Hyaseq DNA Library Prep Kit V2

Cat.No.: HYD111

Product Description

Hyaseq DNA Library Prep Kit V2 is a new generation library preparation kit specially developed and designed for Illumina&MGI sequencing platform. On the basis of the previous generation of library preparation kit, this product exhibits higher efficiency in end repair, dA-tailing, and adapter ligation than the previous versions. The high-fidelity enzyme significantly improves the uniformity and fidelity of amplification. The kit is compatible with most DNA sample types, including standard genomic DNA from animals/plants/microorganisms, FFPE samples, cfDNA, and ChIP DNA.

Components

Components	24T	96T
DNA End Repair Buffer V2	168 μL	672 μL
DNA End Repair Enzyme Mix V2	72 μL	288 μL
Ligation Enhancer V2	720 μL	3×960 μL
Rapid T4 DNA Ligase V2	240 μL	2×480 μL
2 × NGS Library Amplification Mix	600 μL	3×800 μL

Note: If the recommended short adapters are used, the Primer Mix is not required. However, if complete adapters are selected, the Primer Mix is essential but not included in this kit; it must be prepared separately. While the components of this kit are compatible with both Illumina and MGI platforms, a platform-specific Primer Mix must be purchased separately: Cat.No.: HYD313 Primer Mix for Illumina or Cat.No.: HYD317 Primer Mix for MGI.

Storage

Store at -25 ~ -15°C, valid for 1 year.

Notes

1. About the operation

1) Please operate with lab coats and disposable gloves， for your safety.
2) Thaw components at room temperature. After thawing, mix thoroughly by vortexing, spin the tube briefly and place them on ice for later use.
3) When preparing the reaction solution of each step, it is recommended to use a pipette to mix well or gently shake. Vigorous shaking may cause a decrease in library output.
4) It is highly recommended to use filtered pipet tips to avoid cross-contamination. Be sure to change pipet tips when processing different samples.
5) Improper operations may very likely cause aerosol contaminations, impacting the accuracy of result. Mandatory physical isolation of PCR reaction mixing regions and PCR product purification assay regions is recommended. Equipped with equipment such as specialized pipettes for library preparation.Perform routine cleaning for each area by wiping the surfaces with 0.5% sodium hypochlorite or 10% bleach
6) This product is for research use only.

2. About DNA Fragmentation

1) This kit is compatible with either mechanically fragmented DNA or enzymatically fragmented DNA.
2) The kit is compatible with 100 pg - 1000 ng of input DNA. It is highly recommended to use high-quality input DNA with A260/A280 = 1.8-2.0. Table 1 lists the recommended amount of Input DNA.

Table 1 The recommended amount of Input DNA

Application	Sample types	Input DNA
WGS	Complex genome	50 ng-1000 ng
Targeted capture sequencing	Complex genome	10 ng-1000 ng
WGS, Targeted capture sequencing	FFPE DNA	50 ng-1000 ng
Targeted capture sequencing	cfDNA/ctDNA	≥500 pg
WGS	Microbial genomes	≥1 ng
WGS (PCR-free)	High-quality input DNA	≥50 ng

Note: When the input DNA is with poor quality or DNA size selection is required, the input DNA amount should be increased accordingly.

3) "Input DNA" refers specifically to the DNA added to the end repair/dA-tailing step.

4) A beads-purification/size-selection step is recommended post fragmentation if the input DNA sample contains high concentrations of salts like the metal-chelating agent. The salts might impact the efficiency of the following reactions, including end repair and dA-tailing. Please elute the DNA samples in TE Buffer instead of sterilized ultra-pure water for fragmentation if using the mechanical fragmentation method. If using the enzymatic fragmentation method without performing beads clean-up or size-selection before proceeding to library preparation, please ensure that the stop buffer used doesn't contain exceeding metal-chelating agent. Otherwise, please clean-up or size select the fragmented samples and elute them in TE buffer or sterilized ultra-pure water (≤50 μL) before proceeding to library preparation.

3. About Adapter Ligation

1) Illumina or MGI Long Adapter (Barcoded Adapter) kits and short Adapter kits are available for customers to choose according to their experimental requirements.

2) Selecting high-quality, commercial adapters was recommended. If self-made adapters are selected, please entrust a company with experience in NGS primer synthesis and remark the need for strict contamination control.

3) In addition, it is recommended to prepare DNA annealing solution in a clean bench and only operate one type of adapter each time to prevent cross-contamination. 3. Please thaw the adapters on the ice or at 4°C; when operating at room temperature, the laboratory temperature should not exceed 25°C to prevent the adapters from denaturing.

