YEASEN

Hieff NGS™ OnePot II DNA Library Prep Kit for MGI

13321

INSTRUCTIONS FOR USE

Ver. HB220406

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Product description

Hieff NGS™ OnePot II DNA Library Prep Kit for MGI is a new generation of library prepare kit specially designed for MGI sequencing platform, which contains high quality enzyme for DNA fragmentation. This kit combines fragmentation, end-repair, and dA-tailing into one step, significantly reducing time and cost of library preparation. The kit has a high library conversion rate and can be applied for most DNA samples from animals, plants, and microorganisms, including low-quality samples such as FFPE samples.

Components

Components No.	Name		13321ES16	13321ES96
13321-A		Smearase™ Mix	160 μL	960 μL
13321-B		Ligation Enhancer	480 μL	4×720 μL
13321-C		Fast T4 DNA Ligase	80 μL	480 μL
13321-D		2×Ultima HF Amplification Mix	400 μL	4×600 μL
13321-E		Primer Mix for MGI	80 μL	480 μL

Specifications

Product Type	Library Preparation Kit
Libraries	Fragment Library
Fragmentation method	Smearase
Input amount	10 ng-1 μg
For Use With (Equipment)	MGI Platforms
Sample Type	gDNA, cDNA and FFPE samples
Sequencing Type	Genome & DNA Sequencing
Product Line	DNA library construction
For Use With (Application)	NGS library preparation
Quantity	16/96 Reactions

Storage

-25°C ~ -15°C storage, valid for one year.

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 contact MGI or our company.

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

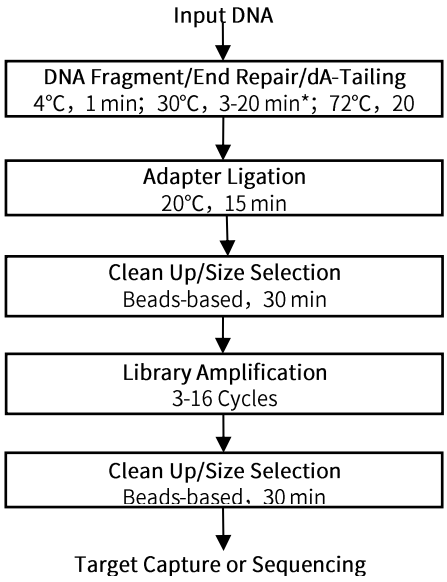

Figure 1. Workflow of library preparation using OnePot II DNA library prep kit

3. Operation steps

3.1 DNA Fragmentation/End Preparation/dA-Tailing

During this step, the genomic DNA samples will be fragmented, end-repaired, and dA-tailed at the 3' ends.

3.1.1 Thaw the reagents mentioned in Table1. Invert to thoroughly mix the reagents and place them on ice for later use.

3.1.2 Assemble the reagents according to Table1 on ice.

Table 1 Reaction Assembly for DNA Fragmentation/ End Repair/ dA-Tailing

Components	Volume (μL)
Input DNA	x
Smearase™ Mix	10
TE	Up to 60

3.1.3 Mix thoroughly by vortexing or pipetting several times, briefly centrifuge to collect the liquid to the bottom of the tube.

3.1.4 Place the tube in a thermocycler and run the program according to Table 2 to process DNA fragmentation, end-repair, and dA-tailing.

Table 2 Program setup for DNA Fragmentation/ End Repair/ dA-Tailing

Temperature	Time
Hot lid 105°C	On
4 °C	1 min*
30 °C	3-20 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 3 for the fragmentation of intact genomic DNA. We recommend extending the fragmentation time for another 2-4 minutes if the input DNA amount is 500-1000 ng. For FFPE DNA samples with uncertain quality, please kindly refer to Table 4.

Table 3 Fragmentation time for standard DNA

Insert peak size	Fragmentation Time	Modification Range
350 bp	8 min	5-12 min
250 bp	10 min	8-15 min
200 bp	13 min	10-20 min
150 bp	20 min	12-25 min

Table 4 Fragmentation time for FFPE DNA

Insert peak size	Fragmentation Time	DIN*
250 bp	9-13 min	> 8.0
250 bp	8-11 min	6.5-8.0
250 bp	4-8 min	4.2-6.5
250 bp	3-6 min	2.5-4.2

Note: *DIN is short for DNA Integrity Number, is an approach by Agilent to measure the degradation level of FFPE DNA samples. Please refer to figure 2 for details.

