HB220412

Hieff NGS™ Ultima Dual-mode mRNA Library Prep Kit for MGI®

Cat# 13330

INSTRUCTION FOR USE

Yeasen Biotechnology (Shanghai) Co., Ltd.

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Product Information

Product Name	Cat#	Specification
Hieff NGS™ Ultima Dual-mode mRNA Library Prep Kit for MGI ®	13330ES24	24 T
	13330ES96	96 T

Product Description

Hieff NGS™ Ultima Dual-mode mRNA Library Prep Kit for MGI® is a new mRNA library construction kit specially developed and designed for MGI ® high-throughput sequencing platform. Compared with the traditional library construction method, this product combines cDNA two-strand synthesis with Endprep and dA-tailing, which greatly reduces the time for library construction and simplifies the operation process. The two types of buffers in the cDNA synthesis module can be used for constructing strand-specific or conventional library according to the needs. This product is compatible with 10 ng-4 μg of input total RNA from eukaryotic organism. After mRNA isolation, fragmentation, double-stranded cDNA synthesis, end repair, dA-tailing, adaptor ligation, and library amplification, the total RNA sample is finally converted into a library suitable for sequencing on the MGI® platform.

The kit contains two independent modules. The core of BOX-I is oligo (dT) magnetic beads required for mRNA purification. BOX-II contains mRNA fragmentation reagents, reverse transcription reagents, conventional and strand-specific ds-cDNA synthesis, and all reagents required for subsequent library construction. Among them, dTTP is replaced with dUTP in the strand-specific two-strand synthesis Buffer, so that dUTP is incorporated into the second strand of cDNA. Then, the high-fidelity DNA polymerase used in this kit cannot amplify the DNA template containing uracil, achieving strand specificity. All reagents provided have undergone strict quality control and functional verification, ensuring the stability and reproducibility of library construction to the greatest extent.

Product Components

Components				13330ES24	13330ES96
BOX-I	12603-A	○	mRNA Capture Beads	1.2 mL	4.8 mL
	12603-B	○	Beads Binding Buffer	1.2 mL	4.8 mL
	12603-C	◎	Beads Wash Buffer	15 mL	60 mL
	12603-D	○	Tris Buffer	1.2 mL	4.8 mL
BOX-II	13333-A	🔴	Frag/Prime Buffer	450 μL	2×900 μL
	13333-B	🔴	1st Strand Enzyme Mix	48 μL	192 μL
	13333-C	🟤	Strand Specificity Reagent	150 μL	580 μL
	13333-D	🟢	2nd Strand Buffer (dNTP)	720 μL	2×1440 μL
	13333-E	🟣	2nd Strand Buffer (dUTP)	720 μL	2×1440 μL
	13333-F	🟢	2nd Strand Enzyme Master Mix	120 μL	480 μL
	13333-G	🔵	Ligation Enhancer	720 μL	2×1440 μL
	13333-H	🔵	Novel T4 DNA Ligase	120 μL	480 μL
	13333-I	◌	2×Super Canace™ II High-Fidelity Mix	600 μL	2×1200 μL
	13333-J	◌	Primer Mix for MGI	120 μL	480 μL

Shipping and Storage

All components in Box I are shipped with ice packs and can be stored at 2-8°C for one year.

All components in Box II 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.

1.2 Thaw components at room temperature. Once the components are thawed, mix thoroughly by vortexing, spin the tube briefly and place on ice for later use.

1.3 It is recommended to perform each step reaction in a PCR machine with a heated lid. The PCR machine should be preheated to the set temperature before use.

1.4 Please use consumables that are free of RNase contamination and clean the experimental area regularly. It is recommended to use ThermoFisher's RNAZap™ high-efficiency nucleic acid removal spray to remove RNase contamination.

1.5 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 (It is recommended to use ThermoFisher's DNAZapTM high-efficiency nucleic acid removal spray).

1.6 For research use only!

2 Application

This kit is suitable for high-quality total RNA from eukaryotes such as animals, plants, and fungi with a starting input of 10 ng-4 μg (volume≤50 μL). If the initial RNA concentration is low and the volume exceeds 50 μL, it is recommended to condensed the DNA with Hieff NGS™ RNA Cleaner magnetic beads. To ensure that the mRNA has a complete poly(A) tail structure, RNA needs to be detected by the Agilent 2100 Bioanalyzer RNA 6000 Nano/Pico chip and the RIN value must be greater than 7.

