Hieff NGS™OnePot II DNA Library Prep Kit for MGI®

Cat# 13321

INSTRUCTION FOR USE

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Product Information

Product Name	Cat#	Specification
Hieff NGS™ OnePot II DNA Library Prep Kit for MGI®	13321ES16	16 T
	13321ES96	96 T

Product Description

Hieff NGS™ OnePot II DNA Library Prep Kit for MGI® developed for the MGI® platform utilize a patent non-restrictive endonuclease which fragments the DNA to targeted sizes in a time-dependent manner. This kit enables the single-step reaction of fragmentation, end-repair, and A-tailing without the requirement of beads clean-up, significantly reducing material loss, experimental costs, and hands-on time. 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. By sequencing, all samples with different GC content can obtain excellent sequencing results, with high library coverage, good uniformity and low preference, which makes library construction simple and efficient.

- Compatible with 10 ng-1 μg DNA samples of most types, including cfDNA and FFPE samples
- High-quality fragmentation enzyme which randomly fragments ds DNA without bias
- Combining fragmentation, end-repair, and A-tailing in one single step
- High fidelity polymerase with high amplification efficiency, significantly increasing library quality and yield
- Compatible with FFPE DNA samples
- Stringent quality control and batch effect elimination

Product Components

Components		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

Shipping and Storage

All the components are shipped with ice packs 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.

1.2 Please thaw each component of the kit at room temperature before use. Please invert the thawed reagents several times, briefly spin down, and put them on ice until use.

1.3 It is highly recommended to mix the reagents by pipetting up-and-down or by gentle vortexing when setting up the reactions. Vigorous vortexing may impact the library yield.

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 highly recommended to pre-heat the lid of the thermocycler for each reaction step.

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 For research use 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 5 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 6 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 construction 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 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 1 and the actual DNA input. If the joint dilution is required, please dilute the joint with TE buffer.

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

Input DNA	A dilution of 10 μM Adapter	Diluted input amount（μL）
31ng-1 μg	Do not dilute	5
10-30 ng	5	5

4 Beads 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 use by vortexing or pipetting up-and-down.

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

4.7 Please prepare fresh 80% Ethanol for each time of beads clean-up or size-selection to ensure the recovery rate.

4.8 For accurate size-selection, it is recommended to start with a pre-purification volume of more than 100 μL. If less, we recommend bringing the volume up to 100μL with ultra-pure water.

4.9 Please thoroughly dry but not overly dry the beads before elution. Ethanol residue may affect the following reactions; overdried

beads may reduce the recovery rate. It usually takes about 3-5 mins to dry the beads at room temperature.

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

Table 2 Recommended cycle numbers targeting the library yield of 1 μg

Input DNA(ng)	Number of cycles required to generate
	1 μg
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 construction, please refer to the higher cycle number for amplification.

6 Library Quality Analysis

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

6.2 Library concentrations can be measured by fluorescent-based methods such as QubitTMand PicoGreenTMor qPCR

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

6.4 We recommend the qPCR method for library quantification: fluorescent-based methods such as QubitTMand PicoGreenTMcan'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 and be analyzed using Agilent Bioanalyzer 2100, 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 material: Absolute ethanol, sterilized ultra-pure water, TE Buffer (10 mM Tris-HCl, pH 8.0-8.5+0.1 mM EDTA), Eppendorf LoBind tubes, PCR tubes, magnetic stands, thermocyclers, etc.

2 Workflow

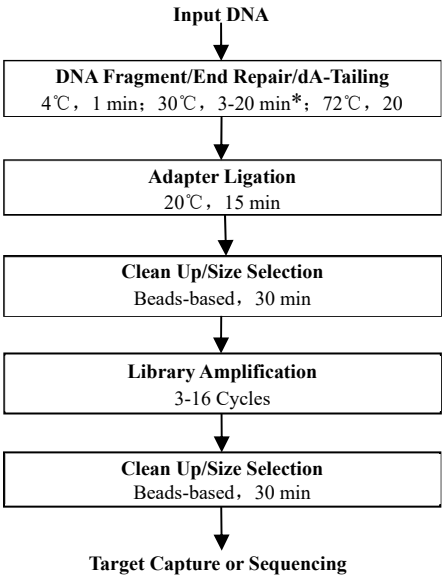

Figure 1 Workflow of library construction using OnePot II DNA library prep kit

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 Table 3. Invert to thoroughly mix the reagents and place them on ice for later use.

