Fast DNA Library Prep Set for Illumina & MGI

Rapid DNA Library Preparation Kit (Illumina and MGI platforms)

Catalog number:

F666063 (24 rxns)

F666063 (96 rxns)

Storage condition: -20℃ storage, dry ice transportation.

Products Content:

Component	24 rxns	96 rxns
ERAT Mix	48 μl	192 μl
10×ERAT Buffer	120 μl	480 μl
T4 DNA Ligase	72 μl	288 μl
T4 DNA Ligase Buffer	336 μl	672 μl×2 tubes
2×PCR Mix	1.2 ml	1.2 ml x 4 tubes
DNA Control (50 ng/ul, 200 bp)	20 μl	20 μl

Products Introduction

This kit is suitable for both Illumina and MGI sequencing platforms. It provides pre-mixed enzyme modules for DNA end modification, 5'-end phosphorylation modification, 3'-end A-addition, and Adaptor ligation in DNA library construction, and it can be used with primer kits for different sequencing platforms to prepare DNA libraries specific for Illumina or MGI sequencing platforms. DNA libraries can be prepared for Illumina or MGI sequencing platforms by combining with primer kits for different sequencing platforms. The use of high-fidelity DNA polymerase for library enrichment and preference-free PCR amplification extends the coverage of the sequence and allows for the preparation of high-quality DNA libraries. All the reagents provided in the kit have been subjected to strict quality control and functional validation to maximize the stability of library construction.

Product Features

1. End flattening, phosphorylation, and addition of A in one step.
2. No need to purify after end repair, add joints directly.
3. Ultra-fidelity amplification minimizes amplification preference
4. The resulting libraries are suitable for multiple sequencing platforms.

MGI sequencing platform: MGISEQ-2000, MGISEQ-200, BGISEQ-500 and other MGI platform sequencers Illumina sequencing platform: Illumina Gallx, HiSacnSQ, HiSeq 2500/2000/1000, MiSeqsequencing and other Illumina platforms. platform sequencers.

5. Applicable to gDNA library and cfDNA library after physical interruption.

Provide your own instruments, reagents and consumables

1. Magnetic field: DynaMagTM-2 (Cat. No. 12321D) is recommended.

2. Anhydrous ethanol (100% ethanol, analytically pure): deionized water (pH between 7.0 and 8.0).

3. Junction primer kit.

MGI platform: Kang for MGI second-generation sequencing multi-sample junction primer kit II!IIumina platform: Kang for illumina second-generation sequencing junction primer kit W!

4. DNA purification and recovery kit: It is recommended to use the magnetic bead method for DNA purification and recovery kit.

5. Reaction tubes: it is recommended to use low adsorption PCR tubes and 1.5 ml centrifuge tubes; gun head: it is recommended to use high-quality filtration gun head to prevent contamination of reagent kits and library samples.

Pre-experiment preparation and precautions

1. In order to avoid repeated freezing and thawing of reagents affecting the library yield, it is recommended to store the reagents in portions at the time of first use.

2. PCR products are easily contaminated due to improper operation, resulting in inaccurate results, it is recommended to isolate the PCR reaction system preparation area from the PCR product purification area, and use special pipettes to clean each experimental area regularly.

3. Sample preparation

The fragment size of the DNA sample should be concentrated in a range: magnetic beads can be double-selected when the interrupting product fragments are more diffuse. -The recommended number of cycles for this kit is adjusted according to the input volume, please refer to the manual of each platform for the specific program.

-cfDNA input needs to be greater than lng.

4 Reagent preparation

Remove the corresponding reagents in the kit, centrifuge briefly, put the enzyme mixture on ice for use: buffer needs to be dissolved at room temperature before use, then shake and centrifuge, put it on ice for use, deionized water is put at room temperature for use: please prepare the mixture on ice. -The buffer in the kit may

precipitate after dissolving on ice, the precipitate does not affect the function of the reagent, please shake and mix well until the precipitate disappears and then use the reagent.

Schematic diagram of DNA banking process

Illumina platform DNA library construction operation program

* Please read these instructions carefully before performing the experiment and select the protocol according to the type of sequencing platform to be used.

