Tinzyme Co.,Limited

Email:sales@tinzyme.com Website:www.tinzyme.com
Tel:+86-755-86134126 WhatsApp/Facebook/Twitter:+86-189-22896756

Fast NGS Library Kit for Illumina&MGI

Product Number:PCK85M

Shipping and Storage

-20°C,dry ice transport.

Components

Component	PCK85M (24rxns)	PCK85M (96rxns)
ERAT Mix	48μL	192μL
10×ERAT Buffer	120μL	480μL
T4 DNA Ligase	72μL	288μL
T4 DNA Ligase Buffer	336μL	672μL×2
2×PCR Mix	1.2mL	1.2mL×2
DNA Control(50ng/μL,200bp)	20μL	20μL

Description

This kit is suitable for both Illumina and MGI sequencing platforms. It provides the premixed enzyme modules required for DNA end repair,5'-end phosphorylation modification,3'-end A addition and Adaptor ligation in DNA library preparation. It can be used with different sequencing platform adapters. Primer kits can prepare DNA into specific DNA libraries for Illumina or MGI sequencing platforms. Using high-fidelity DNA polymerase for library enrichment and unbiased PCR amplification,the coverage area of the sequence is expanded,and high-quality DNA libraries can be prepared. All reagents provided in the kit have undergone strict quality control and functional verification to ensure the stability of library preparation to the greatest extent.

Features

1. End repair and phosphorylation and adding A to complete in one step;
2. After the end repair without purification,directly add the Adaptor;
3. Ultra fidelity amplification,the maximum extent to reduce the amplification bias;
4. The library is suitable for multiple sequencing platforms:MGI sequencing platform:MGISEQ-2000, MGISEQ-200, BGISEQ-500 and other MGI sequencing platform lllumina GAllx, HiSacnSQ, HiSeq 250/2000/1000, MiSeqsequencing and other lllumina platform sequencers;
5. It is suitable for preparing gDNA and cDNA libraries after physical interruption.

Self-provided reagents and consumables

1. Magnetic rack:DynaMagTM-2 is recommended;
2. Anhydrous ethanol (100% ethanol,analytically pure);Deionized water (pH 7.0-8.0);
3. Adaptor index kit:
   MGI platforms.
   llumina platform.
4. DNA purification recycling kit;
   Reaction tube:Low adsorption PCR tube and 1.5mL centrifuge tube are recommended;
   Tips:It is recommended to use high quality filter gun head to prevent contamination of kit and library samples.

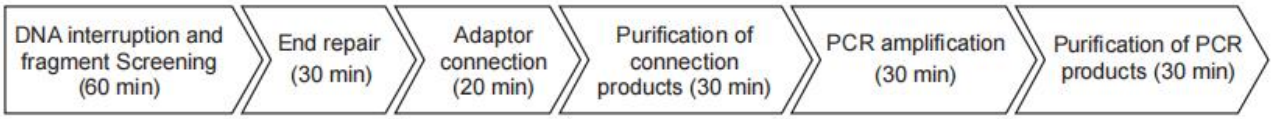
Preparation and important things before the experiment

1. In order to avoid repeated freezing and thawing of reagents affecting the yield of the library,it is recommended to store the

reagents in separate packaging when they are used for the first time;

2. Due to improper operation of PCR products,it is easy to pollute,resulting in inaccurate experiment results.It is suggested to isolate the preparation area of PCR reaction system from the purification area of PCR products,and use a special pipette to clean each experiment area regularly;
3. Sample preparation
   (1) The fragment size of DNA sample should be concentrated:magnetic bead double selection can be carried out when the fragment of interrupted product is more dispersed;
   (2) The recommended number of library cycles for this kit is adjusted according to the amount of input. For specific schemes,please refer to the instructions of each platform;
   (3) The input of cfDNA must be greater than 1ng.
4. Reagent preparation
   (1) Take out the corresponding reagent in the kit,centrifuge briefly,and put the enzyme mixture on ice for use:before use,dissolve the buffer solution at room temperature and then centrifuge it in shock,put it on ice for use,and put deionized water at room temperature for use; Please make the mixture on the ice.
   (2) The buffer solution in the kit may precipitate after freezing and dissolving. The precipitation will not affect the function of the reagent. Please fully shake and mix until the precipitation disappears.