The mRNA isolation module of this kit uses oligo (dT) magnetic beads. Only mRNA with poly(A) tail can be extracted; other RNAs without poly(A) tail, such as non-coding RNA, no poly(A) Tail mRNA etc. cannot be applied to this kit. In addition, this kit is not compatible with FFPE samples since the mRNA in the FFPE sample is severely degraded and usually does not have a complete poly(A) tail structure.

The library prepared by this kit can be used for a variety of RNA-Seq applications, including:

- Gene expression
- Single nucleotide variation discovery
- Gene fusion identification
- Splice variant analysis

3 Adapter Ligation

3.1 Currently, MGI only has two kinds of serial adapters:1-128 and 501-596. User Requirement Specification can be seen or known from MGI or Yeasen. In addition, MGI states: Due to the different design process of the two joints, it is forbidden to use them together, otherwise the sequencing data cannot be separated!

3.2 It is recommended to use high-quality commercial adapters. If self-made adapters are used, please entrust a company with experience in NGS primer synthesis and strict pollution control is required. In addition, it is recommended to prepare DNA annealing solution in a clean bench and only operate one type of adapter at a time to prevent cross-contamination.

3.3 The concentration of the adapter directly affects the ligation efficiency and library yield. In this kit, the volume of the adapter is fixed at 5 μL. Please dilute the adapter for the different amounts of input RNA according to Table 1. 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 1 The recommended adapter amount for different amounts of input RNA

Input Total RNA	Adapter stock concentration
100-499 ng	2 μM
500-4000 ng	5 μM

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

4 Bead-based DNA Clean up and Size Selection

4.1 There are multiple steps in the library construction process that require the use of DNA purification magnetic beads. We recommend using 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 before use, otherwise the yield will decrease and the sorting effect will be affected.

4.3 The magnetic beads should be mixed well by vortexing or pipetting before use.

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

4.5 The 80% ethanol used for magnetic bead rinsing should be freshly prepared, otherwise it will affect the recovery efficiency.

4.6 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 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 2 days or at -20°Cfor about a month.

5 Library Amplification

5.1 On the basis of the first-generation DNA polymerase, the high-fidelity DNA polymerase in the kit has greatly improved its amplification uniformity and exhibits no amplification bias.

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 2 lists the recommended cycle numbers for PCR amplification.

Table 2 The recommended Input Total RNA Amount and Number of Amplification Cycles*

Input Total RNA	Number of cycles	
	Non-stranded	Stranded
10 ng	15	15
100 ng	14	14
500 ng	12	13
1 μg	11	12

[Note**]:** *The yield of library is not only related to the input amount and the number of amplification cycles, but also affected by sample quality, fragmentation conditions and sorting conditions. In the process of library construction, consider the actual situation comprehensively and select the most appropriate library construction conditions.

Instructions

1 Required Materials Not Included

1.1 Purified magnetic beads: Hieff NGS™ DNA Selection Beads (Cat#12601) or AMPure XP Beads (Cat#A63880) or other equivalent products.

1.2 RNA quality control: Agilent 2100 Bioanalyzer RNA 6000 Nano/Pico Chip or other equivalent products.

1.3 Adapters: For details, please consult MGI or Yeasen.

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

1.5 Other materials: absolute ethanol, sterile ultrapure water, low adsorption pipette tip, PCR tube, magnetic stand, PCR instrument, etc.