1 Sample processing

1.1 Sample requirements

This kit is suitable for library preparation of genomic DNA extracted from all animal, plant and bacterial species. It is recommended to use high-quality genomic DNA with good integrity and A260/A280=1.8~2.0 for interruption. If the DNA of the physically broken samples is concentrated and of high purity, end repair can be performed directly, and the splice products can be recovered completely. If the interrupted product is more dispersed, fragment screening is required, see 2.1 for the specific program.

1.2 DNA interruption methods and fragment screening

1.2.1 Interruptions

Please interrupt the genomic DNA to the desired primary band range and set the interruption parameters according to the different Covairs models.

1.2.2 Clip filtering

The wide distribution of DNA after interruption usually requires fragment screening to control the concentration of fragments in the final library. A magnetic bead fragment screening protocol is recommended (Table 1), and fragment screening can also be performed by cut-gel purification.

Table 1 Suggested amount of magnetic beads for obtaining different DNA backbands (100 μl reaction system)

DNA fragment size	DNA fragment size	210 bp	260 bp	300 bp	360 bp	430 bp	470 bp	500 bp
Magnetic bead dosage	First choice	100	90	80	70	64	60	55
Magnetic bead dosage	Second choice	50	25	25	25	25	25	25

Selective recovery of DNA fragments is recommended using the CombiVision Magnetic Bead DNA Purification and Recovery Kit.

Note: Selective recovery of DNA fragments is an optional step, and DNA loss is approximately 60-95% for magnetic bead fragment screening. If the interrupted fragments are concentrated, the library can be constructed directly. If the starting sample size is less than 50ng, selective

recovery of DNA fragments is not recommended. In addition, the amount of magnetic beads used for selective recovery of DNA fragments is different for different sizes of DNA libraries. The specific amount of magnetic beads used can be found in Table 1 (if you use magnetic beads from manufacturers other than CombiVision, you need to find out the optimal amount of beads by yourself).

In the following steps, a peak length of 210 bp can be selected for the recovery of DNA fragments, and the starting volume of the reaction is 100 μl, with less than 100 μl made up with deionized water.

1. Before use, CMPure should be left at room temperature for 30 min, and then vortex shaken for 20 s to make it a homogeneous solution.

2. Add deionized water to the interrupting system to replenish the reaction volume to 100 μl.

3. Transfer the above reaction system to a new 1.5 ml centrifuge tube.

4. Add 100 μl of well-mixed CMPure, vortex and shake for 5 s, and leave for 5 min at room temperature.

5. Centrifuge briefly, place the tube on a magnetic rack to separate the beads from the supernatant solution until the solution is clear (about 5 min), carefully transfer the supernatant solution to a new 1.5 ml tube, and discard the beads.

Note: Do not discard the top clear.

6. Add 50 μl of well-mixed CMPure to the supernatant, vortex and shake for 5 s and leave for 5 min at room temperature.

7. Centrifuge the tube briefly on a magnetic rack to separate the beads from the supernatant solution until the solution is clear (about 5 min), carefully aspirate the supernatant and discard it, avoiding contact with the beads that have bound the target DNA.

Note: Do not discard the beads.

8. Keeping the centrifuge tube fixed on the magnetic rack, add 250 μl of freshly prepared 80% ethanol to the centrifuge tube and allow to stand at room temperature for 30 s. When the suspended beads are fully adsorbed, carefully discard the supernatant.

9. Repeat step 8.

10. Keep the centrifuge tube fixed on the magnetic rack and let it stand at room temperature for 10 min to allow the magnetic beads to dry in the air.

11. Remove the centrifuge tube from the magnetic rack, add 46 μl of deionized water (supplied), vortex to completely resuspend the beads in the eluent, and allow to stand at room temperature for 5 min.

12. Centrifuge briefly, place the tube on a magnetic rack until the solution is clear (about 5 min), and transfer 43 μl of eluate to a new PCR tube for downstream end-recovery use.

Note: Be sure not to transfer the magnetic beads; trace bead contamination can affect the proper performance of subsequent DNA library construction.

1.3 Quantification and quality control of DNA from constructed samples

Library sample DNA refers to the DNA in the end repair step, this kit is compatible with cfDNA and physically interrupted gDNA, the sample DNA amount ranges from 0.5-1 μg in a volume of ≤43 μl.

