Hieff NGS™ Ultima Pro DNA Library Prep Kit for Illumina V2

12197

INSTRUCTIONS FOR USE

Ver. HB221028

CONTENT

Product description

Hieff NGS™ Ultima Pro DNA Library Prep Kit for Illumina V2 is a new generation library construction kit specially developed and designed for the Illumina high-throughput sequencing platform. On the basis of the previous generation of library construction 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. Compatible with 100 pg - 1000 ng DNA samples of most types, including cfDNA and FFPE samples.With an industry-leading library conversion rate of more than 70%.Proven to provide high-quality libraries and sequencing data.Strict batch stability.

Components

Components No.	Name		12197ES08 (8T)	12197ES24 (24T)	12197ES96 (96T)
12197-A		Endprep Buffer 2.0	100 μL	400 μL	4 mL
12197-B		Endprep Enzyme 2.0	48 μL	144 μL	576 μL
12197-C		Ligation Enhancer 2.0	32 μL	96 μL	384 μL
12197-D		Rapid T4 DNA Ligase 2.0	240 μL	720 μL	3×960 μL
12197-E		CanaceTM Pro Amplification Mix 2.0	40 μL	120 μL	480 μL
12197-F		Primer Mix	200 μL	600 μL	3×800 μL

Specifications

Product Type	Library Preparation Kit
Libraries	Fragment Library
Fragmentation method	Ultrasonic
Input amount	100 pg - 1000 ng
For Use With (Equipment)	Illumina Platforms
Sample Type	gDNA
Sequencing Type	Genome & DNA Sequencing
Product Line	DNA library constrction
For Use With (Application)	NGS library preparation
Quantity	8 /24/96 Reactions

Shipping and Storage

Dry ice shipping. -15°C ~ -25°C storage, valid for one years.

Instructions

1. Required Materials Not Included

1.1 Purification beads: Hieff NGS™ DNA Selection Beads (Cat#12601), AMPure XP Beads (Cat#A63880), or equivalent products.

1.2 DNA size analysis: Agilent Technologies 2100 Bioanalyzer or equivalent devices.

1.3 DNA Adapter: Choose YEASEN 96 Single index complete Adapters (Cat#13519-Cat#13520); 384 Dual CDI Primers (Cat#12412~Cat#12413)； 384 Unique Dual Index (UDI) Primers(Cat#12404~Cat#12407);96 UMI UDI Adapters for illumina(Cat#13370~Cat#13371)

1.4 Other materials: ethanol, sterilized ultra-pure water, TE Buffer (10 mM Tris-HCl, pH 8.0-8.5; 1 mM EDTA), Eppendorf tubes, PCR tubes, magnetic stands, thermocyclers, etc.

2. Operation flowchart

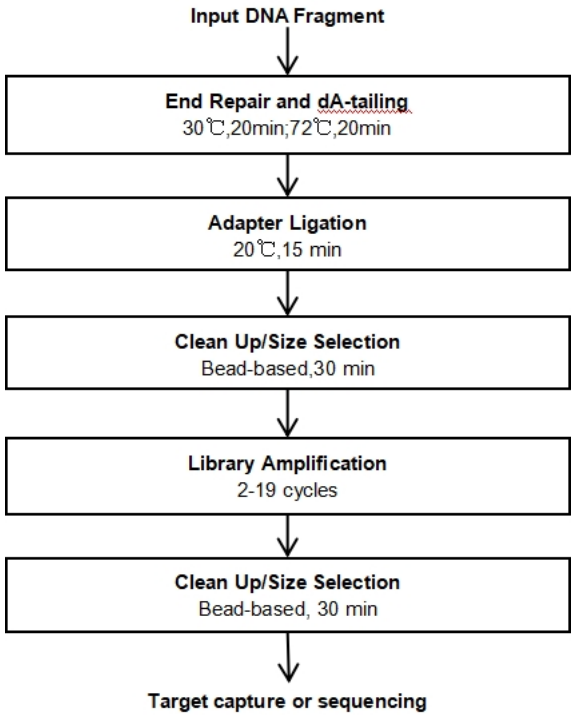

Figure 1 Flowchart of library construction using Ultima Pro DNA library prep kit for Illumina

3. Operation steps

3.1 End Repair/dA-Taling

During this step, the input DNA fragments will be blunted, 5’ phosphorylated, and dA-tailed at the 3’ ends.

3.1.1 Thaw the reagents mentioned in Table 4. Invert to thoroughly mix the reagents and place them on ice;

3.1.2 Assemble the reagents in Table 1 on ice:

Table 1 The reaction system for End Repair/ dA-Tailing

Components	Volume (μL)
Fragmented DNA	x
Endprep Buffer 2.0	6
Endprep Enzyme	4
ddH_2O	Up to 60

3.1.3 Mix thoroughly by vortexing or pipetting several times. Spin the reaction solution briefly to the bottom of the tube.

3.1.4 Place the PCR tube in a thermocycler and run the reaction programs mentioned in Table 2.

Table 2 The reaction programs for End Repair/dA-Tailing

Temperature	Time

Hot lid 105°C	On
30°C	20 min
72°C	20 min
4°C	Hold

3.2 Adapter Ligation

During this step, the dA-tailed templates will be ligated with Illumina adapters.

