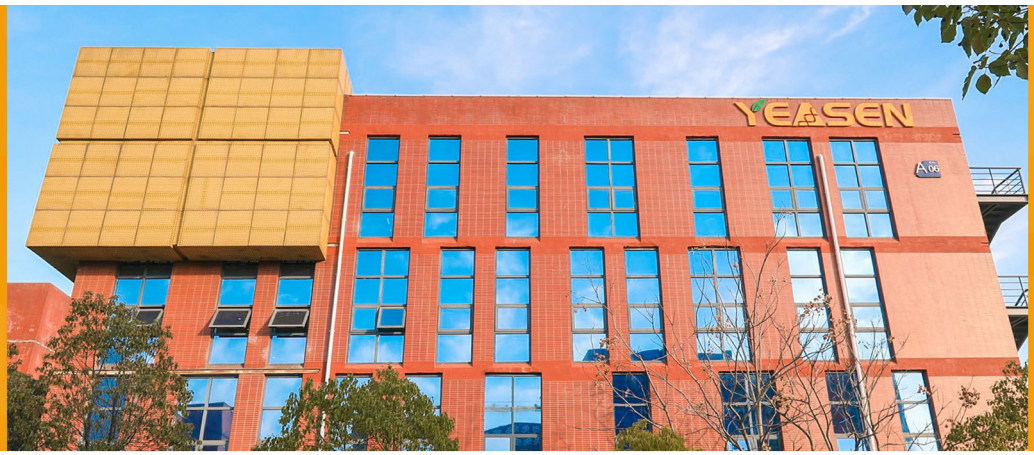
HB220331

Hieff NGS™ Ultima DNA Library Prep Kit for MGI®

Cat# 13310



INSTRUCTION FOR USE

Yeasen Biotechnology (Shanghai) Co., Ltd.

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Product Information

Product Name	Cat#	Specification
Hieff NGS™ Ultima DNA Library Prep Kit for MGI®	13310ES16	16 T
	13310ES96	96 T

Product Description

Hieff NGS™ Ultima DNA Library Prep Kit for MGI® is a new generation library construction kit specially developed and designed for the MGI® high-throughput sequencing platform. As a new upgrade version, the high-quality enzymatic composition and simplified operation process included in this kit can significantly improve the library conversion rate and amplification efficiency. Hieff NGS™ Ultima DNA Library Prep Kit for MGI® has a wide range of sample adaptability, while compatible with FFPE, cfDNA, ChIP DNA and other samples, help to obtain excellent sequencing data.

- Suitable for 500 pg-1μg DNA samples
- Compatible with cfDNA, FFPE and other low-quality samples
- Efficient library conversion rate and amplification efficiency
- Excellent library and sequencing data obtainment verified by multi-sample experiments
- Strict batch performance and stability and quality control

Product Components

Component Number and Name			13310ES16	13310ES96
13310-A	🟡	Endprep Mix	160 μL	960 μL
13310-B	🔵	Ligation Enhancer	480 μL	4×720 μL
13310-C	🔵	Fast T4 DNA Ligase	80 μL	480 μL
13310-D	⚪	2×Ultima HF Amplification Mix	400 μL	4×600 μL
13310-E	⚪	Primer Mix for MGI™	80 μL	480 μL

Shipping and Storage

All the components are shipped with dry ice and can be stored at -20°C for one year.

Cautions

1 Operation

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

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.

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 500 pg-1 μg 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

Applications	Sample type	Input DNA
WGS	Complex genome	50 ng-1 μg
Targeted capture sequencing	Complex genome	10 ng-1 μg
WGS, Targeted sequencing	FFPE DNA	≥50 ng
Targeted sequencing	cfDNA/ctDNA	≥500 pg
WGS	microbial genomes	≥1 ng
WGS (PCR-free)	High-quality input DNA	≥100 ng
Immunoprecipitation sequencing	ChIP-DNA	≥500 pg
Targeted sequencing	amplicon	≥500 pg

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 It is recommended to process a beads-purification/size-selection after the fragmentation for the high concentrations of salts like the metal-chelating agent in the DNA sample might impact the efficiencies 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 At present, MGI has two kinds of serial number joints: 1-128 and 501-596. For its use requirements, please refer to the "use of Adapter" or consult the company. In addition, MGI stated that the two kinds of joints are prohibited mixed use due to different design processes, otherwise the sequencing data cannot be split!

3.2 The adapters’ quality and concentration will directly affect the ligation efficiency and the library yield. Please indeed determine the joint amount according to Table 2 and the actual DNA input. If the joint dilution is required, please dilute the joint with TE buffer.

