Hieff NGS® DNA&RNA Library Co-Prep Kit V2

12305ES

INSTRUCTIONS FOR USE

Ver. EN20230608

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Product description

Hieff NGS® DNA&RNA Library Co-Prep Kit V2 is a DNA&RNA co-library kit for Illumina®&MGI® sequencing platform, which contains efficient cDNA synthesis reagent and enzyme digestion reagent. Compared with the traditional library construction method, this product can efficiently complete cDNA synthesis and DNA&RNA library construction in one tube.The kit contains high quality enzyme for DNA fragmentation and combines DNA fragmentation, end-repair, and dA-tailing into one step, which reduce time significantly and cost of library preparation. 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. This upgraded kit using the latest optimized ligase greatly decreases the self-ligation rate during adapter ligation. Moreover, the introduction of a new high-fidelity polymerase further improves the homogeneity and fidelity of amplification.

All the components provided by the kit have undergone strict quality control and functional verification, which ensures the stability and repeatability of library construction to the greatest extent.

Specifications

Cat.No.	12305ES08 / 12305ES24 / 12305ES96
Size	8 T / 24 T / 96 T

Components

Components No.		Name	12305ES08	12305ES24	12305ES96
12305-A	○	Random Primer	20 μL	60 μL	240 μL
12305-B	●	cDNA Reaction Buffer	64 μL	192 μL	768 μL
12305-C	●	cDNA Enzyme Mix	16 μL	48 μL	192 μL
12305-D	●	Smearase Buffer	80 μL	240 μL	960 μL
12305-E	●	Smearase Enzyme Mix	40 μL	120 μL	480 μL
12305-F	●	Ligation Enhancer	240 μL	720 μL	2×1440 μL
12305-G	●	Novel T4 DNA Ligase	40 μL	120 μL	480 μL
12305-H	●	2×Super Canace® II High-Fidelity Mix	200 μL	600 μL	2×1200 μL
*		Primer Mix*	40 μL	120 μL	480 μL

Note: * indicates that this reagent is not included in this kit and additional reagents are required.The kit is compatible with dual platforms of Illumina®&MGI®, but additional primer mix (CAT # 13334 Primer Mix for MGI® and Cat# 13335 Primer Mix for Illumina®) is required.

Storage

This product should be stored at -25~-15℃ for 1 years.

Notes

1. About the operation

1.1. Please operate with lab coats and disposable gloves，for your safety.

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

1.4. Please use consumables without RNase contamination., and cleaning the experimental area regularly. ThermoFisher's RNAZap™ high-efficiency nucleic acid removal spray was recommended to remove RNase contamination.

1.5. 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.

1.6. This product is for research use only.

2. Adapter Ligation

2.1 Illumina or MGI Long Adapter (Barcoded Adapter) kits and short Adapter kits are available for customers to choose according to their experimental requirements.

2.2 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.

2.3 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.

2.4 The concentration of the adapter directly affects the ligation efficiency and library yield. The adapter volume added to the kit is fixed to 5 μl. The adapters are recommended to be diluted with 0.1×TE buffer and the diluted adapters can be stored at 4°C for 48 hours. Table 25 lists the recommended adapter amount for different amounts of input RNA.

Table 1-1 The recommended Illumina® adapter amount for different input DNA&RNA

Input Total DNA&RNA	Illumina® Adapter stock concentration
<10 ng	3 μM
⩾10 ng	15 μM

Table 1-2 The recommended MGI® adapter amount for different input DNA&RNA

Input Total DNA&RNA	MGI® Adapter stock concentration
<10 ng	5 μM
⩾10 ng	10 μM

*The Adapter usage can be adjusted according to different types of Total RNA samples and input amount.

3. 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 2 The recommended number of cycles to generate DNA&RNA library *

Input Total DNA&RNA	Number of cycles
<1 ng	10~12
1 ng	9~10
10 ng	6~7
50 ng	4~5
100~1000 ng	4

Note：*The yield of the library is not only related to the input quantity and the number of amplification cycles, but also affected by the quality of samples, fragmentation conditions and sorting conditions. In the process of library construction, choose the most appropriate conditions according to the actual situation.