It should be ensured that the DNA fragments of the library samples are concentrated as much as possible; the more concentrated the fragments are, the better the sequencing quality will be; on the contrary, the sequencing quality will be reduced.

2 Library construction process

2.1 Sample Screening Program

For library sample DNA: If the sample DNA after physical interruption is well distributed and of high purity, it can be directly end-repaired, and the splice products can be fully recovered. If the DNA of the physically broken sample has a wide range of distribution, length sorting is usually required. Dual sorting with magnetic beads is recommended, but can also be performed by cutting and purification. There are two options for the length sorting execution position:

Before Ⅰ end repair: the magnetic bead double-selection process can be referred to 1.2 Physical interruption and magnetic bead double-selection, and the magnetic bead dosage can be referred to Table 1. This scheme is suitable for samples with sufficient inputs and poor purity.

After Ⅱ connector connection: bead double selection process can refer to 1.2 Physical interruption and bead double selection, bead dosage can refer to the table 6. This program is suitable for samples with sufficient inputs and high purity.

Note: Ensure the uniqueness of the double-selection step, as double-selection twice will result in a severe degradation of the library quality, and bead double-selection is not recommended when the sample input is less than 50 ng.

2.2 End-of-pipe repair

2.2.1 According to the sample concentration, take an appropriate amount of sample (100 ng is recommended) into a new PCR tube and replenish with deionized water to a total volume of 43 μl (see Table 2). For each batch of library construction, DNA Control can be added as the library quality control, the sample volume of DNA Control is 2 μl, and 41 μl of deionized water should be added to a total volume of 43 μl, and the following end repair reaction mix should be prepared in the PCR tube:

Table 2 Formulation of end-repair reaction solution

individual parts making up a compound	volumetric
fragmented DNA	X
ERAT Mix	2 μl
10×ERAT Buffer	5 μl
deionized water	43-X
Total	50 μl

2.2.2 Shake and mix for 5 s. Instantaneous centrifugation collects the reaction solution to the bottom of the tube, ensuring that there are no air bubbles in the reaction solution;

2.2.3 Place the PCR tube containing the reaction mixture from the previous step on the PCR instrument and react under the following conditions:

Table 3 End-repair reaction conditions

temp	timing
Hot cover (85° C)	
37° C	15 min
65° C	15 min
4° C	Hold

2.3 Joint connections

To construct DNA libraries for the Illumina sequencing platform, we recommend the use of CombiSense II Sequencing Multi-Sample Junction Primer Kit I/II (Illumina platform).

2.3.1 Add the following reaction mixture directly to the above reaction solution where DNA end repair has been completed

Table 4 Preparation of joint connection reaction mixture

individual parts making up a compound	volumetric
T4 DNA Ligase Buffer	14 μl
T4 DNA Ligase	3 μl
Adaptor for Illumina	5 μl
deionized water	8 μl
Total	30 μl

Attention:

1. If the amount of sample input for library construction is less than 50ng, please dilute Adaptor for Illumina 10 times with deionized water and then use it.

2. Shake and mix for 5 s. Ensure adequate mixing and collect the reaction solution to the bottom of the tube by centrifugation;

3. Place the PCR tube containing the reaction solution from the previous step on the PCR instrument and perform the reaction according to the conditions in the table below:

Table 5 Connector Connection Reaction Procedure

temp	timing
Hot cover (30° C)	
23° C	20 min
4° C	Hold

2.4 Connection product purification

There are two options for ligation product purification, selective recovery and complete recovery. Option 2 (complete recovery of DNA fragments) is recommended if the starting sample size is less than 50 ng or if double-selected interruptions from magnetic beads are used; option 1 (selective recovery of DNA fragments) is recommended if non-double-selected interruptions are used.

Scheme I. DNA Fragment Selection and Recovery The operation procedure refers to 1.2.2 Fragment Screening, and the amount of double-selected magnetic beads can be referred to Table 6.

