UltraClean Universal Plus DNA Library Prep Kit for MGI V3

UNDM637

Vazyme

Instruction for Use

Version 24.1

Contents

01/Product Description

UltraClean Universal Plus DNA Library Prep Kit for MGI V3 is an ultra-fast DNA library preparation kit based on enzymatic fragmentation for MGI sequencing platform. The kits are charac terized by high library conversion rate and low interference from background, and perfectly compatible with various samples from 100 pg - 500 ng. It is suitable for the PCR and PCR-Free libraries. This kit simplifies the process of library preparation and shortens the operation time, it takes about 2 h to prepare a single library. All reagents provided in the kit have undergone strict control of background nucleic acids and functional verification, which is for minimizing interference from background to ensure the stability of library preparation and the reliability of pathogen detection.

02/Components

Components	UNDM637-01 (24 rxns)	UNDM637-02 (96 rxns)
FEA Enzyme Mix V3	240 μl	960 μl
Rapid Ligation Buffer V3	720 μl	4 × 720 μl
Rapid DNA Ligase V3	120 μl	480 μl
VAHTS HiFi Amplification Mix	600 μl	4 × 600 μl
PCR Primer Mix 3 for MGI	120 μl	480 μl
Enhancer Buffer	240 μl	960 μl

03/Storage

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

04/Applications

This kit is suitable for preparing a dedicated library for MGI sequencing platform. It can be used with an initial input of 100 pg - 500 ng and is suitable for various DNA samples such as alveolar lavage fluid, blood cells, serum, plasma, cerebrospinal fluid, etc.

05/Self-prepared Materials

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

DNA quality control: Agilent Technologies 2100 Bioanalyzer or equivalent;

Equalbit 1 × dsDNA HS Assay Kit (Vazyme #EQ121);

DNA adapter:

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

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

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

#NM34401-NM34404 are adapters with an 8 bp Unique Indexed at two ends, which can prepare 384 different libraries by adapter combinations.

Other materials: Absolute ethanol, Nuclease-free ddH_2O, 0.1 × TE, Elution buffer (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.

06-1/Input DNA and Fragmentation

◇ 100 pg - 500 ng gDNA can be used for library preparation. It is strongly recommended to use high-quality genomic (A260/A280 = 1.8 - 2.0).

06-2/DNA Adapter

◇ For the MGI sequencing platform, Vazyme provides two sets of Indexed Adapters, which can be selected according to the different applications and pooling strategy.

◇ The quality and amount of adapters directly affect the preparation efficiency and library quality. If the adapter input is too more, it may lead to residual adapters or adapter dimers. If the adapter input is too llittle, it may affect ligation efficiency and reduce library yield. Table 1 lists the recommended adapter usage for different Input DNA.

Table 1. Recommended adapter concentration for 100 pg - 500 ng input DNA

Input DNA	UDB Adapter dilution ratio	Volume
100 pg	1:200	5 μl
1 ng	1:100	5 μl
100 ng	1:2	5 μl
500 ng	Undiluted	5 μl

▲ Increasing the usage of adapter can improve the library yield to some extent. When optimizing the preparation efficiency, you may increase the adapter input (by 2 - 10 folds, preferably) under the recommended conditions in the table above. For example, with a default adapter volume of 5 μl, when the input DNA is 500 ng, Vazyme adapter usage can be increased to 10 μl to increase the library yield by 5% - 15%. However, it should be noted that increasing the adapter concentration may increase the adapter residue in the library, resulting in sequencing data wasting.

06-3/Adapter Ligation Product Purification

◇ Excessive adapters must be removed before library amplification (for PCR Library) or sequencing (for PCR-Free library). The recommended purification condition of 0.8 × (100 μl products, 80 μl beads) is suitable for most cases. To obtain libraries with larger insert sizes, the amount of beads can be reduced to lower the content of small DNA fragments. However, this adjustment only changes the main peak size of the library. If you need to accurately control the library distribution, you can carry out a size selection after this purification process.

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

◇ If the result shows the purification products are heavily contaminated with adapter or adapter dimer, they can be further purified with beads: the first purified 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 of the adapter or adapter dimer, especially when preparing PCR-Free libraries. It may also be necessary to reduce the amount of adapter in order to completely eliminate the adapter or adapter dimer residue.