2 Operation flowchart

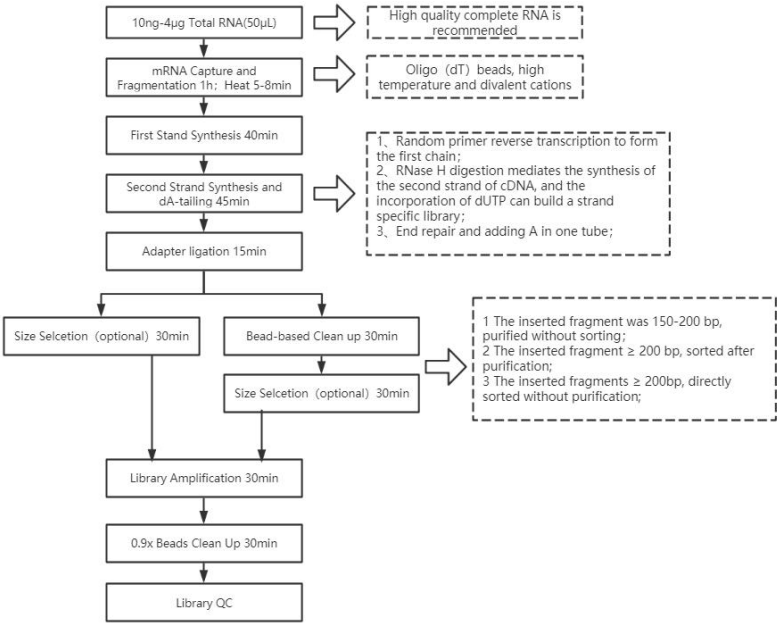

Figure 1 Operation process of mRNA library construction kit

3 Operation steps

3.1 mRNA Purification and Fragmentation

3.1.1 Take out the mRNA Capture Beads from 2-8°C, and equilibrate at room temperature for at least 30 min. Dilute 10 ng-4 μg of total RNA with Nuclease-free Water to a final volume of 50 μL in a PCR tube and place on ice for later use.

3.1.2 Mix the magnetic beads upside down or vortex. Add 50 μL of the magnetic bead suspension to 50 μL of total RNA sample and pipette 6 times to mix well. Spin the reaction solution briefly to the bottom of the tube. Place the mixture of magnetic beads and RNA in a PCR machine and run the following program: 65°C, 5 min; 25°C, 5 min; 25°C, hold.

3.1.3 Place the tube in a magnetic stand and let it stand at room temperature for 5 minutes to separate mRNA from total RNA. Carefully remove the supernatant.

3.1.4 Remove the tube from the magnetic stand and resuspend the magnetic beads with 200 μL Beads Wash Buffer. Pipette repeatedly 6 times to mix thoroughly. Place the tube in a magnetic stand and let it stand at room temperature for 5 min, and carefully remove the supernatant.

3.1.5 Repeat step 3.1.4 for a total of two washes.

3.1.6 Remove the tube from the magnetic stand. Add 50 μL Tris Buffer to resuspend the magnetic beads and pipette 6 times to mix thoroughly.

3.1.7 Put the sample in the PCR machine and run the following program to elute the mRNA: 80°C, 2 min; 25°C, hold. Take the sample out from the PCR machine. Add 50μL Beads Binding Buffer and pipette repeatedly 6 times to mix thoroughly.

3.1.8 Incubate at room temperature for 5 minutes to allow mRNA to bind to the magnetic beads. Place the tube in a magnetic stand, let it stand at room temperature for 5 minutes, and carefully remove the supernatant.

3.1.9 Remove the tube from the magnetic stand, resuspend the magnetic beads with 200 μL Beads Wash Buffer, pipette repeatedly 6 times to mix thoroughly. Place the tube in a magnetic stand and let it stand at room temperature for 5 minutes. Aspirate all the supernatant.

[Note]: a 10 μL pipette is needed to aspirate the remaining liquid.

3.1.10 Remove the tube from the magnetic stand and resuspend the magnetic beads with 18.5 μL Frag/Prime Buffer. Pipette 6 times to mix thoroughly and place the tube in the PCR machine (preheated at 94°C). Table 3 lists the recommended program for mRNA fragmentation. However, different species have different fragmentation effects. A gradient of fragmentation time can be made according to your situation, such as 94℃, 5 min. Use Agilent 2100 to analyze the mRNA purified product size.

Table 3 The recommended program for mRNA fragmentation

Inserted DNA library size (bp)	Fragmentation Program
200-300	94°C,10 min
300-400	94°C,7 min
400-500	94°C,5 min

3.1.11 In order to prevent the poly(A) tail RNA from binding to the magnetic beads, the sample should be placed in the magnetic stand immediately after the fragmentation is finished. When the solution is clear, transfer 17 μL of the supernatant to a new Nuclease Free tube and proceed to the step 'Synthesis of the first strand cDNA' immediately.