4. Bead-based DNA Cleanup and Size Selection

4.1. There are multiple steps in the library construction process that require DNA purification magnetic beads. We recommend Hieff NGS™ DNA Selection Beads (Yeasen Cat#12601) or AMPure® XP magnetic beads (Beckman Cat#A63880) for DNA purification and size-selection.

4.2. 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.

4.3. The magnetic beads should be mixed well by vortex or pipetting prior to use.

4.4. Do not aspirate the beads when transferring the supernatant, even trace amounts of the beads may impact the following reactions.

4.5. The 80% ethanol should be freshly prepared, otherwise it will affect the recovery efficiency.

4.6. 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.

4.7. 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.

5. Library Quality Analysis

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

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

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

5.4. 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.

5.5. The size distribution of libraries can be analyzed using Agilent Bioanalyzer or other devices based on the principles of capillary electrophoresis or microfluidics.

6. Other Materials

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

6.2. Adapters: Complete Adapter for Illumina (Yeasen Cat#13519-13520 or other equivalent products) or Complete Adapter for MGI (Yeasen Cat#13360-13362 or other equivalent products).

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

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

Workflow

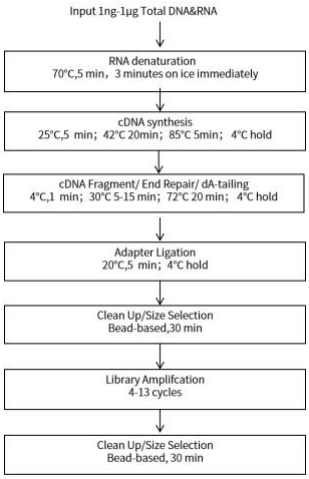

Figure 1. The workflow of DNA&RNA Library Co-Prep Kit

Instructions

Step 1. RNA denaturation

1.Thaw Random Primer at room temperature. Invert to thoroughly mix the reagents and place them on ice for later use. Assemble the reagents according to Table3 on ice.

Table 3 RNA denaturation reaction system

Components	Volume (μL)
Random Primer	2.5
DNA&RNA（1 ng-1 ug）	32.5
Total	35

2.Mix thoroughly by gently pipetting up and down , and then spin down briefly.

3.Place the tube in a thermocycler and set the program according to table 4 to perform RNA denaturation.

Table 4 RNA denaturation reaction program

Temperature	Time
Heat lid to 75°C	On
70°C	5 min
On ice	3 min

Step 2. cDNA Synthesis

1.Take the first-strand synthesis reagents from -20°C, invert and mix well, and then spin down briefly. As shown in Table 5, prepare the first-strand cDNA synthesis reaction.

Table 5 cDNA synthesis reaction system

Components	Volume (μL)
Denatured DNA&RNA	35
cDNA Reaction Buffer	8
cDNA Enzyme Mix	2
Total	45

2.Mix thoroughly by gently pipetting up and down , and then spin down briefly.

3.Place the tube in a thermocycler and set the program according to table 6 to perform cDNA synthesis.

Table 6 cDNA synthesis reaction program

Temperature	Time
Heat lid to 75°C	On
25°C	5 min
42°C	20 min
85°C	5 min
4°C	Hold

Step 3. cDNA Fragmentation/End Repair/dA-Tailing

This step performs cDNA samples fragmentation, end-repair and dA-tailing in one reaction.

1. Thaw the reagents list in Table 7. Invert and mix thoroughly, and place them on ice for later use.

2. Prepare the reactions on ice according to Table 7.

Table 7. Reaction Assembly for DNA Fragmentation/ End Repair/ dA-Tailing

Components	Volume (μL)
cDNA	45
Smearase Buffer	10
Smearase Enzyme Mix	5
Total	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 8 to perform DNA fragmentation, end-repair, and dA-tailing reaction.

Table 8. Program setup for DNA Fragmentation/ End Repair/ dA-Tailing

Temperature	Time
Heat lid to 105°C	on
4°C	1 min*
30°C	5-15 min**
72°C	20 min
4°C	Hold

Note: *Pre-set the program to 4°C to effectively control the fragmentation performance 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 Table9 for the fragmentation of intact genomic DNA.

Table 9. Guideline for choosing fragmentation time for gDNA

Insert peak size	Fragmentation Time
150~200 bp	5~15 min

Step 4. Adapter Ligation

This step enables the product from step 3.1ligated with special Illumina® or MGI® adapters.