3.1.2 Assemble the reagents according to Table3 on ice.

Table 3 Reaction Assembly for DNA Fragmentation/ End Repair/ dA-Tailing

Components	Volume(μL)
Input DNA	x
Smearase™Buffer	10
Smearase™Enzyme	5
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 Table4 to process DNA fragmentation, end-repair, and dA-tailing.

Table 4 Program setup for DNA Fragmentation/ End Repair/ dA-Tailing

Temperature	Duration
Heat lid to 105°C	On
4 °C	1 min*
30 °C	3-20 min**
65 °C	20 min
4 °C	Hold

Note: *Pre-set the program to 4℃ 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℃.

**Please refer to table 5 for the fragmentation of intact genomic DNA. We recommend extending the fragmentation time for another 2-4 minutes if the input DNA amount is 500-1000ng. For FFPE DNA samples with uncertain quality, please kindly refer to table 6.

Table 5 Fragmentation time for standard DNA

Insert peak size	Fragmentation Time	Modification Range
600 bp	8 min	6-12 min
350 bp	10 min	8-14 min
250 bp	12 min	10-15 min
200 bp	15 min	13-18 min
150 bp	20 min	15-25 min

Table 6 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.

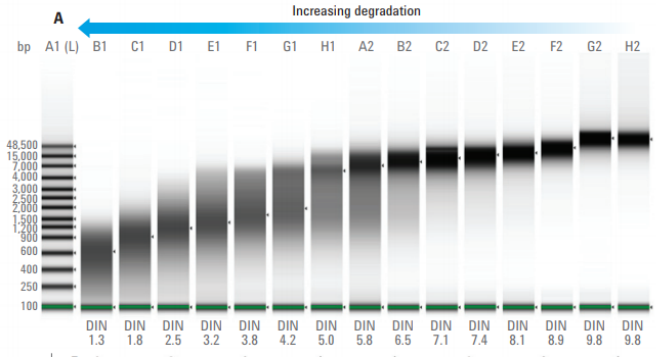

Figure 2 The Agilent 2200 DIN values of DNA samples with different degrees of degradation

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 1.

3.2.2 Thaw the reagents mentioned in Table 7, invert to mix thoroughly, and place them on ice for later use.

3.2.3 Add the following reagents (according to table 7) into the same tube from the last step.

Table 7 Adapter Ligation Reaction Assembly

Component	Volume(μL)
dA-tailed DNA (from last step)	60
Ligation Enhancer	30*
DNA Adapter	5
Novel T4 DNA Ligase	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 8 to process the adapter ligation reaction.

Table 8 Adapter Ligation Program setup

Temperature	Duration
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.

3.3 Post-ligation Clean-up or Size-selection

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.

Clean-up:

3.3.1 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.

3.3.2 Thoroughly mix the beads by inverting or vortexing.

3.3.3 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.

3.3.4 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).

3.3.5 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.

3.3.6 repeat the previous step for a total of 2 washes.

3.3.7 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).

3.3.8 Take the tube off the magnetic stand for elution:

1) 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.

2) 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.

Size-selection:

3.3.1 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.

3.3.2 Thoroughly mix the beads by inverting or vortexing.

3.3.3 Based on the targeted sizes, add the first round of beads to the 100 μL purified DNA templates according to Table 9. Mix by vortexing or pipetting up-and-down 10 times.

Table 9 Recommended Beads: DNA ratios

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×

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 NGS™DNA Selection Beads (Cat#12601).

3.3.4 Incubate at room temperature for 5 minutes.

3.3.5 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).

3.3.6 Add the second round of beads to the supernatant according to table 9.

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

3.3.8 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).

3.3.9 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.

3.3.10 repeat the previous step for a total of 2 washes.

3.3.11 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).

3.3.12 Take the tube off the magnetic stand, add 21 µL ddH2O. Thoroughly mix by vortexing or pipetting up-and-down and incubate at room temperature for 5 minutes.

3.3.13 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 10, 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 10 Reaction Assembly for Amplification

Component	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 11 to start the amplification.

Table 11 Program Setup for Amplification

Temperature	Duration	Cycle number
98°C	1 min	1
98°C	10 sec	Refer to table 1 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^{TM}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 Quality Control of the Final Libraries

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.

Memo:

BRING VALUES TO CUSTOMERS
BUILD A HEALTHIER AND HAPPIER WORLD

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