3.2 Synthesis of the first strand cDNA

3.2.1 Take the first-strand synthesis reagents from -20°C, invert and mix well, and then leave in a flash. As shown in Table 4, prepare the first-strand cDNA synthesis reaction solution.

Table 4 First-strand cDNA synthesis reaction system

Components	Volume (μL)
Fragmented mRNA	17
Strand Specificity Reagent	6
1st Strand Enzyme Mix	2

3.2.2 Use a pipette to mix gently, and then centrifuge the reaction solution to the bottom of the tube.

3.2.3 Place the above PCR tube in a PCR machine, set up the reaction program as shown in Table 5, and synthesize the first strand cDNA. Immediately after the reaction, the second-strand cDNA synthesis was performed.

Table 5 First-strand cDNA synthesis reaction program

Temperature	Duration
Hot lid 105°C	On
25°C	10 min
42°C	15 min
70°C	15 min
4°C	Hold

3.3 Synthesis of the second strand cDNA/end repair/add A

3.3.1 Remove the second-strand synthesis reagents from -20°C, thaw and mix them upside down; follow Table 6 to prepare the second-strand cDNA synthesis/end repair/addition A reaction solution.

Table 6 Second-strand cDNA synthesis reaction system

Components	Volume (μL)
1st Strand cDNA	25
2nd Strand Buffer (dNTP or dUTP)*	30
2nd Strand Enzyme Master Mix	5

[Note]: *For construction a normal mRNA library, please use a buffer containing dNTP; for construction a strand-specific mRNA library, please use a buffer containing dUTP.

3.3.2 Use a pipette to mix gently, and then centrifuge the reaction solution to the bottom of the tube.

3.3.3 Place the PCR tube in a PCR machine and set up the reaction program as shown in Table 7 to synthesize the second strand cDNA.

Table 7 Second-strand cDNA synthesis reaction program

Temperature	Duration
Hot lid 105°C	on
16°C	30 min
72°C	15 min
4°C	Hold

3.4 Adapter Ligation

In this step, specific MGI® adaptors can be connected to the ends of the end repair and dA tailing products.

3.4.1 Refer to Table 1 in Note 3 and dilute the Adapter to an appropriate concentration according to the amount of Input RNA.

3.4.2 Thaw the reagents in Table 8 and mix them upside down. Place them on ice for later use.

3.4.3 Continue to prepare the reaction system shown in Table 8 in the PCR tube after step 3.3.

Table 8 Adapter Ligation system

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

[Note]: *Ligation Enhancer should be turned upside down, shaken, mixed thoroughly and centrifuged briefly before use.

**The original concentration of the adapter of our company is 10 μM. Please dilute the adapter according to the input amount according to the tips in Table 1 of Note 2 to make the volume of the adapter fixed at 5 μL.

3.4.4 Use a pipette to mix gently, and centrifuge briefly to collect the reaction solution to the bottom of the tube.

3.4.5 Place the PCR tube in the PCR machine, set the reaction program shown in Table 9 and perform the adapter connection reaction:

Table 9 Adapter Ligation reaction program

Temperature	Duration
Hot lid	Off
20°C	15 min
4°C	Hold

3.5 Post Ligation Clean Up

This scheme is suitable for fragments <200 bp, to remove the linker residue in the system by two purifications; when the inserted fragments are ≥200 bp, refer to the sorting scheme in Appendix II to obtain the target length by purification, sorting or direct sorting library.

Suitable for libraries with inserts <200 bp (two rounds of purification are required):

3.5.1 Preparation: Take out the Hieff NGS™ DNA Selection Beads magnetic beads from the refrigerator and equilibrate at room temperature for at least 30 minutes. Prepare 80% ethanol. Vortex or fully invert the magnetic beads to ensure adequate mixing.

3.5.2 Pipette 60 μL Hieff NGS™ DNA Selection Beads (0.6×, Beads:DNA=0.6:1) into the Adapter Ligation product, vortex or pipette to mix, and incubate at room temperature for 5 min. Centrifuge the PCR tube briefly and place it in a magnetic stand to separate the magnetic beads and liquid. After the solution is clear (about 5 minutes), carefully remove the supernatant.