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!

◇ General precautions for magnetic beads usage:

▲ 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 range 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 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 separation time may 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 library yield. 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 at 37°C in an oven).

06-5/Size Selection

◇ If the input DNA distribution range is wide, size selection is usually required to control the final library size. It is recommended to use Two Rounds Beads Selection or selection can also be performed using gel extraction.

◇ Size selection can be carried out after Adapter Ligation or Library Amplification. The size selection steps are not included in the standard experiment protocol. Refer to **Appendix I: Tow Rounds Beads Selection** for more information.

◇ There is a large amount of DNA loss in size selection. 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 little, it must be guaranteed that the size selection occurs only once. Two or more size selections can lead to a significant reduction in library complexity and yield.

◇ Over-amplification typically results in the 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 over-amplification by the mean of size selection.

◇ The high concentration of PEG in Rapid Ligation Buffer V3 has a significant impact on Two Rounds Beads Selection and gel extraction. Therefore, if size selection is performed after Adapter Ligation, the Adapter Ligation product purification steps (**08/Experiment Process/08-2/Adapter Ligation/6. Clean up the reaction product using VAHTS DNA Clean Beads**) must not be omitted and the purified product must be eluted in a suitable volume of eluent, followed by Two Rounds Beads Selection or gel extraction. If selection must be performed after Adapter Ligation, the selection condition should be explored separately. If size selection takes place after Library Amplification, the original purification step can be replaced with Two Rounds Beads Selection or gel extraction.

06-6/Library Amplification

◇ PCR Primer Mix 3 for MGI is designed to amplify the library based on MGI sequencers with full length adapters. Replace the amplification primers for stubby adapters or other platform libraries, recommended the final concentration for each primer is 5 - 20 μM.

◇ During the late stage of PCR, primers are usually depleted before dNTP. At this time, excessive cycles will cause non-specific annealing after the amplification products unwind, 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-stranded libraries that have the correct length, which can be normally combined with Flow Cell and sequenced after denaturation. The existence of these products has no significant impact on sequencing. 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 requires strictly control of the number of amplification cycles. An insufficient number of cycles will lead to a lower library yield, while excessive cycles will lead to various adverse effects such as over amplification, increased amplification bias, PCR duplicates, chimeric products and amplification mutations. Table 2 specifies the recommended number of amplification cycles to obtain a 100 ng or 1 μg library yield when using 100 pg - 500 ng of high-quality Input DNA.

Table 2. Recommended amplification cycles for 100 pg - 500 ng input DNA

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

▲ The above table shows the number of cycles measured for high-quality 293 gDNA which was fragmented for 5 min at 30°C. When DNA quality is poor, the number of cycles must be adjusted to obtain sufficient library yield.

06-7/Library Quality Control

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

◇ Library length 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 (Advanced Analytical), etc.

◇ Library concentration analysis:

There are common methods for determining library concentration based on dsDNA fluorescent dyes, such as Qubit, Equalbit 1 × dsDNA HS Assay Kit (Vazyme #EQ121) and PicoGreen, etc.

06-8/Further Precautions

◇ The size and distribution range of DNA fragments are determined by a time-dependent enzyme-based reaction, thus the fragmentation reaction should be carried out on ice.

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

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

◇ It is recommended to use a PCR instrument with a heated lid when carrying out the reaction in each step. Choose the preheated lid option and set 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 reaction 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