Schematic diagram of DNA library construction process



Illumina platform DNA library preparation protocol

* Please read the instructions carefully before the experiment and select the operation plan according to the type of sequencing platform to be used.

1. Sample processing

1.1. Sample Requirements

This kit is suitable for library preparation from genomic DNA extracted from all animal,plant,bacterial and other species samples. It is recommended to use high-quality genomic DNA with good integrity and A260/A280=1.8~2.0 for interruption. If the DNA distribution of the samples after physical interruption is concentrated and the purity is high,end repair can be carried out directly,and the complete recovery scheme of the adapter ligation products is used. If the interrupted product is more diffuse,fragment screening needs to be performed. For specific protocols,see 2.1.

1.2. DNA fragmentation method and fragment screening

1.2.1. Interruption

Please interrupt the genomic DNA to the desired main band range,and set the corresponding interrupt parameters according to different Covairs models.

1.2.2. Fragment screening

After fragmentation,the DNA distribution range is wide,and fragment screening is usually required to control the fragment concentration of the final library. The magnetic bead fragment screening protocol is recommended (Table 1),and fragment screening can also be performed by gel-cutting purification.

Table 1 Recommended dosage of magnetic beads for obtaining different DNA main bands (100μL reaction system)

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

Note:Selective recovery of DNA fragments is an optional step,and the amount of DNA loss is approximately 60-95% for magnetic bead fragment screening. If the fragments of the interrupted products are concentrated,the library can be built directly. The amount of starting sample is less than 50ng,and selective recovery of DNA fragments is not recommended. In addition,when constructing DNA libraries of different sizes,the amount of magnetic beads used for selective recovery of DNA fragments is different. For the specific amount of magnetic beads,please refer to Table 1.

In the following steps,the peak length of recovered DNA fragments can be selected to be 210bp,and the initial volume of the reaction is 100μL.

1.2.2.1. Before using the Magnetic Bead,let it stand at room temperature for 30 minutes,and vortex the Magnetic Bead for 20s to thoroughly mix it into a homogeneous solution.

1.2.2.2. Add deionized water to the interrupted system to bring the reaction volume up to 100μL.

1.2.2.3. Transfer the above reaction to a new 1.5mL centrifuge tube.

1.2.2.4. Add 100μL of well-mixed Magnetic Bead,vortex for 5s,and let stand for 5min at room temperature.

1.2.2.5. Briefly centrifuge,place the centrifuge tube on a magnetic stand,and separate the magnetic beads from the supernatant solution until the solution is clear (about 5minutes),carefully transfer the supernatant solution to a new 1.5mL centrifuge tube,and place Discard the magnetic beads.

Note:Do not discard the supernatant.

1.2.2.6. Add 50μL of mixed Magnetic Bead to the supernatant,vortex for 5s,and place at room temperature for 5min.

1.2.2.7. Briefly centrifuge,put the centrifuge tube on the magnetic stand,and separate the magnetic beads from the supernatant solution until the solution is clear (about 5minutes). Carefully aspirate and discard the supernatant,avoiding contact with the magnetic particles that have bound the target DNA beads.

Note:Do not discard the magnetic beads.

1.2.2.8. Continue to keep the centrifuge tube fixed on the magnetic stand,add 250μL of freshly prepared 80% ethanol to the centrifuge tube,and place at room temperature for 30s. After the suspended magnetic beads are completely adsorbed,carefully discard the supernatant.

1.2.2.9. Repeat step 8.

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

1.2.2.11. Remove the centrifuge tube from the magnetic stand,add 46μL of deionized water (self-provided),vortex to completely resuspend the magnetic beads in the eluent,and let stand at room temperature for 5minutes.

1.2.2.12. Centrifuge briefly,place the tube on the magnetic stand until the solution is clear (about 5minutes),and transfer 43μL of the eluate to a new PCR tube for downstream end repair.

Note:Be sure not to transfer the magnetic beads. Contamination of a small amount of magnetic beads can affect the normal progress of subsequent DNA library preparation.