3.5.3 Keep the PCR tube in the magnetic standstand at all times, add 200 μL of freshly prepared 80% ethanol to rinse the magnetic beads, incubate at room temperature for 30 sec, carefully remove the supernatant. Repeat step 5 for the twice rinsing.

3.5.4 Keep the PCR tube in the magnetic standstand at all times, open the lid and air dry the magnetic beads until cracks just appear (no more than 5 minutes).

3.5.5 Remove the PCR tube from the magnetic stand, add 52 μL ddH_2O, vortex or pipette gently to mix well, and let it stand at room temperature for 5 minutes. Centrifuge the PCR tube briefly and place it in a magnetic stand. After the solution is clarified (about 3 minutes), carefully transfer 50 μL of supernatant to a new PCR tube, and perform another round of purification.

3.5.6 Pipette 40 μL Hieff NGS™ DNA Selection Beads (0.8×, Beads:DNA=0.8:1) into the product of the previous step, vortex or pipette to mix, and incubate at room temperature for 5 min. Centrifuge the PCR tube briefly and place it in a magnetic stand to separate the magnetic beads and liquid. After the solution is clear (about 3 minutes), carefully remove the supernatant.

3.5.7 Keep the PCR tube in the magnetic standstand at all times, add 200 μL of freshly prepared 80% ethanol to rinse the magnetic beads, incubate at room temperature for 30 sec, carefully remove the supernatant.

3.5.8 Repeat step 3.5.7 for a total of two rinses.

3.5.9 Keep the PCR tube in the magnetic standstand at all times, open the lid and air dry the magnetic beads until cracks just appear (no more than 5 minutes).

3.5.10 Take the PCR tube out of the magnetic stand, add 21 μL ddH2O, vortex or pipette gently until fully mixed, and let it stand at room temperature for 5 minutes. Centrifuge the PCR tube briefly and place it in a magnetic stand. After the solution clarifies (about 3 minutes), carefully transfer 20 μL of supernatant to a new PCR tube for PCR amplification.

3.6 Library Amplification

This step will carry out PCR amplification and enrichment on the adapter ligation products after purification or length sorting.

3.6.1 Thaw the reagents in Table 10 and mix them upside down. Place them on ice for later use.

3.6.2 Prepare the reaction system shown in Table 10 in a sterile PCR tube.

Table 10-A PCR reaction system for products connected with short adapter

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

3.6.3 Use a pipette to gently pipette or shake to mix, and centrifuge briefly to collect the reaction solution to the bottom of the tube.

3.6.4 Place the PCR tube in the PCR machine, set the reaction program shown in Table 11, and perform PCR amplification.

Table 11 PCR amplification reaction program

Temperature	Time	Cycle
98°C	1 min	1
98°C	10 sec	11~15 *
60°C	30 sec	
72°C	30 sec	
72°C	5 min	1
4°C	Hold	-

[Note]: *The number of library amplification cycles needs to be adjusted according to the sample quality, input volume and other conditions for library construction. See Table 2 for details.

3.7 Post Amplification Clean Up

3.7.1 Preparation: Take out the Hieff NGS™ DNA Selection Beads magnetic beads from the refrigerator and equilibrate at room temperature for at least 30 minutes. Prepare 80% ethanol.

3.7.2 Vortex or fully invert the magnetic beads to ensure adequate mixing.

3.7.3 Pipette 45 μL Hieff NGS™ DNA Selection Beads (0.9×, Beads: DNA=0.9:1) into the Adapter Ligation product, vortex or pipette to mix, and incubate at room temperature for 5 min.

3.7.4 Centrifuge the PCR tube briefly and place it in a magnetic stand to separate the magnetic beads and liquid. After the solution is clear (about 5 minutes), carefully remove the supernatant.

3.7.5 Keep the PCR tube in the magnetic stand at all times, add 200 μL of freshly prepared 80% ethanol to rinse the magnetic beads, incubate at room temperature for 30 sec, carefully remove the supernatant.

