Hieff NGS™ OnePot Pro DNA Library Prep Kit V3

12194ES

INSTRUCTIONS FOR USE

Ver. EN20240605

CONTENT

Product description

Hieff NGS™ OnePot Pro DNA Library Prep Kit V2 is a new generation enzymatic fragmentation-based library prep kit specially developed and designed for Illumina &MGI sequencing platform. Compared to traditional library construction methods, this product employs high-quality fragmentation enzymes, eliminating the cumbersome ultrasonic process. It simplifies the operation by combining the fragmentation and end-repair modules into one. Additionally, the enzymes and buffer for the ligation module are pre-mixed, significantly reducing the time and cost of library construction. This makes it more suitable for automated library construction.. This library prep kit has an excellent library conversion rate and is applicable for samples from all common animals, plants, microorganisms, etc., and also the FFPE samples. On the basis of the previous generation of library construction kit, this product exhibits higher efficiency in fragmentation, end repair, dA-tailing, and adapter ligation than the previous versions. The high-fidelity enzyme significantly improves the uniformity and fidelity of amplification.

Specifications

Cat.No.	12194ES08 / 12194ES24 / 12194ES96
Size	8 T / 24 T / 96 T

Components

Components No.		Name	12194ES08	12194ES24	12194ES96
12194-A		Smearase™ Buffer 3.0	80 µL	240 µL	960 µL
12194-B		Smearase™ Enzyme 3.0	80 µL	240 µL	960 µL
12194-C		Ligation Ready Mix	200 µL	600 µL	3×800 µL
12194-D		2× Ultima HF Amplification Mix	200 µL	600 µL	3×800 µL

[**Note**]: The kit components are compatible with both Illumina &MGI sequencing platform, if the complete adapter was used, Hieff NGS™ Primer Mix (Yeasen Cat#12190 or Cat#12191) in needed.

Storage

This product should be stored at -25~-15°C for 1 year.

Notes

一、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 construction.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.

二、DNA Fragmentation

1. 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.

2. Following experiments could be impacted if high concentrations of salts like the metal chelating agent were introduced with the input DNA. We recommend eluting the DNA sample in ddH_2O for fragmentation.

3. Please refer to table 6 for the fragmentation time of standard DNA samples. The kit has low fragmentation bias and provides uniform GC coverage for DNA samples with a wide range of GC compositions. Please adjust the fragmentation time based on your experimental requirements.

4. For accurate fragmentation, please prepare the reaction on ice.

三、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. 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 1-2 lists the recommended dilution methods for conventional and UMI Adapters for different amounts of Input DNA using this kit for the Illumina or MGI sequencing platforms.

Table 1 The recommended Illumina adapter amount for different input DNA

Input DNA	Conventional adapter dilution ratio	Concentration	UMI adapter dilution ratio	Concentration
＜1 ng	7.5-Fold	2 μM	15-Fold	1 μM
1 ng ~ 10 ng	3-Fold	5 μM	3-Fold	5 μM
10 ng ~ 200 ng	1.5-Fold	10 μM	2-Fold	7.5 μM
＞200 ng	0-Fold	15 μM	0-Fold	15 μM

Table 2 The recommended MGI adapter amount for different input DNA

Input DNA	Conventional adapter dilution ratio	Concentration	UMI adapter dilution ratio	Concentration
＜1 ng	5-Fold	2 μM	10-Fold	1 μM
1 ng ~ 10 ng	2-Fold	5 μM	2-Fold	5 μM
10 ng ~ 200 ng	0-Fold	10 μM	1.25-Fold	8 μM
＞200 ng	0-Fold	10 μM	0-Fold	10 μM

四、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.

五、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 3 lists recommended cycle numbers targeting the library yield of 1 µg.

Table 3 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
1000-2000 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.

六、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.

七、Other Materials

1. DNA purification magnetic beads: Hieff NGS™ DNA Selection Beads (Yeasen Cat#12601) or AMPure® XP Beads (A63880) or other equivalent products.