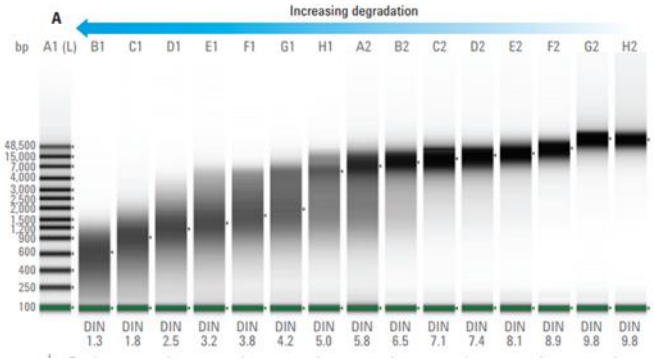

Figure 2. The Agilent 2200 DIN values of DNA samples with different degrees of degradation

3.2 Adapter Ligation

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

3.2.1 Dilute the adapters to recommended concentrations according to Table 10.

3.2.2 Thaw the reagents mentioned in Table 5, invert to mix thoroughly, and place them on ice for later use.

3.2.3 Add the following reagents (according to Table 5) into the same tube from the last step.

Table 5 Adapter Ligation Reaction Assembly

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

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

3.2.4 Mix the assembled reaction by pipetting up-and-down or gently vortexing, briefly spin the solution to the bottom.

3.2.5 Put the tube into a thermocycler and set up the program according to Table 6 to process the adapter ligation reaction.

Table 6 Adapter Ligation Program setup

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 with magnetic beads to remove unligated adapters, adapter dimers, or other unusable templates.

3.3.1 Cleanup

1) Preparation: take the Hieff NGS™ DNA Selection Beads out of the fridge, allow it to equilibrium to room temperature for at least 30 minutes. Prepare 80% ethanol freshly.

2) Thoroughly mix the beads by inverting or vortexing.

3) Add 80 μL Hieff NGS™ DNA Selection Beads (0.8×, Beads: DNA=0.8:1) to the reaction tube containing the adapter-ligated templates, vortex mix or pipette for 10 times to mix, incubate at room temperature for 5 minutes.

4) Briefly spin down the sample and place it on a magnetic stand to separate the beads from the liquid. Carefully remove the supernatant when the liquid becomes clear (about 5 minutes).

5) Add 200 μL freshly prepared 80% ethanol to the tube while it is placed on the magnetic stand. Incubate at room temperature for 30 seconds and carefully remove the supernatant.

6) Repeat the previous step for a total of 2 washes.

7) While keeping the tube on the magnetic stand, open the lid to air-dry the beads until the beads start to crack (no more than 5 minutes).

8) Take the tube off the magnetic stand for elution:

a) If size-selection is not required, 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.

b) If need to perform a size-selection afterward, add 102 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 100 μL supernatant to a new PCR tube without touching the beads.

3.3.2 Size Selection

1) Preparation: take the Hieff NGS™ DNA Selection Beads out of the fridge, allow it to warm to 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 7. Mix by vortexing or pipetting up-and-down 10 times.

Table 7 Recommended Beads: DNA ratios

Inserted DNA library size	150 - 250 bp	200-300 bp	300-400 bp
Final DNA library size	230 - 330 bp	280-380bp	380-480bp
Volume ratio in the 1st round (Beads:DNA)	0.78×	0.68×	0.58×

Volume ratio in the 2nd round (Beads:DNA)	0.20×	0.20×	0.20×

Note: "×" in the above table refers to the volume of the purified DNA templates. For example, if targeting 250 bp insert size for a DNA sample with 100 μL, then the volume of the first round of the beads is 0.78×100 μL=78 μL, and the volume of the second round of the beads is 0.20×100 μL=20 μL. The recommended ratios mentioned in this table are for adapter-ligated DNA templates. If performing size selection before adapter ligation, please refer to the recommended ratios mentioned in the user manual of Hieff NGSTMDNA Selection Beads (Cat#12601).

4) Incubate at room temperature for 5 minutes.

5) Briefly spin down the sample and place it on a magnetic stand to separate the beads from the liquid. Carefully transfer the supernatant to a new tube when the liquid becomes clear (about 5 minutes).

6) Add the second round of beads to the supernatant according to Table 7.

7) Thoroughly mix by vortexing or pipetting up-and-down 10 times. Incubate at room temperature for 5 minutes.