3.7.6 Repeat step 3.7.5 for a total of two rinsing.

3.7.7 Keep the PCR tube in the magnetic stand at all times, open the lid and air dry the magnetic beads until cracks just appear (no more than 5 minutes).

3.7.8 Take the PCR tube out of the magnetic stand, add 21 μL ddH2O, vortex or pipette gently to mix well, and let it stand at room temperature for 5 minutes. Centrifuge the PCR tube briefly and place it in a magnetic stand. After the solution clarifies (about 3 minutes), carefully transfer 20 μL of supernatant to a new PCR tube for library quantification and quality inspection.

3.8 DNA Library Quality Control

Generally, the quality of the constructed library can be evaluated by concentration detection and length distribution detection.

Appendix 1: Demonstration of mRNA Fragmentation Effects

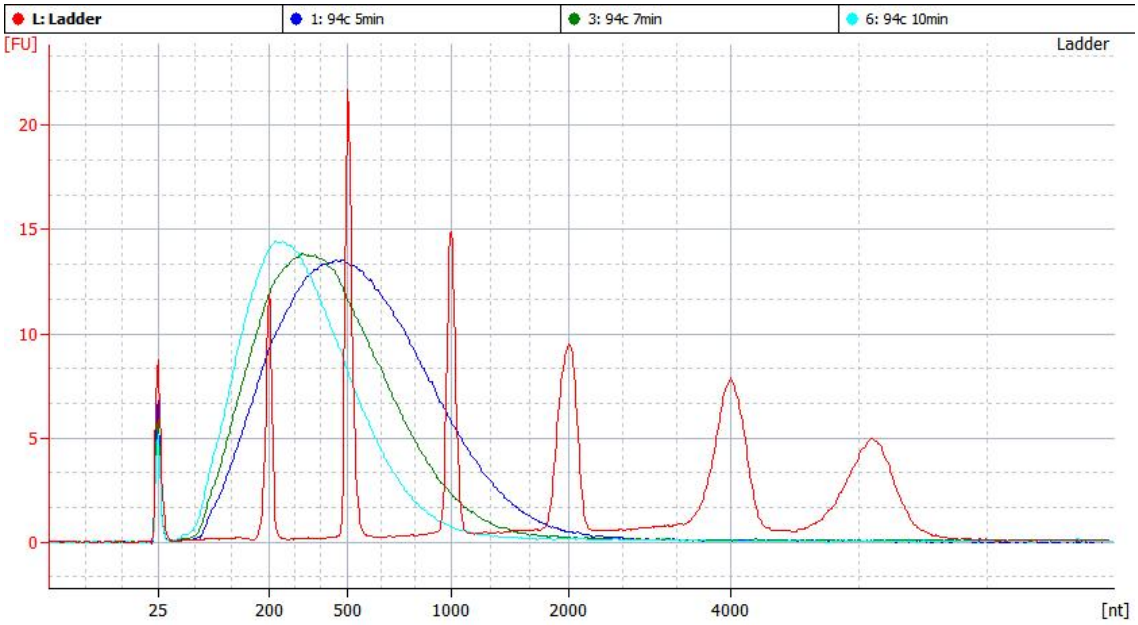

Figure 2. The range of RNA fragments corresponding to different fragmentation times of mRNA. There were treated at 94°C for 10 min, 94°C for 7 min and 94°Cfor 5 min, respectively. After fragmentation, mRNA was purified by 2.2x magnetic beads and detected by Agilent 2100 Bioanalyzer.

[Note]: The RNA used in this result is Agilent's Universal Human Reference RNA. If other sources of RNA are used, it is best to optimize the fragmentation time.

Appendix 2: Explanation of Sorting Conditions

The sorting scheme is suitable for the construction of a library with fragmented RNA at 94°C for 10 min, 94°C for 7 min and 94°C for 5 min, and a library with an insert larger than 200 bp can be obtained:

Scheme 1: Sorting after purification of the linker ligation product

0.6×Hieff NGS™ DNA Selection Beads adapter ligation product

1. Preparation: Take out the Hieff NGS™ DNA Selection Beads magnetic beads from the refrigerator and equilibrate at room temperature for at least 30 minutes. Prepare 80% ethanol.
2. Vortex or fully invert the magnetic beads to ensure adequate mixing.
3. Pipette 60 μL Hieff NGS™ DNA Selection Beads (0.6×, Beads: DNA=0.6:1) into the Adapter Ligation product, vortex or pipette to mix, and incubate at room temperature for 5 min.
4. Centrifuge the PCR tube briefly and place it in a magnetic stand to separate the magnetic beads and liquid. After the solution is

clear (about 5 minutes), carefully remove the supernatant.