Table 2 Recommended adapter: insert molar ratios for 500 pg-1 μg Input DNA

Input DNA	10μM Adapter dilution ratio	Adapter input（μL）
31ng-1 μg	/	5
11-30 ng	5	5
3-10 ng	10	5
0.5-2 ng	20	5

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 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 The necessity of library amplification depends on the amount of DNA input, the sequencing data applications, etc. The amplification step is required if using partial 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 3 lists the recommended cycle numbers to obtain 100 ng or 1000 ng of library yield.

Table 3 The recommended number of cycles to generate 500 pg-1 μg of library yield

Input DNA	Number of cycles required to generate	
	100 ng	1,000 ng
1 μg	0	3 - 4
500 ng	0	4 - 5
250 ng	1 - 4	5 - 7
100 ng	2 - 5	6 - 8
50 ng	4 - 7	8- 10
10 ng	6 - 8	9 - 12
5 ng	7 - 10	11 - 14
1 ng	9 - 11	12 - 15
500 pg	11 - 13	14 - 16

Note: If size-selection is performed before amplification, choose the higher end of the recommended cycle numbers.

6 Library Quality Analysis

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

6.2 Library 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™ can't 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 libraries that can be sequenced), thus providing a more accurate measurement for loading.

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

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: For details, please consult Huada Intelligent or our company.

1.4 Other materials: ethanol, sterilized ultra-pure water, TE Buffer (10 mM Tris-HCl, pH 8.0-8.5; 0.1 mM EDTA), Eppendorf tubes, PCR tubes, magnetic stands, thermocyclers, etc.

2 Operation flowchart

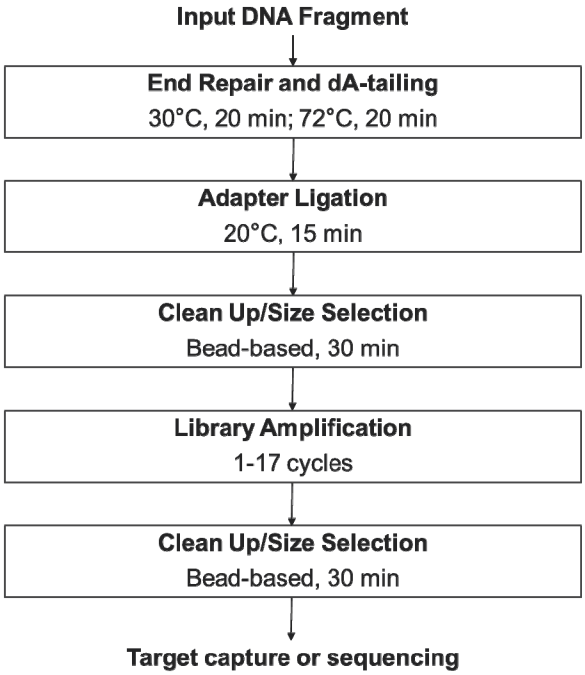

Figure 1 Flowchart of library construction using Ultima Pro DNA library prep kit for Illumina

3 Operation steps

3.1 End Repair/dA-Taling

After 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 for later use.

3.1.2 Assemble the reagents in Table 4 on ice:

Table 4 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 5.

Table 5 The reaction programs for End Repair/dA-Tailing

Temperature	Duration
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 2.

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 6 The reaction system for Adapter Ligation

Components	Volume (μL)
dA-tailed DNA	60
Ligation Enhancer	30*
Fast T4 DNA Ligase	5
DNA Adapter	5

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

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 7.

Table 7 The reaction programs for Adapter Ligation

Temperature	Duration
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% ethonal.

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

3) Add 80 μL Hieff NGS™ DNA Selection Beads (0.8×, Beads: DNA=0.8:1) 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 8. Mix thoroughly by vortexing or pipetting 10 times.

Table 8 Recommended Beads:DNA ratios for beads-based size selection

Average length of Input DNA	**150 - 250 bp**	**200-300 bp**	**300-400 bp**
Average length of DNA Library	**230 - 330 bp**	**280-380bp**	**380-480bp**
1st volume ratio（Beads:DNA）	0.78×	0.68×	0.58×
2nd volume ratio（Beads:DNA）	0.20×	0.20×	0.20×

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 in 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 the 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 9. 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 9 Reaction system for PCR amplification

Components	Volume (μL)
2×Ultima HF Amplification Mix	25
Primer Mix for MGI™	5
Adapter Ligated DNA（Product from step 3.3）	20

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 10.

Table 10 Reaction programs for PCR amplification

Temperature	Duration	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.

3.7 Library cyclization

Library cyclization reaction was performed using Hieff NGS™ Fast-Pace DNA Cyclization Kit for MGI™ (Cat # 13341) or other equivalent products.

Memo:

BRING VALUES TO CUSTOMERS
BUILD A HEALTHIER AND HAPPIER WORLD

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