4) The adapters' quality and concentration will directly affect the ligation efficiency and the library yield. Too high concentration of adapters favors adapter dimer formation while too little adapter reduces ligation rate and library yield. Corresponding dilutions with TE Buffer according to Input DNA amount when using Adapter.Table 2 -3 lists the recommended Adapter dilution methods for different Input DNA amounts using this kit.

Table 2 The recommended Illumina adapter amount for different input DNA

Input DNA	Adapter Dilution(Volume of Adapter : Total Volume)	Concentration
0.1ng	150-Fold (1 : 150)	0.1 μM
1 ng	30-Fold (1 : 30)	0.5 μM
10 ng	7.5-Fold (1 : 7.5)	2 μM

100 ng	3-Fold (1 : 3)	5 μM
1000 ng	1.5-Fold (1 : 1.5)	10 μM

Table 3 The recommended MGI adapter amount for different input DNA

Input DNA	Adapter Dilution(Volume of Adapter : Total Volume)	Concentration
0.1ng	100-Fold (1 : 100)	0.1 μM
1 ng	20-Fold (1 : 20)	0.5 μM
10 ng	5-Fold (1 : 5)	2 μM
100 ng	2-Fold (1 : 2)	5 μM
1000 ng	undiluted	10 μM

4. About Bead-based DNA Cleanup and Size Selection

1) DNA size-selection can be performed before end repair/dA-tailing, after adapter ligation, or after amplification.
2) It is recommended to perform size-selection right after adapter ligation if the input DNA amount is more than 50 ng; otherwise, please perform size-selection after amplification.
3) The Ligation Enhancer contains a high concentration of PEG, which may cause a significant impact on accurate size-selection. Thus, if size-selection is to be performed right after adapter ligation, it is strongly recommended to add a beads clean-up step before the size-selection. Size selection step can be performed directly if it is performed before the end repair/dA-tailing or after the library amplification.
4) The magnetic beads should be equilibrated at room temperature prior to use, otherwise the yield will decrease and the size selecting effect will be affected.
5) The magnetic beads should be mixed well by vortex or pipetting prior to use.
6) Do not aspirate the beads when transferring the supernatant, even trace amounts of the beads may impact the following reactions.
7) The 80% ethanol should be freshly prepared, otherwise it will affect the recovery efficiency.
8) For accurate size-selection, it is recommended to start with a volume of more than 100 μL. If less, it is recommended to bring the volume up to 100 μL with ultra-pure water.
9) The magnetic beads should be dried at room temperature before eluting the product. Insufficient dryness will easily cause ethanol residual to affect subsequent reactions; excessive dryness will cause the magnetic beads to crack and reduce the purification yield. Normally, drying at room temperature for 3-5 minutes is enough to allow the beads to fully dry.

10) If needed, the purified or size-selected DNA samples eluted in 0.1× TE buffer can be stored at 4°C for 1-2 weeks or at -20°C for a month.

5. About Library Amplification

1) Whether or not to perform library amplification depends on the amount of DNA input, types of the adapters, the sequencing data applications, etc. The amplification step is required if using partial adapters. When using full-length adapters, if the input DNA<200 ng, it is recommended to perform amplification; otherwise, amplification is not necessary.
2) Amplification cycle numbers should be strictly controlled. Insufficient amplification may lead to low library yield; Over-amplification may introduce increased bias, errors, duplicated read, and chimeric products. Table 4 lists recommended cycle numbers targeting the library yield of 1 μg.

Table 4 The recommended number of cycles to generate 1 μg of library yield

Input DNA	Number of cycles required to generate 1 μg of library yield
1000 ng	2 - 4
500 ng	2 - 4
250 ng	4 - 6
100 ng	5 - 7
50 ng	7 - 9
10 ng	9 - 11
5 ng	10 - 12
1 ng	12 - 15
100 pg	16 - 18

Note:

1. Table 4 shows the number of loop parameters using high-quality Input DNA tests of around 200 bp.The FFPE DNA quality varies greatly, and when the DNA quality is poor or the library length is long, the number of cycles needs to be appropriately increased to obtain sufficient libraries.
2. If size selection is required during the library building process, higher cycle number for Library Amplification is recommended; otherwise, lower cycle number is recommended.
3. If incomplete adapters are used, at least 2 cycles need to be amplified to form a complete adapter.