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

6. Repeat step 5 for a total of two rinses.

7. Keep the PCR tube in the magnetic stand at all times, open the lid and air dry the magnetic beads until cracks just appear (no more than 5 minutes).

8. Take the PCR tube out of the magnetic stand, add 102 μL ddH2O, vortex or pipette gently to mix well, and let it stand at room temperature for 5 minutes. Centrifuge the PCR tube briefly and place it in a magnetic stand. After the solution clarifies (about 5 min), carefully transfer 100 μL of supernatant to a new PCR tube, ready to perform double rounds of sorting.

Two-round sorting (94°C, 7 min interruption, the size of the sorting library is 380 bp~480 bp as an example, other library sizes are sorted by magnetic beads according to the recommended ratio)

1. Please vortex or fully invert the magnetic beads to ensure uniform mixing.

2. According to the DNA fragment length requirements, refer to Table 12, add 65 μL (0.65×) of the first round of sorting magnetic beads to the above 100 μL DNA, vortex or pipette 10 times to mix.

3. Incubate at room temperature for 5 minutes.

4. Centrifuge the PCR tube briefly and place it in a magnetic stand. After the solution is clarified (about 5 minutes), carefully transfer the supernatant to a clean centrifuge tube, leaving 1-2 μL of solution at the bottom of the tube.

5. Refer to Table 12 and add 15 μL (0.15×) of the second round of sorting magnetic beads to the supernatant.

6. Vortex to mix or pipette 10 times to mix, and let stand at room temperature for 5 minutes.

7. Centrifuge the PCR tube briefly and place it in a magnetic stand. After the solution is clear (about 3 minutes), carefully remove the supernatant.

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

9. Repeat step 8.

10. Keep the PCR tube in the magnetic stand, open the lid and dry the magnetic beads until cracks just appear (about 3 minutes).

11. Take the PCR tube out of the magnetic stand, add 21 μL ddH2O, vortex or pipette gently to mix well, and let it stand at room temperature for 5 minutes.

12. Centrifuge the PCR tube briefly and place it in a magnetic stand to separate the magnetic beads and liquid. After the solution is clear (about 3 minutes), carefully transfer 20 μL of supernatant to a clean tube.

Table 12 Recommended ratio of magnetic beads for short linker library sorting

Inserted DNA library size (bp)	**200~300**	**250~350**	**350~450**	**450~550**
Library size (bp)	280~380	380~480	480~580	580~680
Fragmentation	94°C 10 min	94°C 7 min	94°C 7 min	94°C 5 min
1st volume ratio（Beads:DNA）	70 (0.7×)	65 (0.65×)	58 (0.58×)	50 (0.5×)
2nd volume ratio（Beads:DNA）	20 (0.2×)	15 (0.15×)	15 (0.15×)	15 (0.15×)

[note]: The recommended two-round sorting ratio in this table was suit for Hieff NGS™ DNA Selection Beads；"×" in the table represents sample DNA volume. For example, the main peak of the required library insertion fragment is needed to be 300 bp, if the sample DNA volume is 100 μL after the short connector connection, the volume of magnetic beads used in the first round of separation is 0.65×100 μL=65 μL, and that of magnetic beads used in the second round is 0.15×100 μL=15 μL.