1.3. Quantification and quality control of DNA from library samples

The DNA of the library preparation sample refers to the DNA in the end repair step. This kit is compatible with cfDNA and gDNA after physical interruption. The amount of sample DNA is 0.5-1μg,and the volume is ≤43μL. It should be ensured that the DNA fragments of the library building samples are concentrated as much as possible. The more concentrated the fragments,the better the sequencing quality;otherwise,the sequencing quality will decrease.

2. Library preparationprocess

2.1. Sample Screening Scheme

About the sample DNA for library preparation:If the sample DNA after physical interruption is concentrated and high in purity,end repair can be performed directly,and the adapter ligation product can be completely recovered. Length sorting is usually required if the DNA of the physically disrupted sample has a wide distribution. It is recommended to use the magnetic bead double-selection scheme,and it can also be sorted by gel-cutting purification. There are two options for where the length sorting is performed:

2.1.1. Before end repair:Please refer to 1.2 Physical Interruption and Magnetic Bead Double Selection for the magnetic bead double selection process,and the magnetic bead dosage can refer to Table 1. This scheme is suitable for samples with sufficient input and poor purity.

2.1.2. After the adaptor is connected:Please refer to 1.2 Physical Interruption and Magnetic Bead Dual Selection for the magnetic bead dual selection process,and the magnetic bead dosage can refer to Table 6. This scheme is suitable for samples with sufficient input and high purity.

Note:To ensure the uniqueness of the double-selection step,performing double-selection twice will lead to a serious decline in the quality of the library. When the sample input amount is less than 50ng,magnetic bead double-selection is not recommended.

2.2. End Repair

2.2.1. Depending on the sample concentration,take an appropriate amount of sample (100ng recommended) into a new PCR tube and add deionized water to a total volume of 43μL (see Table 2). DNA Control can be added to each batch of library preparationas a quality control substance for library preparation. The sampling volume of DNA Control is 2μL,and 41μL of deionized water is added to make the total system 43μL. The following end repair reaction mixture is prepared in a PCR tube.

Table 2 Preparation of end repair reaction solution

Component	Volume
Fragmented DNA	X
ERAT Mix	2μL
10×ERAT Buffer	5μL
Deionized water	43-X
Total	50μL

2.2.2. Shake and mix for 5s,and centrifuge briefly to collect the reaction solution to the bottom of the tube to ensure that there are no air bubbles in the reaction solution;

2.2.3. Place the PCR tube containing the reaction mixture in the previous step on the PCR machine and react according to the following conditions (Table 3):

Table 3 End Repair Reaction Conditions

Temperature	Time
Hot Lid (85℃)	
37℃	15min
65℃	15min
4℃	Hold

2.3. Adaptor connection

2.3.1. Add the following reaction mixture directly to the above reaction solution that has completedDNA end repair.

Table 4 Preparation of linker ligation reaction mixture

Component	Volume
T4 DNA Ligase Buffer	14μL
T4 DNA Ligase	3μL
Adaptor for Illumina	5μL
Deionized water	8μL
Total	30μL

Note:1)If the input amount of the library sample is less than 50ng,please dilute the Adaptor for Illumina 10 times with deionized water before use.

2)Shake and mix for 5s,please ensure that the mixing is sufficient,and centrifuge briefly to collect the reaction solution to the bottom of the tube;

3)Place the PCR tube containing the reaction solution in the previous step on the PCR machine,and perform the reaction according to the conditions in the following table:

Table 5. Adaptor ligation reaction procedure

Temperature	Volume
Hot Lid(30℃)	
23℃	20min
4℃	Hold

2.4. Purification of ligation products

There are two methods for purification of products,selective recovery and complete recovery. If the initial sample size is less than 50ng or interruption products after magnetic bead double selection are used,option 2 (complete DNA fragment recovery) is recommended for this step. Option 1 (DNA fragment selection recovery) is recommended if non-double-selected interrupting products are used.

Scheme 1. DNA fragment selection and recovery

Refer to 1.2.2 Fragment Screening for the operation process,and Table 6 for the amount of double-selection magnetic beads.