6. About Library Quality Analysis

1) The constructed libraries quality is generally analyzed by measuring the concentrations and size distributions.
2) Libraries'concentrations can be measured by fluorescent-based methods such as Qubit and PicoGreen or qPCR.
3) It is NOT recommended to use absorbance-based quantification methods such as NanoDrop.
4) It is recommended to use qPCR method for library quantification: fluorescent-based methods such as Qubit and PicoGreen cannot differentiate the incomplete dsDNA structures (inserts with no adapter or with only one of the ends ligated with adapter) from the complete libraries. The qPCR method will only amplify and measure the complete libraries with both ends ligated with adapters (the sequencable libraries), thus providing a more accurate measurement for loading.
5) The size distribution of libraries can be analyzed using Agilent Bioanalyzer or other devices based on the principles of capillary electrophoresis or microfluidics.

7. Other Materials

1) Purification Beads: NGS DNA Selection Beads (Cat.No.: HYD511); or AMPure® XP Beads (A63880); or other equivalent products.
2) DNA Quality Control: Agilent 2100 Bioanalyzer or other equivalent products.
3) Adapters: For detailed information, refer to Section 3: Adapter Ligation in the Notes above.
4) Other materials: absolute ethanol, sterile ultrapure water, low retention pipette tips, PCR tube, magnetic stands, thermal cycler, etc.

Instructions

Step 1. End Repair/dA-Tailing

1. Thaw the reagents mentioned in Table 5 . Invert to thoroughly mix the reagents and place them on ice for later use.
2. Assemble the reagents according to Table 5 on ice.

Table 5 End Repair/ dA-Tailing reaction system

Components	Volume
Fragmented DNA	x μL
DNA End Repair Buffer V2	7 μL
DNA End Repair Enzyme Mix V2	3 μL
ddH_2O	Up to 60 μL

3. Gently mix by pipetting or shaking, Centrifuge briefly to get the solution down.
4. Place the tube in a thermocycler and set the program according to table 6 .

Table 6 End Repair/dA-Tailing reaction program

Temperature	Time
Heat lid to 105 °C	On
30 °C	30 min
72 °C	30 min
4 °C	Hold

Step 2. Adapter Ligation

1. Dilute the adapter to the appropriate concentration according to Table 2-3.
2. Thaw the reagents mentioned in Table 7. Invert to thoroughly mix the reagents and place them on ice for later use.
3. Assemble the reagents according to Table 7 on ice.

Table 7 Adapter Ligation reaction system

Components	Volume
dA-tailed DNA（Product from step 1）	60 μL
Ligation Enhancer V2	30 μL*

DNA Adapter	5 μL**
Rapid T4 DNA Ligase V2	10 μL
ddH_2O	5 μL

*The Ligation Enhancer is viscous. Please mix thoroughly by inverting or vortexing and briefly centrifuge before use.

**The original concentration of the Illumina adapter of Hyasen is 15 μM.The original concentration of the MGI adapter of Hyasen is 10 μM. Please dilute the adapter according to the input amount and make the volume of the adapter fixed at 5 μL.

4. Mix thoroughly by gently pipetting up and down , and spin down briefly to collect all liquid from the sides of the tube.
5. Incubate the sample in a preheated thermal cycler as shown in Table 8 and perform the adapter connection reaction.

Table 8 Adapter Ligation reaction program

Temperature	Time
Heat lid to 105°C	Off
20°C	15 min
4°C	Hold

Step 3. Clean-up or Size-selection post Adapter Ligation

This step is to purify or size-select the product in step 2 with magnetic beads. The purification can remove residue adapters, adapter dimers, or other unusable products.

Clean up of Adapter-ligated DNA

1. Preparation: take the NGS DNA Selection Beads out of the fridge, and equilibrate at room temperature for at least 30 minutes. Prepare 80% ethanol freshly.
2. Thoroughly mix the beads by inverting or vortexing.
3. Add 88 μL NGS DNA Selection Beads (0.8×, Beads : DNA = 0.8:1) to the tube containing the adapter-ligated product, shake and mix well, and incubate at room temperature for 5 minutes.
4. Centrifuge briefly to get the solution down, and place the centrifuge tube on the magnetic rack. After the magnetic beads are completely adsorbed (about 5 min), carefully remove the liquid.
5. Keep the tube on the magnetic stand, directly add 200 μL freshly prepared 80% ethanol to the tube. Incubate at room temperature for 30 seconds and carefully remove the liquid.

6. Repeat step 5 again.
7. Keep the tube on the magnetic stand, open the cap and dry the beads until the beads are just cracked (no more than 5 minutes).
8. Take the tube off the magnetic stand for elution and elute the DNA

1) If the product does not need to be size selected, add 21 μL ddH_2O directly. Mix thoroughly by vortexing or pipetting up and down 10 times. Incubate at room temperature for 5 min. Spin the tube briefly and place it on magnetic stand. When the solution is clear (about 5 min), transfer 20 μL of supernatant to a new PCR tube carefully without touching the magnetic beads.
2) If the product needs to be size selected, add 102 μL ddH_2O directly. Mix thoroughly by vortexing or pipetting up and down 10 times. Incubate at room temperature for 5 min. Spin the tube briefly and place it on magnetic stand. When the solution is clear (about 5 min), transfer 100 μL of supernatant to a new PCR tube carefully without touching the magnetic beads.
   Note: If the purified product needs to be stored, it can be eluted with TE Buffer.