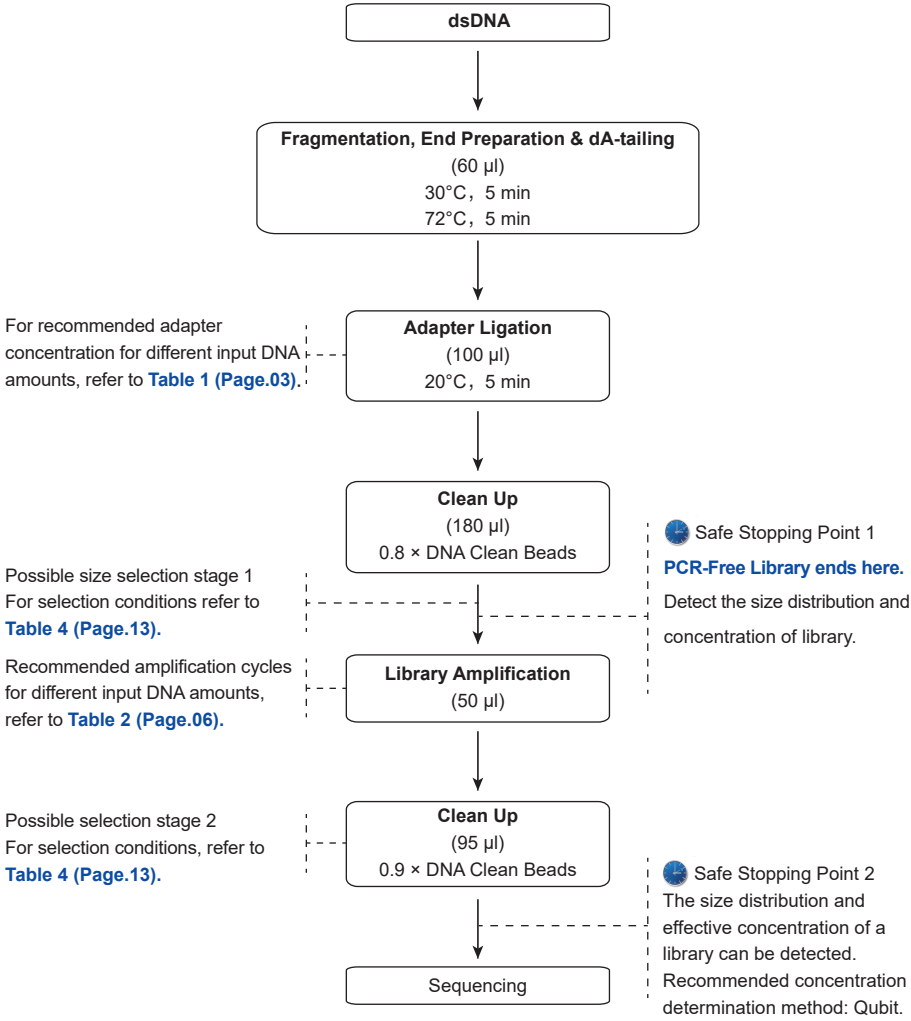

Fig 1. Workflow of UltraClean Universal Plus DNA Library Prep Kit for MGI V3.

08/Experiment Process

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

This step is for fragmenting the Input DNA while simultaneously repairing the fragmented DNA end, as well as the 5' end phosphorylation and the 3' end dA tailing.

1. Thaw the FEA Enzyme Mix V3. Mix gently and centrifuge briefly. Then put it on ice before use. All of the following steps are kept on ice.

2. Prepare the following solution in the PCR tube:

Components	Volume
Input DNA	x μl
ddH_2O	To 50 μl

3. Add 10 μl of FEA Enzyme Mix V3 to each sample, pipetting up-and-down or vortexing to mix, and centrifuge to collect the reaction solution at the bottom of the tube and **place it in the PCR instrument immediately for reaction! ! !**

▲ Fragmentation reaction is a time-dependent enzyme-based reaction, and the size of the fragment product depends on the reaction time. Therefore, it is recommended to add the FEA Enzyme Mix V3 to the reaction solution separately at the end. Mix immediately, and then carry out the follow-up reaction.

▲ When the DNA contains impurities and inhibitor, it is recommended to add Enhancer Buffer to the reaction system to enhance fragmentation ability. The amount of Enhancer Buffer added can be adjusted in 1 μl units.

4. Perform the following program in a PCR instrument:

Temperature	Time
105°C (Preheated lid)	On
30°C	5 min
72°C	5 min
4°C	Hold

▲ Properly extending the time for adding a poly(A) tail, such as 72°C for 20 min, which can improve the efficiency of polyadenylation and enhance library conversion rate to some extent.

▲ When using high-quality human gDNA for library preparation, different inputs within the recommended input range (100 pg - 500 ng) with the same reaction time resulted in little variation in the distribution of fragmentation product (the distribution range is essentially consistent, but the main peak size may vary slightly, **refer to Fig 2**). The fragmentation time is 5 min and adapter size is approximately 120 bp, the main peak size of the library is 280 - 320 bp after amplification. If the input DNA is not within the expected range, it is recommended to increase or decrease the fragmentation time by 2 - 5 min.