1.Please refer to table 1 to dilute the adapter to appropriate concentration according to the amount of input DNA.

2. Thaw the reagents list in table 10, invert and mix thoroughly, and place them on ice for later use.

3. Add the reagents list in table 10 to step 3 PCR tube.

Table 10. Reaction Assembly for Adapter Ligation

Components	Volume (μL)
dA-tailed DNA	60
Ligation Enhancer	30*
Novel T4 DNA Ligase	5
DNA Adapter	5**
Total	100

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

**The original concentration of the Illumina® adapter of YEASE 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 YEASE 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 11 and perform the adapter connection reaction.

Table 11 Adapter Ligation reaction program

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

Step 5. Clean Up Post Ligation

This step is to purify or size-select the product in step 4 with magnetic beads. The purification can remove residue adapters, adapter dimers, or other unusable products.

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 45 μL Hieff NGS™ DNA Selection Beads (0.45 ×, Beads : DNA = 0.45:1) to the tube containing the adapter-ligated product in step 3.2, 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 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 6. Library Amplification

This step can enrich the purified or size-selected products by PCR amplification.

1. Thaw the reagents list in table 12, invert and mix thoroughly, and place them on ice for later use.

2. Assemble the following reaction in a sterilized PCR tube.

Table 12-A/B adapter-ligated DNA PCR reaction with Illumina® short adapter（left）and Illumina® completed adapter（right）

Components	Volume (μL)	Components	Volume (μL)
2×Super Canace® II High-Fidelity Mix	25	2×Super Canace® II High-Fidelity Mix	25
Universal Primer/ i5 Primer*	2.5	Primer Mix**	5
Index Primer/ i7 Primer*	2.5		
Adapter Ligated DNA	20	Adapter Ligated DNA	20
Total	50	Total	50

[Note]: * If you use an adapter without index, commonly known as a short adapter (small Y adapter), the Index primer provided in the short adapter reagent (Cat#12414~ Cat#12415) is recommended for amplification.

**If you use an adapter with index (Cat#13519~ Cat#13520), commonly known as long adapter (big Y adapter), Hieff NGS™ Primer Mix for Illumina (Yeasen Cat#13335) in needed.

Table 13 adapter-ligated DNA PCR reaction with MGI® completed adapter

Components	Volume (μL)
2×Super Canace® II High-Fidelity Mix	25
Primer Mix for MGI®*	5
Adapter Ligated DNA	20
Total	50

[Note]: * The primer mix for MGI is not included in this kit, Hieff NGS™ Primer Mix for MGI (Yeasen Cat#13334) in needed.

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 14 to start the amplification.

Table 14 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 2
60°C	30 sec	
72°C	30s	
72°C	1 min	1
4°C	Hold	-

Step 7. Post-Amplification Clean-up/Size Selection

1. Preparation: Take out the Hieff NGS™ DNA Selection Beads from the 4°C and equilibrate at room temperature for at least 30 minutes. Prepare 80% ethanol.

2. Vortex or fully invert the magnetic beads.

3. Add 45 μL Hieff NGS™ DNA Selection Beads (0.9×, Beads:DNA=0.9:1) to Adapter-ligated DNA and mix well on a vortex mixer or by pipetting up and down at least 10 times, and incubate at room temperature for 5 min.

4. Spin the PCR tube briefly and place it on a magnetic stand to separate the magnetic beads and liquid. After the solution is clear (about 5 minutes), carefully discard the supernatant.

5. Keep the PCR tube on the magnetic stand, add 200 μL of freshly prepared 80% ethanol to the tube, incubate at room temperature for 30 sec, carefully remove the supernatant.

6. Repeat step 3.7.5 once for a total of 2 washing steps.

7. Keep the PCR tube on the magnetic stand at all times, completely remove the residual ethanol, and air-dry beads for 5 minutes while the tube is on the magnetic stand with the lid open (no more than 5 minutes).

8. Remove the PCR tube from the magnetic stand, Elute DNA target from the beads with 52 μl ddH2O. Mix well on a vortex mixer or by pipetting up and down, and incubate for 5 minutes at room temperature. Briefly spin the tube, and put the tube back on the magnetic stand until the solution is clear, carefully transfer 50 μL of supernatant to a new PCR tube for library quantification and quality analysis.

Step 8. 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 5.

Note:

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