3.2.1 Dilute the adapter to the appropriate concentration according to Table 3.

3.2.2 Thaw the reagents mentioned in Table 6. Mix thoroughly and place them on ice for later use.

3.2.3 Add the following reagents to the product of step 3.1:

Table 3The reaction system for Adapter Ligation

Components	Volume (μL)
dA-tailed DNA (Product from step 3.1)	60
Ligation Enhancer	30*
DNA Adapter	5**
Novel T4 DNA Ligase	5

Note: *The Ligation Enhancer is viscous. Please mix thoroughly by inverting or vertexing and briefly spin down before use.

**The concentration of the Adapter that comes with this kit is 15 μM, which is the same as most commercialized adapters currently available. Please dilute the adapters according to Table 2 and make the volume up to 5 μL with ultra-pure water.

3.2.4 Mix thoroughly by vortexing at low speed or pipetting several times. Spin the reaction solution briefly to the bottom of the tube.

3.2.5 Place the PCR tube in a thermocycler and run the reaction programs mentioned in the Table 4.

Table 4 The reaction programs for Adapter Ligation

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

Note: If low ligation efficiency is observed for low input DNA, the ligation time can be doubled for better performance.

3.3 Clean-up or Size-selection post Adapter Ligation

This step is to clean-up or size-select the product from the previous step (step 3.2) with magnetic beads to remove unligated adapters, adapter dimers, or other unusable templates.

3.3.1 Cleanup of Adapter-ligated DNA

1) Equilibrate the Hieff NGS™ DNA Selection Beads at room temperature (~ 30 min). Prepare 80% ethanol.

2) Resuspend the beads thoroughly by vortexing or shaking the bottle.

3) Add 60 μL Hieff NGS™ DNA Selection Beads (0.6×, Beads:DNA=0.6:1) to the adapter ligation product. Mix thoroughly by vortexing or pipetting up and down 10 times. Incubate at room temperature for 5 min.

4) Spin the tube briefly and place it onto magnetic stand. When the solution is clear (about 5 min), aspirate the supernatant and discard.

5) Keep the tube on the magnetic stand, add 200 μL of freshly prepared 80% ethanol to rinse the magnetic beads, and incubate at room temperature for 30 sec. Aspirate the ethanol and discard.

6) Repeat step 5) once for a total of two washes.

7) Remove residual ethanol with a 10 μL - pipette tip. Keep the tube in the magnetic stand, open the lid and air-dry the beads until cracks just appear (about 5 min).

8) Remove the tube from the magnetic stand and elute the DNA:

- 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.
- 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.

3.3.2 Size Selection of Adapter-ligated DNA

1) Equilibrate the Hieff NGS™ DNA Selection Beads at room temperature for at least 30 min. Prepare 80% ethanol.

2) Resuspend the beads thoroughly by vortexing or shaking the bottle.

3) Based on the targeted sizes, add the first round of beads to the 100 μL purified DNA templates according to Table 5. Mix thoroughly by vortexing or pipetting 10 times.

Table 5 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 manufactural protocols of Hieff NGS™ DNA Selection Beads (Cat#12601).

4) Incubate at room temperature for 5 min.

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 8. Mix thoroughly by vortexing or pipetting up and down at least 10 times.

7) Incubate at room temperature for 5 min.

8) Spin the tube briefly and place it onto magnetic stand. When the solution is clear (about 5 min), aspirate the supernatant and discard.

9) Keep the tube on the magnetic stand. Add 200 μL of freshly prepared 80% ethanol to rinse the magnetic beads and incubate at room temperature for 30 sec. Aspirate the ethanol and discard.

10) Repeat step 9) once for a total of two washes.

11) Remove residual ethanol with a 10 μL - pipette tip. Keep the tube in the magnetic stand, open the lid and air-dry the selection beads until cracks just appear (about 5 min).

12) Remove the tube from the magnetic stand. Add 21 μL ddH_2O and mix thoroughly by vortexing or pipetting up and down 10 times. Incubate at room temperature for 5 min.

13) 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.

3.4 Library Amplification

In this step, the adapter-ligated DNA is amplified with PCR amplification after cleanup or size selection.

3.4.1 Thaw the reagents mentioned in Table 6. Mix thoroughly and place them on ice for later use.

3.4.2 Add the following reagents to the product of step 3.3:

Table 6 Reaction system for PCR amplification

Components	Volume (μL)
Adapter Ligated DNA (Product from step 3.3)	20
Canace™ Pro Amplification Mix	25
Primer mix	5

【Note】:* if the complete adapter (Cat#13519~Cat#13520) was used, Primer Mix in the kit was used for amplification; If an incomplete adapter is used (Cat#12412~Cat#12413, Cat#12404~Cat#12407, Cat#13370~Cat#13371), please refer to the kit instructions and use the Index Primer provided in the kit for amplification.

3.4.3 Mix thoroughly by vortexing or pipetting several times. Spin the reaction solution briefly to the bottom of the tube.