Table 6 Suggested amount of magnetic beads for obtaining DNA master bands (100 μl reaction system)

DNA Clip size	Insert clip +Adaptor+primer	290 bp	340 bp	440 bp	460 bp	500 bp	720 bp
Magnetic	First choice	85	70	55	50	45	35
Magnetic	Second choice	25	25	20	20	20	15

Scheme II. Complete recovery of DNA fragments

1. Remove the CMPure beads 30 min in advance and place at room temperature, shake well before use;

2. Pipette 80 μl of CMPure into 80 μl of ligated product, shake and mix for 5 s, incubate for 5 min at room temperature;

3. For instantaneous centrifugation, place the centrifuge tube on a magnetic rack and let it stand for 5 min until the liquid is clarified, pipette and discard the supernatant;

4. Keeping the centrifuge tube fixed on a magnetic rack, add 250 μl of freshly prepared 80% ethanol and discard the ethanol completely after the suspended beads are fully adsorbed (about 1 min);

5. Repeat step 4 once, the last time sucking up as much liquid as possible from the bottom of the tube. If a small amount remains on the wall of the tube, centrifuge the tube momentarily, separate it on a magnetic rack, and then suck up the liquid from the bottom of the tube with a small-volume pipette;

Note: Do not suck up the magnetic beads as this may affect the yield.

6. Keep the centrifuge tube fixed on the magnetic rack, open the cap of the centrifuge tube and dry at room temperature for 5~6 min until the beads are free of reflection and cracks;

7. Remove the centrifuge tube from the magnetic rack, add 25 μl of deionized water for DNA elution, shake and mix for 5 s, and dissolve for 5 min at room temperature;

8. Instantaneous centrifugation, place the centrifuge tube on a magnetic rack, let it stand for 5 min until the liquid is clarified, transfer all 23 μl of supernatant to a new PCR tube and carry out the PCR amplification reaction or store it at -20℃.

2.5 PCR amplification

2.5.1 Refer to Table 7 for the preparation of PCR reaction mix, and it is recommended to use CombiVision Illumina Sequencing Platform Second Generation Sequencing Multi-Sample Junction Primer Kit I/II.

Table 7 Preparation of PCR amplification reaction mixes

individual parts making up a compound	volumetric	volumetric
2×PCR Mix	25 μl	50 μl
Universal primer	1 μl	2 μl
Index primer	1 μl	2 μl
Purification of recovered connector connection products	23 μl	23 μl
deionized water	\windshield	25 μl
Total	50 μl	100 μl

Note: This kit provides a sufficient amount of 2×PCR Mix to increase the amount of libraries while controlling the number of cycles, and 100 μl of PCR amplification system can be selected.

2.5.2 Shake and mix for 5 s. Centrifuge instantaneously and collect the reaction solution to the bottom of the tube.

2.5.3 Place the above PCR tubes on the PCR instrument and run the reaction according to the following conditions

Table 8 PCR reaction program

temp	timing	cycle number (math.)
thermal cover 98° C	on 3 min	
98° C 60° C 72° C	10 s 15 s 30 s	Refer to table 9
72° C 4° C	5 min Hold	

Note: The number of cycles should be adjusted according to the amount of starting DNA. The exact number of cycles can be found in Table 9.

Table 9 Recommended number of amplification cycles for obtaining 100 ng and 1 μg libraries

sample DNA	Number of cycles required for the corresponding	Number of cycles required for the corresponding
sample DNA	100 ng	1 μg
100 pg	14-16	19-20
1 ng	9-12	14-15
5 ng	7-10	12-14
10 ng	6-8	10-12
50 ng	4-6	8-10
100 ng	2-5	6-9
500 ng	0*/1-3	3-6
1000 ng	0*/1-3	3-4

Attention:

1 Refer to the lowest number of cycles if the bead double-selection was performed before end-repair of the sample DNA, or the highest number of cycles if the bead double-selection was performed after junction attachment.

2 FFPE samples are of poor quality and can be increased by 3 cycles from the recommended maximum number of cycles.

3 *When a full-length Adaptor is used for junction ligation and the library output meets the application requirements, the PCR-Free library can be obtained directly without PCR amplification; if an incomplete Adaptor is used, 1-3 rounds of PCR amplification are required to obtain the complete Adaptor sequence required for sequencing.