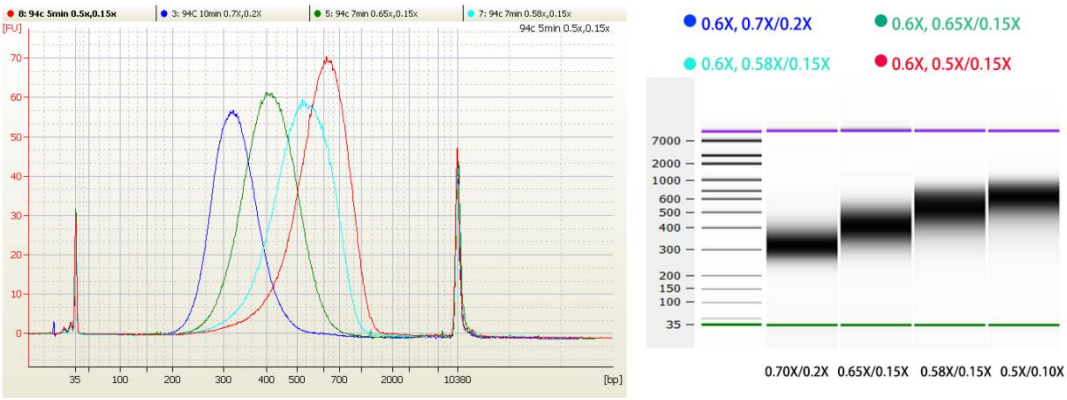

Figure 3. Library size of 1 μg 293 total RNA after fragmentation at 94°Cfor 10 min, 94°Cfor 7 min and 94°Cfor 5 min, according to the recommended ratio of magnetic beads in Table 12

Solution 2: Direct sorting of linker ligation products (take 94°C, 7 min fragmentation, sorting library size of 410 bp ~ 510 bp as an example, other library sizes are sorted by magnetic beads according to the recommended ratio)

It is recommended to sort directly for total RNA of 500 ng or more which used for mRNA capture and library construction. The regents are viscous and needs to be added carefully. Samples with slightly poor RNA quality may have residual adapters.

1. Please vortex or fully invert the magnetic beads to ensure uniform mixing.
2. According to the DNA fragment length requirements, refer to Table 14, add 20 μL (0.20×) of the first round of sorting magnetic beads to the above 100 μL ligation system, vortex or pipette 10 times to mix. Incubate at room temperature for 10 min.
3. Centrifuge the PCR tube briefly and place it in a magnetic stand. After the solution is clarified (about 5 minutes), carefully transfer 100 μL of the supernatant to a clean centrifuge tube.
4. Refer to Table 13 and add 20 μL (0.20×) of magnetic beads for the second round of sorting to the supernatant.
5. Vortex to mix or pipette 10 times to mix, and let stand at room temperature for 10 minutes.
6. Centrifuge the PCR tube briefly and place it in a magnetic stand. After the solution is clarified (about 3 minutes), carefully remove the supernatant.
7. Keep the PCR tube in the magnetic stand, add 200 μL of freshly prepared 80% ethanol to rinse the magnetic beads, incubate at room temperature for 30 sec, and carefully remove the supernatant.
8. Repeat step 7.
9. Keep the PCR tube in the magnetic stand, open the lid and dry the beads until cracks just appear (about 3 minutes).
10. Take the PCR tube out of the magnetic stand, add 21 μL ddH2O, vortex or pipette gently to mix thoroughly, and let it stand at room temperature for 5 minutes.
11. Centrifuge the PCR tube briefly and place it in a magnetic stand to separate the magnetic beads and liquid. After the solution is clear (about 3 minutes), carefully transfer 20 μL of supernatant to a clean tube.

Table 13 Recommended ratio of magnetic beads for short linker library sorting

Inserted DNA library size (bp)	200~300	300~400	400~500	500~600
Library size (bp)	280~380	380~480	480~580	580~680
Fragmentation	94°C 10 min	94°C 7 min	94°C 7 min	94°C 5 min
1st volume ratio（Beads:DNA）	25 (0.25×)	20 (0.20×)	15 (0.15×)	15 (0.15×)
2nd volume ratio（Beads:DNA）	10 (0.1×)	10 (0.1×)	10 (0.1×)	10 (0.1×)

【note】: The recommended two-round sorting ratio in this table was suit for Hieff NGS™ DNA Selection Beads; "×" in the table represents sample DNA volume. For example, the main peak of the required library insertion fragment is needed to be 300 bp, if the sample DNA volume is 100 μL after the short connector connection, the volume of magnetic beads used in the first round of separation is 0.20×100 μL=20 μL, and that of magnetic beads used in the second round is 0.1×100 μL=10 μL.

Memo：

Memo：

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