).

Clean up: Use 0.6 × beads for purification.

- ▲ If two rounds of size selection is not performed: the DNA is eluted with 22.5 μl eluent. 20 μl supernatant is removed.
- ▲ If two rounds of size selection is performed: the DNA is eluted with 105 μl eluent. 100 μl supernatant is removed, and the library length was selected according to the two round beads selection conditions in Table 5 (Page.15).

Step 3: Library Amplification (refer to 08-3/Library Amplification)

Number of cycles: Refer to Table 3 (Page.07) and adjust according to sample quality.

Clean up:

- ▲ If the amplified products are not performed two rounds of size selection: 0.9 × beads purification is used and the DNA is eluted with 22.5 μl eluent. 20 μl supernatant is transferred to a new EP tube, and is stored at -20°C.
- ▲ If the amplified products are performed two rounds of size selection: Fill it with ddH_2O to 100 μl, and the library length was selected according to the two round beads selection conditions in Table 5 (Page.15).

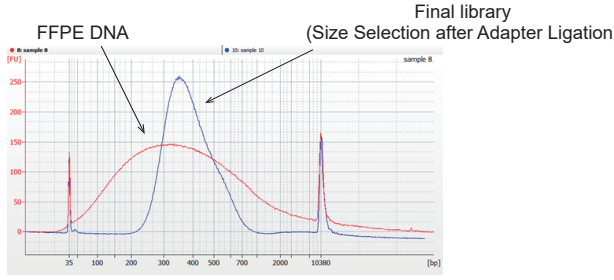
Step 4: Library Quality Control

Library concentration detection:

Equalbit 1 × dsDNA HS Assay Kit (Vazyme #EQ121) that is based on Fluorescent dye method is recommended to detect library concentration.

Library size distribution analysis:

Size distribution analysis using Agilent 2100 Bioanalyzer.



FFPE DNA Library (size selection)

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