8) Briefly spin down the sample and place it on a magnetic stand to separate the beads from the liquid. Carefully remove the supernatant when the liquid becomes clear (about 5 minutes).

9) Add 200 μL freshly prepared 80% ethanol to the tube while it is placed on the magnetic stand. Incubate at room temperature for 30 seconds and carefully remove the supernatant.

10) Repeat the previous step for a total of 2 washes.

11) While keeping the tube on the magnetic stand, open the lid to air-dry the beads until the beads start to crack (no more than 5 minutes).

12) Take the tube off the magnetic stand, add 21 μL ddH_2O. Thoroughly mix by vortexing or pipetting up-and-down and incubate at room temperature for 5 minutes.

13) 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.

3.4 Library Amplification

This step amplifies the purified or size-selected libraries.

3.4.1 Thaw the reagents mentioned in Table 8, invert to mix thoroughly, and place them on ice for later use.

3.4.2 Assemble the following reaction in a sterilized PCR tube.

Table 8 Reaction Assembly for Amplification

Components	Volume (μL)
2×Ultima HF Amplification Mix	25
Primer Mix for MGI	5
Adapter Ligated DNA（3.3 Step product）	20

3.4.3 Mix the assembled reaction by pipetting up-and-down or gently vortexing, briefly spin the tube to collect the liquid to the bottom.

3.4.4 Put the tube into a thermocycler and set up the program according to Table 9 to start the amplification.

Table 9 Program Setup for Amplification

Temperature	Time	Number of cycles
98°C	1 min	1
98°C	10 sec	Refer to table 11 in the important notes section
60°C	30 sec	
72°C	30 sec	

72°C	5 min	1
4°C	Hold	-

3.5 Post-Amplification Clean-up/Size Selection

Operation is the same as the "Post-ligation Clean-up". Please use the Hieff NGS™DNA Selection Beads to purify the amplified libraries with 0.9× beads (Beads:DNA=0.9:1).

If size-selection is needed, please refer to the "Post-ligation Size-selection" protocols (Can skip the purification step).

3.6 DNA Library Quality Control

The constructed library quality is generally analyzed by measuring the concentrations and size distributions. Please refer to the important notes section for more details.

3.7 Library Cyclization

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

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. After thawing, mix thoroughly by vortexing, spin the tube briefly and place them on ice for later use.

1.3 It is recommended to pipette or gently shake during the reaction solution preparation of each step. 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 experiments 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 10 ng – 1 μg of input DNA. High quality input DNA (A260/A280 = 1.8-2.0) is highly recommended.

2.2 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 TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) for fragmentation.

2.3 Please refer to Table 3 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 refer to Table 4 for the fragmentation time of FFPE samples with different degrees of degradation. Please adjust the fragmentation time

based on your experimental requirements.

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

2.5 If experiencing unsatisfied library preparation performance of heavily degraded FFPE samples, we recommend using Yeasen FFPE DNA Repair Reagent (Cat#12606) to repair the FFPE DNA samples. This reagent can be added to the fragmentation/end-repair/A-tailing reaction, thus does not require additional steps of operations.

3. Adapter Ligation

3.1 MGI has two kinds of adapters: 1-128 and 501-596. For adapter instruction, please refer to MGI "Use of Adapter" or consult Yeasen. In addition, MGI stated that the two kinds of adapters 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 adapter amount according to Table 10 and the actual DNA input. If the adapter dilution is required, please dilute the adapter with TE buffer.

Table 10 Recommended adapter: insert molar ratios for 10ng-1 μg Input DNA

Input DNA	Adapter Dilution(Volume of Adapter : Total Volume)	Concentration
10-30 ng	5-Fold (1 : 5)	2 μM
31ng-1 μg	Do not dilute	10 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 We recommend performing 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. If size-selection is to be completed before end repair/dA-tailing or after amplification, you can directly proceed to size-selection without any previous purification.

4.4 Please allow the magnetic beads to equilibrium to room temperature for at least 30 mins before use for ideal performance and recovery rate.

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

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 11 lists recommended cycle numbers targeting the library yield of 1 μg.

Table 11 Recommended cycle numbers targeting the library yield of 1 μg

Input DNA	Number of cycles required to generate 1 μg of library yield
1 μg	3 - 5
500 ng	4 - 6
200 ng	5 - 7
50 ng	8 - 10
10 ng	10 - 12

Note: If fragment sorting is performed during library preparation, please refer to the higher cycle number for amplification.

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 microfluidics.

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