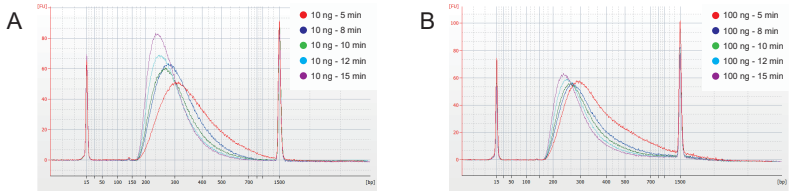

Fig 2. Library quality analysis of Input DNA and fragmentation time

08-2/Adapter Ligation

This step is to add adapter to the products after fragmenting in the previous step.

1. Dilute adapter to appropriate concentration according to Table 1 (Page.03).
2. Thaw Rapid Ligation Buffer V3 and Rapid DNA Ligase V3. Mix thoroughly and centrifuge briefly. Put them on ice before use.
3. Prepare the reaction solution as follows:

Components	Volume	
Products from previous step	60 µl	
Rapid Ligation Buffer V3	30 µl	■
Rapid DNA Ligase V3	5 µl	■
DNA Adapter X	5 µl	

4. Mix gently by pipetting and avoid vortexing, briefly centrifuge to collect the solution at the bottom of the tube.
5. Perform the following program in a PCR instrument:

Temperature	Time
105°C (Preheated lid)	On
20°C	5 min
4°C	Hold

▲ Properly extend the ligation time, such as 20°C 15 min, which can improve the efficiency of adapter ligation to some extent.

6. 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 80 µl VAHTS DNA Clean Beads to 100 µl of Adapter Ligation solution. Vortexing or pipetting 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 (this step can be omitted, wash once in total).
   g. Always keep the PCR tubes on the magnetic rack and air-dry the beads for 5 min.
   h. Remove the PCR tube from the magnetic rack for elution:

▲ If the purification products do not perform Two Rounds Beads Selection: Add 22.5 μl of Elution buffer (10 mM Tris-HCl, pH 8.0 - 8.5) or ddH_2O for elution, vortex or pipette gently to resuspend beads and incubate at room temperature for 2 min. Briefly centrifuge the PCR tube and place it on the magnetic rack until the supernatant is clear (~ 5 min), carefully transfer 20 μl of supernatant to a new EP tube. Do not disturb the magnetic beads.

▲ If the purification products perform Two Rounds Beads 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 2 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. Perform size selection according to the Two Rounds Beads Selection criteria in **Table 4** (Page 13).

Samples can store at -20°C for long-term preservation. 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. Whether to proceed with this step depends on the amount of input DNA, whether adapters are full length, and downstream application. If adapters are not full length (e.g., Vazyme #NM34401-NM34404), this step is necessary. If adapters are full length (e.g., Vazyme #NM108), for input DNA <50 ng, library amplification is recommended. If input DNA is ≥50 ng or there is no need for library amplification, skip this step and proceed directly to 08-4/Library Quality Control.

1. Thaw the PCR Primer Mix 3 for MGI and the VAHTS HiFi Amplification Mix. Mix by inversion and briefly centrifuge to collect the solution at the bottom of the tube. Prepare the following solution in the PCR tube:

Components	Volume	
Purified or selected Adapter Ligation products	20 μl	
PCR Primer Mix 3 for MGI	5 μl	■
VAHTS HiFi Amplification Mix	25 μl	■
Total	50 μl	

2. Mix gently by pipetting and avoid vortexing, briefly centrifuge to collect the solution at the bottom of the tube.
3. Perform the following program in a PCR instrument:

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 2 (Page.06)
72°C	1 min	1
4°C	Hold	

4. For size selection, refer to **Appendix I: Two Rounds Beads Selection**. If size selection is not required, using VAHTS DNA Clean Beads to purify the reaction products:

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

   b. Add 45 μl VAHTS DNA Clean Beads to 50 μl of Library Amplification solution. Vortexing or pipetting to resuspend.

   c. Incubate for 5 min at room temperature .

   d. Briefly centrifuge the 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 (this step can be omitted, wash once in total).

   g. Always keep the PCR tubes on the magnetic rack and air-dry the beads for 5 min.

   h. Remove the PCR tube from the magnetic rack and add 22.5 μl of Elution buffer (10 mM Tris-HCl, pH 8.0 - 8.5) or ddH_2O for elution, vortex or pipette gently to resuspend beads and incubate at room temperature for 2 min. Briefly centrifuge the PCR tube and place it on the magnetic rack until the supernatant is clear (~ 5 min), carefully transfer 20 μl supernatant to a new EP tube. Do not disturb the magnetic beads.