3.4.4 Place the PCR tube to a thermocycler and run the reaction programs in Table 7.

Table 7 Reaction programs for PCR amplification

Temperature	Time	Number of cycles
98°C	1 min	1
98°C	10 sec	Refer to Table 3
60°C	30 sec	
72°C	30 sec	
72°C	5 min	1
4°C	Hold	-

3.5 Cleanup and Size-Selection of PCR product

The cleanup steps refer to 3.3.1. Hieff NGS™ DNA Selection Beads (0.9×, Beads:DNA=0.9:1) is used to purify the PCR product. If size selection is needed, please refer to 3.3.2.

3.6 DNA Library Quality Control

The quality of the constructed DNA library can be evaluated by concentration detection and size distribution detection. For details, please refer to Note 6.

Notes

1. Operation

1.1 For your safety and health, please wear lab coats and disposable gloves for operation.

1.2 Thaw components at room temperature. Once the components are thawed, mix thoroughly by vortexing, spin the tube briefly and place them on ice for later use.

1.3 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.

1.4 It is highly recommended to use filtered pipet tips to avoid cross-contamination. Be sure to change pipet tips when processing different samples.

1.5 It is recommended to perform each reaction step in a thermocycler with a heated lid. The thermocycler should be preheated to the set temperature before use.

1.6 Improper operations may very likely cause carry-over contaminations through aerosols, impacting the experiment's accuracy. It is highly recommended to divide the experiment environment into the pre-PCR and post-PCR regions, with separate sets of devices and disposables in each area. Perform routine cleaning for each area by wiping the surfaces with 0.5% sodium hypochlorite or 10% bleach.

1.7 This product is used for scientific research purposes only!

2. DNA Fragmentation

2.1 This kit is compatible with either mechanically fragmented DNA or enzymatically fragmented DNA;

2.2 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 8 lists the recommended amount of Input DNA.

Table 8 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 sequencing	FFPE DNA	50 ng-1000 ng
Targeted 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.

2.3 "Input DNA" specifically refers to the DNA samples ready for end repair/dA tailing.

2.4 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. Adapter Ligation

3.1 YEASEN provides the following barcoded adapters and index primers developed for the Illumina platform: 96

Single index complete Adapters (Cat#13519-Cat#13520); 384 Dual CDI Primers(Cat#12412~Cat#12413)；384 Unique Dual Index (UDI) Primers(Cat#12404~Cat#12407);96 UMI UDI Adapter（Cat#13370~Cat#13371）.

3.2 The quality and concentration of adapters 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 9 lists the recommended Adapter dilution methods for different Input DNA amounts using this kit.

Table 9 Recommended adapter: insert molar ratios for 100 pg-1000 ng Input DNA

Input DNA	Adapter Dilution(Volume of Adapter : Total Volume)	Concentration
0.1ng ~ 1 ng	150-Fold (1 : 150)	0.1 μM
1 ng ~ 10 ng	75-Fold (1 : 75)	0.2 μM
10 ng ~ 25 ng	15-Fold (1 : 15)	1 μM
25 ng ~ 100 ng	7.5-Fold (1 : 7.5)	2 μM
100 ng ~ 1000 ng	3-Fold (1 : 3)	5 μM

4. Bead-based Clean-Up and Size-Selection

4.1 DNA size-selection can be performed before end repair/dA-tailing, after adapter ligation, or after amplification.

4.2 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.

4.3 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.

4.4 The magnetic beads should be equilibrated at room temperature before use, otherwise the yield will decrease, and the effect will be affected.

4.5 Please thoroughly mix the beads before each use by vortexing or pipetting up-and-down.

4.6 Please be sure not to take the beads when transferring the supernatant, even trace amounts of the beads may impact the following reactions.

4.7 The 80% ethanol used for magnetic bead rinsing should be freshly prepared, otherwise it will affect the recovery efficiency.

4.8 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.

4.9 The magnetic beads should be dried at room temperature before the product is eluted. Insufficient drying will easily cause residual ethanol to affect subsequent reactions; excessive drying will cause the magnetic beads to crack and reduce the purification yield. Normally, drying at room temperature for 3-5 min is enough to allow the beads to fully dry.

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

5. Library Amplification

5.1 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.

5.2 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 10 lists the recommended cycle numbers to obtain 1000 ng of library yield.

Table 10 The recommended number of cycles to generate 1,000 ng 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 3 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. Library Quality Analysis

6.1 The constructed libraries' quality is generally analyzed by measuring the concentrations and size distributions.

6.2 Libraries' concentrations can be measured by fluorescent-based methods such as Qubit and PicoGreen or qPCR

6.3 It is NOT recommended to use absorbance-based quantification methods such as NanoDrop.

6.4. 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.

6.5 The size distribution of libraries can be analyzed using Agilent Bioanalyzer or other devices based on the principles of capillary electrophoresis or micro-control flow.

To enable success of our customers
Together to make a healthier and brighter world

Yeasen Biotechnology (Shanghai) Co., Ltd

Add: No.166, Tianxiong Road, Pudong New Area, Shanghai, China, 201318
Web: www. yeasenbiotech.com Tel: +86-021-34615995
E-mail: overseas@yeasen.com