4 The number of PCR cycles is greater than or equal to 12 when the sample type is cfDNA.

5 DNA Control The number of cycles is set to 7.

2.6 PCR product purification

1. Remove the CMPure 30 min in advance and place it at room temperature. Shake well before use;

2. Add 1x the volume of CMPure to the PCR product, shake for 5 s, and incubate for 5 min at room temperature;

3. For instantaneous centrifugation, place the centrifuge tube on a magnetic rack and let it stand for 5 min until the liquid is clarified, pipette and discard the supernatant;

4. Keeping the centrifuge tube fixed on a magnetic rack, add 250 μl of freshly prepared 80% ethanol and discard the ethanol completely after the suspended beads are fully adsorbed (about 1 min);

5. Repeat step 4 once, the last time try to suck up the liquid at the bottom of the tube, if there is a small amount of residue in the wall of the tube can be centrifuged instantly, after separation on the magnetic rack, use a small-volume pipette to suck up the liquid at the bottom of the tube; Note: Do not suck up the magnetic beads, so as not to affect the yield.

6. Keep the centrifuge tube fixed on the magnetic rack, open the cap of the centrifuge tube and dry at room temperature for 5~6 min until the beads are free of reflection and cracks;

7. Remove the centrifuge tube from the magnetic rack, add 32 μl of deionized water for DNA elution, shake and mix for 5 s, and dissolve for 5 min at room temperature;

8. Instantaneous centrifugation, place the centrifuge tube on a magnetic rack and let it stand for 5 min until the liquid is clarified, then transfer 30 μl of supernatant to a new 1.5 ml centrifuge tube for on-line testing or storage at -20℃.

2.7 Quality control of the library

Usually, the constructed library needs to be tested for library concentration and length distribution. Determination of library concentration: It is recommended to use fluorescent dye method (Qubit or Picogreen) or qPCR absolute quantitative method to determine the library concentration. Library length distribution: Agilent 2100 Bioanalyzer; LabChip GX11 Touch microfluidic capillary electrophoresis and other equipment for length distribution detection.

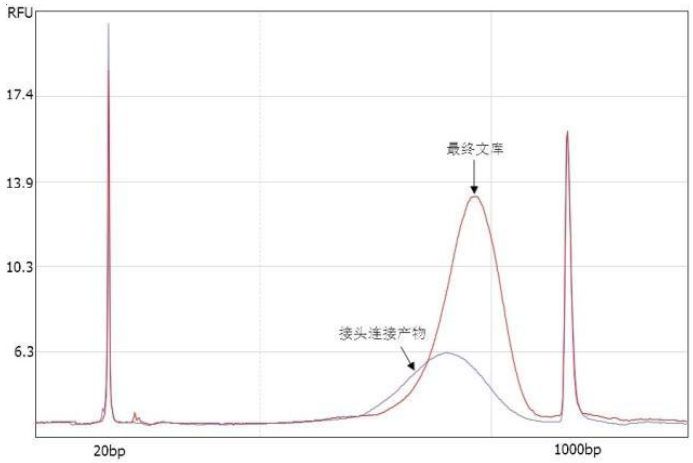

Figure: DNA library construction on Illumina Sequencing Platform by DNA Library Rapid Preparation Kit, and the length distribution of the products in each step of the library construction process.

MGI platform DNA library construction operation program

＊Please read these instructions carefully before performing the experiment and select the protocol according to the type of sequencing platform to be used.

1 Sample processing

1.1 Sample requirements

This kit is suitable for library preparation of genomic DNA extracted from all animal, plant and bacterial species. It is recommended to use high-quality genomic DNA with good integrity and A260/A280=1.8~2.0 for interruption. If the DNA of the physically broken samples is concentrated and of high purity, end repair can be performed directly, and the splice products can be recovered completely. If the interrupted product is more diffuse, fragment screening is required, see 1.2 for specific protocol.

1.2 DNA interruption methods and fragment screening

1.2.1 Interruptions

Please interrupt the genomic DNA to the desired primary band range and set the interruption parameters according to the different Covairs models.

1.2.2 Clip filtering

The wide distribution of DNA after interruption usually requires fragment screening to control the concentration of fragments in the final library. A magnetic bead fragment screening protocol is recommended (Table 1), and fragment screening can also be performed by cutting gel purification.