2. Adapters: Complete Adapter for Illumina: Yeasen Cat#13519-13520; 384 Dual CDI Primers: Yeasen Cat#12412~Cat#12413; 384 Unique Dual Index (UDI) Primers: Yeasen Cat#12312~Cat#12315; UMI UDI Adapters: Yeasen Cat#13370~Cat#13371; Complete Adapter for MGI: Yeasen Cat#13360-13362. DNA Primer Mix：Cat#12190 or Cat#12191.

3. Library quality analysis: Agilent 2100 Bioanalyzer DNA 1000 Chip/ High Sensitivity Chip or other equivalent products; library quantitative reagents.

4. Other materials: absolute ethanol, sterile ultrapure water, low retention pipette tips, PCR tube, magnetic stands, thermal cycler, etc.

八、Workflow

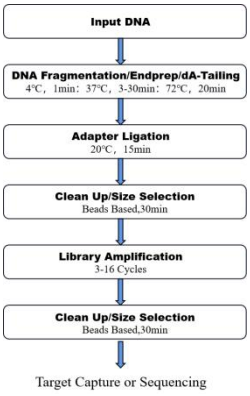

Figure 1. The workflow of OnePot Pro DNA Library Prep Kit

Step 1. DNA Fragment/End Repair/dA-Taling

1. Thaw the reagents mentioned in Table 4 . Invert to thoroughly mix the reagents and place them on ice for later use.
2. Assemble the reagents according to Table 4 on ice.

Table 4 DNA Fragmentation/End Repair/ dA-Tailing reaction system

Components	Volume (μL)
Input DNA	x
Smearase™ Buffer 3.0	10
Smearase™ Enzyme 3.0	10
ddH_2O	Up to 60

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 5 .

Table 5 DNA Fragmentation/End Repair/dA-Tailing reaction program

Temperature	Time
Heat lid to 105°C	On
4 °C	1 min*
37°C/35°C/32°C	3-30 min**
72 °C	20 min
4 °C	Hold

Note: *Pre-set the program to 4°C to control the fragmentation performance effectively and to avoid over-fragmentation. Please place the reaction tube into the thermocycler after the heat block is cooled to 4°C.

**Please refer to table 6 for the fragmentation of intact genomic DNA.

Table 6 Fragmentation time for standard DNA

Time / Insert peak size / Temperature	5min	10min	15min	20min	30min
32°C	>700	300~400	250	200	180
35°C	>700	300~400	200~250	200	180
37°C	500~700	200~300	200	180~200	180

Step 2. Adapter Ligation

1. Dilute the adapter to the appropriate concentration according to Table 1.
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 (μL)
dA-tailed DNA（Product from step 1）	60
Ligation Ready Mix	25*
DNA Adapter	5**
Total	90

Note: *Ligation Ready Mix is viscous. Please mix thoroughly by inverting or vertexing and briefly centrifuge before use.

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

**The original concentration of the MGI adapter of YEASEN 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

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

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 Hieff 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 vertexing.

3. Add 72 μL Hieff NGS™ DNA Selection Beads (0.8 ×, Beads : DNA = 0.8:1) to the tube containing the adapter-ligated product, shack 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 Hieff 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 vertexing.

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	500-600 bp
Final DNA library size	250-350 bp	350-450 bp	450-550 bp	550-650 bp	650-750 bp
Volume ratio in the 1 st round (Beads:DNA)	0.80×	0.70×	0.60×	0.55×	0.50×
Volume ratio in the 2 nd round (Beads:DNA)	0.20×	0.20×	0.20×	0.15×	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 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 9. 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 (μL)
Adapter Ligated DNA	20
2× Ultima HF Amplification Mix	25
Primer mix*	5
Total	50

[Note]: * if the complete adapter was used, Hieff NGS™ Primer Mix (Yeasen Cat#12190 or Cat#12191) in needed; If an incomplete adapter is used (Cat#12412~Cat#12413, Cat#13370~Cat#13371), 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	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	-

Step 5 Post-Amplification Clean-up/Size Selection

The clean up steps refer to step 3. Hieff 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.

Note:

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