   Samples can store at -20°C for long-term preservation. Avoid repeated freezing and thawing.

08-4/Library Quality Control

Reference 06-7/Library Quality Control.

Appendix I: Two Rounds Beads Selection

◇ To meet the needs of different applications, Two Rounds Beads Selection are often required during library preparation to control the range of the library insert size. Refer to **Table 3** for information on choosing when to perform selection, the advantages and disadvantages of the different stage. 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 3. Choosing the stage of the size selection

Stage of size selection	Applicable conditions	Advantages	Disadvantages
After Adapter Ligation	Input DNA distribution is suitable and adequate[b]	Reduction of short fragment DNA loss	Cannot accurately evaluate the library distribution[a]
After Library Amplification	Input DNA is little[b]	Reduce of Input DNA loss during library preparation and increase the complexity of the library	Library size distribution range is slightly broad

a. The effect of Two Rounds Beads Selection is affected by the state of the DNA end. The single-stranded of Input DNA end and the non-complementary region of the adapters will lead to a wider distribution of selected product size.

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

◇ Two Rounds Beads Selection is made by controlling the amount of beads used to perform DNA size selection. The basic principle is: the first round of beads binds 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 Beads Selection effect. Therefore, when the stage of the size selection is different, the amount of beads used for Two Rounds Beads Selection would be different. Select the most appropriate selection parameter according to Table 4 based on the expected library insert size and the stage of the size selection.

Table 4. Library size selection

Stage and conditions for performing size selection	Purification rounds	Expected Insert Size (bp)				
		150	200	250	300	350
After Adapter Ligation (sample volume 100 μl)	1st-Round **X**(μl)	78	68	65	59	56
	2nd-Round **Y**(μl)	20	20	15	15	12
After Library Amplification (fill sample volume up to 100 μl)	1st-Round **X**(μl)	78	70	63	55	50
	2nd-Round **Y**(μl)	20	20	20	20	20

◇ If adapters are not full length (e.g., Vazyme #NM34401-NM34404), please refer to the following table to choose the volume of beads according to expected insert size and selection points.

Stage and conditions for performing size selection	Purification rounds	Expected Insert Size (bp)				
		150	200	250	300	350
After adapter ligation (sample volume 100 μl)	1st-Round **X**(μl)	100	90	75	65	60
	2nd-Round **Y**(μl)	20	20	20	20	20

▲ When magnetic beads are used for size selection, the larger Insert Size, the broader size distribution.

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

◇ Sample pretreatment (important!)

▲ 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 amount of beads 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, directly perform size selection of samples <50 μl is not recommended.

◇ Protocol for selection (Refer to **Table 4 (Page. 13)** to confirm values of **X** and **Y**)

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

b. Add **X** μl of VAHTS DNA Clean Beads to the above 100 μl solution. Vortexing or pipetting to resuspend.

c. Incubate for 5 min at room temperature.

d. 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**.

e. Add **Y** μl of VAHTS DNA Clean Beads to the supernatant. Vortexing or pipetting to resuspend.

f. Incubate for 5 min at room temperature.

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

h. 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**.

i. Repeat step h, wash twice in total.

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

k. Remove the PCR tube from the magnetic rack and add 22.5 μl of Elution buffer (10 mM Tris-HCl, pH 8.0 - 8.5) or ddH_2O for elution, vortex or pipette gently to resuspend beads and incubate at room temperature for 2 min. Briefly centrifuge the PCR tube and place it on the magnetic rack until the supernatant is clear (~5 min), carefully transfer 20 μl supernatant to a new EP tube. Do not disturb the magnetic beads.

▲ If the solution is less than 100 μl. Fill up to 100 μl with ddH_2O.

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