Table 1 Suggested amount of magnetic beads for obtaining different DNA backbands (100 μl reaction system)

DNA fragment	DNA fragment	210 bp	260 bp	300 bp	360 bp	430 bp	470 bp	500 bp
Bead Dosage	First choice	100	90	80	70	64	60	55
Bead Dosage	Second choice	50	25	25	25	25	25	25

Selective recovery of DNA fragments is recommended using the CombiVision Magnetic Bead DNA Purification and Recovery Kit.

Note: Selective recovery of DNA fragments is an optional step, and DNA loss is approximately 60-95% for magnetic bead fragment screening. If the interrupted fragments are concentrated, the library can be constructed directly. If the starting sample size is less than 50ng, selective recovery of DNA fragments is not recommended. In addition, the amount of magnetic beads used for selective recovery of DNA fragments is different for different sizes of DNA libraries, please refer to Table 1 for the specific amount of magnetic beads (if you use magnetic beads from manufacturers other than CombiVision, you need to find out the optimal amount of magnetic beads by yourself).

In the following steps, a peak length of 210 bp can be selected for the recovery of DNA fragments, and the starting volume of the reaction is 100 μl, with less than 100 μl made up with deionized water.

1. Before use, CMPure should be left at room temperature for 30 min, and vortex shaking CMPure for 20 s, so that it can be thoroughly mixed into a homogeneous solution;

2. Add deionized water to the interrupting system to replenish the reaction volume to 100 μl;

3. Transfer the above reaction system to a new 1.5 ml centrifuge tube;

4. Add 100 μl of well-mixed CMPure, vortex and shake for 5 s, and leave for 5 min at room temperature;

5. Centrifuge briefly, place the tube on a magnetic rack to separate the beads from the supernatant solution until the solution is clear (approximately 5 min), carefully transfer the supernatant solution to a new 1.5 ml centrifuge tube, and discard the beads; NOTE: Do not discard the supernatant.

6. Add 50 μl of well-mixed CMPure to the supernatant, vortex and shake for 5 s and leave for 5 min at room temperature;

7. Centrifuge briefly, place the tube on a magnetic rack to separate the beads from the supernatant solution until the solution is clear (about 5 min), carefully aspirate and discard the supernatant, avoiding contact with the beads that have bound the target DNA during this time; NOTE: Do not discard the beads.

8. Keeping the centrifuge tube fixed on the magnetic rack, add 250 μl of freshly prepared 80% ethanol to the centrifuge tube and allow to stand at room temperature for 30 s. When the suspended beads are fully adsorbed, carefully discard the supernatant;

9. Repeat step 8;

10. Keep the centrifuge tube fixed on the magnetic rack and let it stand at room temperature for 10 min to allow the magnetic beads to dry in the air;

11. Remove the centrifuge tube from the magnetic rack, add 46 μl of deionized water (supplied), vortex to completely resuspend the beads in the eluent, and allow to stand at room temperature for 5 min;

12. Centrifuge briefly, place the tube on a magnetic rack until the solution is clear (takes about 5 min), and transfer 43 μl of eluate to a new PCR tube for downstream end-recovery use.

Note: Be sure not to transfer the magnetic beads; trace bead contamination can affect the proper performance of subsequent DNA library construction.

1.3 Quantification and quality control of DNA from constructed samples

Library sample DNA refers to the DNA in the end-repair step, and the amount of compatible sample DNA in this kit ranges from 0.1-1000 ng, with a volume of ≤ 43 μl. It should be ensured that the library sample DNA fragments are concentrated as much as possible, and the more concentrated the fragments are, the better the quality of sequencing will be; on the contrary, the quality of sequencing will be reduced.

2 Library construction process

Regarding library sample DNA: If the sample DNA after physical interruption has a concentrated distribution and is of high purity, it can be directly end-repaired and the splice products can be fully recovered. If the DNA of the physically broken samples is more widely distributed, length sorting is usually required. We recommend the use of magnetic bead double sorting, which can also be carried out by cut-gel purification. Refer to 1.2 for magnetic bead double sorting protocols.