Size Selection of Adapter-ligated DNA

1. Preparation: take the NGS DNA Selection Beads out of the fridge, and equilibrate at room temperature for at least 30 minutes. Prepare 80% ethanol freshly.
2. Thoroughly mix the beads by inverting or vortexing.
3. Based on the targeted sizes, add the first round of beads to the 100 μL purified DNA templates according to Table 9. Mix thoroughly by vortexing or pipetting 10 times.

Table 9 Recommended Beads:DNA ratios for beads-based size selection

Inserted DNA library size	**150 - 250 bp**	**200-300 bp**	**300-400 bp**	**400-500 bp**
Final DNA library size	**250-350 bp**	**350-450 bp**	**450-550 bp**	**550-650 bp**
Volume ratio in the 1st round (Beads:DNA)	0.80×	0.70×	0.60×	0.55×
Volume ratio in the 2nd round (Beads:DNA)	0.20×	0.20×	0.20×	0.15×

Note: "×" in the table indicates the volume of DNA sample. For example, if the insert length of the library is 250 bp and the sample DNA volume is 100 μL, the volume of magnetic beads used in the first round of sorting is 0.7×100 μL=70 μL; the volume of magnetic beads used in the second round of sorting is 0.20×100 μL=20 μL. The recommended bead volume in the table is for the adapter-ligated DNA. If size selection procedure is performed before ligation, please refer to the manufacturer's protocols of NGS DNA Selection Beads.

4. Incubate at room temperature for 5 minutes.

5. Spin the tube briefly and place it onto magnetic stand. When the solution is clear (about 5 min), transfer the supernatant to a new PCR tube.
6. Add the second round of selection beads to the sample from step 5 according to Table 11. Mix thoroughly by vortexing or pipetting up and down at least 10 times.
7. Incubate at room temperature for 5 minutes.
8. Centrifuge briefly to get the solution down, and place the centrifuge tube on the magnetic rack. After the magnetic beads are completely adsorbed (about 5 min), carefully remove the liquid.
9. Keep the tube on the magnetic stand, directly add 200 μL freshly prepared 80% ethanol to the tube. Incubate at room temperature for 30 seconds and carefully remove the liquid.
10. Repeat step 9 again.
11. Keep the tube on the magnetic stand, open the cap and dry the beads until the beads are just cracked (no more than 5 minutes).
12. Take the tube off the magnetic stand for elution, and directly add 21 μL ddH_2O. Thoroughly mix by vortexing or pipetting up-and-down and incubate at room temperature for 5 minutes. (Note: if need to store the purified product, please elute in TE Buffer.) Briefly spin down the tube and place it on a magnetic stand until the liquid becomes clear (about 5 minutes). Carefully transfer 20 μL supernatant to a new PCR tube without touching the beads.

Step 4 Library Amplification

This step can enrich the purified or size-selected products by PCR amplification.

1. Thaw the reagents list in table 10, invert and mix thoroughly, and place them on ice for later use.
2. Assemble the following reaction in a sterilized PCR tube.

Table 10 adapter-ligated DNA PCR reaction system

Components	Volume
Adapter Ligated DNA	20 μL
2 × NGS Library Amplification Mix	25 μL
Primer mix*	5 μL
Total	50 μL

* if the complete adapter was used, DNA Library Prep Primer Mix in needed; If an incomplete adapter is used , please refer to the kit instructions and use the Index Primer provided in the kit for amplification.

3. Gently mix by pipetting or shaking, and centrifuge briefly to get the solution down.

4. Put the tube into a thermocycler and set up the program according to table 11 to start the amplification.

Table 11 PCR amplification reaction program

Temperature	Time	Cycle
Heat lid to 105°C	On	-
98°C	45 s	1
98°C	15 s	Refer to table 4
60°C	30 s	
72°C	30 s	
72°C	1 min	1
4°C	Hold	-

Step 5 Post-Amplification Clean-up/Size Selection

The clean up steps refer to step 3. NGS DNA Selection Beads (1×, Beads:DNA=1:1) is used to purify the PCR product. If size selection is needed, please refer to step 3.

Step 6 Quality Control of the Final Libraries

The quality of the constructed library is generally evaluated by measuring the concentration and size distribution. For details, please refer to Note 6.