2.1 End-of-pipe repair

2.1.1 According to the sample concentration, take an appropriate amount of sample (100 ng is recommended) into a new PCR tube and replenish with deionized water to a total volume of 43 μl (see Table 2). For each batch of library construction, add DNA Control as the library quality control, the sample volume of DNA Control is 2 μl, and replenish 41 μl of deionized water to a total volume of 43 μl, and prepare the following end repair reaction mix in the PCR tube:

Table 2 Formulation of end-repair reaction solution

individual parts making up a compound	volumetric
fragmented DNA	X
ERAT Mix	2 μl
10×ERAT Buffer	5 μl
deionized water	43-X
Total	50 μl

2.1.2 Shake and mix for 5 s. Instantaneous centrifugation collects the reaction solution to the bottom of the tube, ensuring that there are no air bubbles in the reaction solution;

2.1.3 Place the PCR tube containing the reaction mixture from the previous step on the PCR instrument and react under the following conditions:

Table 3 End-repair reaction conditions

individual parts making up a compound	volumetric
T4 DNA Ligase Buffer	14 μl
T4 DNA Ligase	3 μl
Adaptor for MGI	5 μl
deionized water	8 μl
Total	30 μl

2.2 Joint connections

It is recommended to use CombiVision's second-generation sequencing multi-sample junction primer kit Ⅰ, Ⅱ or Ⅲ.

2.2.1 Add the following reaction mixture directly to the above reaction solution where DNA end repair has been completed

Table 4 Preparation of joint connection reaction mixture

temp	timing
Hot cover (85° C)	
37° C	15 min
65° C	15 min
4° C	Hold

Note:

1. If the library sample input is less than 50 ng, please dilute Adaptor for MGI 5-fold with deionized water and then use it.

2. Shake and mix for 5 s, make sure that the mixing is sufficient, and then centrifuge the reaction solution to the bottom of the tube; 3. Place the connected reaction system on the PCR instrument and perform the reaction according to the conditions in the table below:

Table 5 Connector Connection Reaction Procedure

temp	timing
Hot cover (30° C)	
23° C	20 min
4° C	Hold

2.3 Purification of linkage products

1. Remove the CMPure beads 30 min in advance and place them at room temperature, and shake well before use;

2. Pipette 1 times the volume (80 μl) of CMPure into 80 μl of ligated product, shake and mix for 5 s, and incubate for 5 min at room temperature;

3. For instantaneous centrifugation, place the centrifuge tube on a magnetic rack and let it stand for 5 min until the liquid is clarified, pipette and discard the supernatant;

4. Keeping the centrifuge tube fixed on a magnetic rack, add 250 μl of freshly prepared 80% ethanol and discard the ethanol completely after the suspended beads are fully adsorbed (about 1 min);

5. Repeat step 4 once, the last time try to suck up the liquid at the bottom of the tube, if there is a small amount of residue in the wall of the tube can be centrifuged instantly, after separation on the magnetic rack, use a small-volume pipette to suck up the liquid at the bottom of the tube; Note: Do not suck up the magnetic beads, so as not to affect the yield.

6. Keep the centrifuge tube fixed on the magnetic rack, open the cap of the centrifuge tube and dry at room temperature for 5~6 min until the beads are free of reflection and cracks;

7. Remove the centrifuge tube from the magnetic rack, add 46 μl of deionized water for DNA elution, shake and mix for 5 s, and dissolve for 5 min at room temperature;

8. Instantaneous centrifugation, place the centrifuge tube on a magnetic rack and let it stand for 5 min until the liquid is clarified, then transfer all 44 μl of the supernatant to a new PCR tube for the next reaction or store it at -20℃.

2.4 PCR amplification

2.4.1 Prepare PCR reaction mix (Table 6): add 6 μl Index Primer Mix to the PCR tube from the previous step (it is recommended to use the second-generation sequencing multi-sample junction primer kit Ⅰ, Ⅱ, or Ⅲ, and add one Index Primer Mix or a set of Index Primer Mix for each sample, and then add 50 μl 2×PCR Mix.

Note: The Index Primer Mix is composed of Universal primer and Index primer, of which there are 128 Index primers. According to different mixing strategies, different Indexes can be added to each sample, or one set of Indexes can be added to one sample; it is required to ensure that all the Indexes in the final mixed library are assembled in sets. 4 of Indexes 1-16 are in one set, and there are 4 sets in total, which are 1-4, 5-8, 9-12, and 13-16 respectively. 8 of Indexes 17-128 are in one set, and there are 14 sets in total, which are 17-24, 25-25, and 13-16 respectively. In Index17-128, 8 are a set, totaling 14 sets, respectively 17-24, 25-32, 33-40, 41-48, 49-56, 57-64, 65-72, 73-80, 81-88, 89-96, 97-104, 105-112, 113-120 and 121-128.

Table 6 Preparation of PCR reaction mixes

individual parts making up a compound	volumetric
2×PCR Mix	50 μl
Index primer Mix	6 μl
Purification of recovered connector connection products	44 μl
Total	100μl

2. Shake and mix for 5 s. Instantly centrifuge to collect the reaction solution to the bottom of the tube;

3. Place the above PCR tubes on the PCR instrument and perform the reaction according to the conditions in the table below:

Table 7 PCR reaction program

temp	timing	cycle number (math.)
thermal cover 98° C	on 3 min	
98° C 60° C 72° C	10 s 15 s 30 s	Refer to table 8
72° C 4° C	5 min Hold	

Note: The number of cycles should be adjusted according to the amount of starting DNA. The exact number of cycles can be found in Table 8.

sample DNA	Number of cycles	corresponding
	300 ng	1 μg
100 pg	16-18	19-20
1 ng	11-13	15-16
5 ng	9-11	13-15
10 ng	8-10	10-13
50 ng	6-7	8-10
100 ng	5-6	7-9
500 ng	0*/1-3	5-6
1000 ng	0*/1-3	4-5

Attention:

1 FFPE Sample quality is poor and may be increased by 3 cycles from the recommended maximum number of cycles.

2 When DNA quality is poor and libraries are long, the number of cycles can be increased appropriately to obtain a sufficient amount of library.

3 *When a full-length Adaptor is used for junction ligation and the library output meets the application requirements, the PCR-Free library can be obtained directly without PCR amplification; if an incomplete Adaptor is used, 1-3 rounds of PCR amplification are required to obtain the complete Adaptor sequence required for sequencing.

4 The number of PCR cycles is ≥ 12 when the sample type is cfDNA. 5 The number of DNA Control cycles is set to 7.

2.5 PCR product purification

1. Remove the CMPure 30 min in advance and place it at room temperature. Shake well before use;

2. Add 1x the volume of CMPure to the PCR product, shake for 5 s, and incubate for 5 min at room temperature;

3. For instantaneous centrifugation, place the centrifuge tube on a magnetic rack and let it stand for 5 min until the liquid is clarified, pipette and discard the supernatant;

4. Keeping the centrifuge tube fixed on a magnetic rack, add 250 μl of freshly prepared 80% ethanol and discard the ethanol completely after the suspended beads are fully adsorbed (about 1 min);

5. Repeat step 4 once, the last time try to suck up the liquid at the bottom of the tube, if there is a small amount of residue in the wall of the tube can be centrifuged instantly, after separation on the magnetic rack, use a small-volume pipette to suck up the liquid at the bottom of the tube; Note: Do not suck up the magnetic beads, so as not to affect the yield.

6. Keep the centrifuge tube fixed on the magnetic rack, open the cap of the centrifuge tube and dry at room temperature for 5~6 min until the beads are free of reflection and cracks;

7. Remove the centrifuge tube from the magnetic rack, add 32 μl of deionized water for DNA elution, shake and mix for 5 s, and dissolve for 5 min at room temperature;

8. Instantaneous centrifugation, place the centrifuge tube on a magnetic rack and let it stand for 5 min until the liquid is clarified, then transfer 30 μl of supernatant to a new 1.5 ml centrifuge tube for on-line testing or storage at -20℃.

2.6 Quality control of the library

Usually, the constructed library needs to be tested for library concentration and length distribution. Determination of library concentration: It is recommended to use fluorescent dye method (Qubit or Picogreen) or qPCR absolute quantitative method to determine the library concentration. Library length distribution: Agilent 2100 Bioanalyzer; LabChip GX11 Touch microfluidic capillary electrophoresis and other equipment for length distribution detection.