Table 6 Recommended amount of magnetic beads for obtaining DNA main band (100μL reaction system)

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

Scheme 2. Complete recovery of DNA fragments

1. Take out the Magnetic Bead magnetic beads 30 minutes in advance and place them at room temperature,and shake and mix well before use;
2. Pipette 80μL Magnetic Bead into 80μL ligation product,shake and mix for 5 s,and incubate at room temperature for 5 min;
3. Centrifuge briefly,place the centrifuge tube on a magnetic stand,let it stand for 5 minutes until the liquid is clear,pipette and discard the supernatant;
4. Keep the centrifuge tube fixed on the magnetic stand,add 250μL of freshly prepared 80% ethanol,and completely discard the ethanol (about 1 min) after the suspended magnetic beads are completely adsorbed;
5. Repeat step 4 once,and try to suck the liquid at the bottom of the tube as much as possible for the last time. If a small amount remains on the wall of the tube,you can centrifuge the centrifuge tube momentarily. After separating on the magnetic stand,use a small-scale pipette to suck up the liquid at the bottom of the tube;

Note:Do not aspirate the magnetic beads,so as not to affect the yield.

6. Keep the centrifuge tube fixed on the magnetic stand,open the cap of the centrifuge tube,and dry at room temperature for 5-6 min,until the magnetic beads have no reflection or cracks;
7. Remove the centrifuge tube from the magnetic stand,add 25μL of deionized water for DNA elution,shake and mix for 5 s,and dissolve at room temperature for 5 min;
8. Centrifuge briefly,place the centrifuge tube on a magnetic rack,and let it stand for 5 minutes until the liquid is clear. Transfer all 23μL of the supernatant to a new PCR tube for PCR amplification reaction or store at -20°C.

2.5. Purification of ligation products

2.5.1. Refer to Table 7 to prepare PCR reaction mixture.

Table 7 Preparation of PCR amplification reaction mixture

Component	Volume	Volume
2×PCR Mix	25μL	50μL
Universal primer	1μL	2L
Index primer	1μL	2μL

Ligand products (Purified and recovered)	23μL	23μL
Deionized water	\	25μL
Total	50μL	100μL

Note:This kit provides a sufficient amount of 2×PCR Mix to increase the library output on the basis of controlling the number of cycles,and a 100μL PCR amplification system can be selected

2.5.2. Shake and mix for 5 s,centrifuge briefly,and collect the reaction solution to the bottom of the tube.

2.5.3. Place the above PCR tube on the PCR machine and perform the reaction according to the following conditions:

Table 8 PCR reaction program

Component	Time	Cycles
Hot Lid	On	
98℃	3min	
98℃	10s	Refer to Table 9
60℃	15s	
72℃	30s	
72℃	5min	
4℃	Hold	

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

DNA	Cycles	
	100ng	1μg
100pg	14-16	19-20
1ng	9-12	14-15
5ng	7-10	12-14
10ng	6-8	10-12
50ng	4-6	8-10
100ng	2-5	6-9
500ng	0*/1-3	3-6
1000ng	0*/1-3	3-4

Note:1）If the sample DNA is double-selected on magnetic beads before end repair,refer to the minimum cycle number; if the magnetic bead double-select after adapter ligation,refer to the maximum number of cycles.

2）FFPE samples are of poor quality,3 cycles can be added to the recommended maximum number of cycles.

3）When using the full-length Adaptor when the adapter is connected,and the library output meets the application requirements,the PCR-Free library can be obtained directly without the PCR amplification step; if the incomplete adapter is used,1-3 rounds of PCR are required Amplify to obtain the complete adaptor sequence required for sequencing.

4）When the sample type is cfDNA,the number of PCR cycles is greater than or equal to 12.

5）Set the number of DNA Control cycles to 7.

2.6. PCR product purification

2.6.1. Take out the Magnetic Bead 30 minutes in advance and place it at room temperature,and shake and mix well before use; Add 1 volume of Magnetic Bead to the PCR product,shake and mix for 5 s,and incubate at room temperature for 5 min;

2.6.2. Centrifuge briefly,place the centrifuge tube on a magnetic stand,let it stand for 5 minutes until the liquid is clear,pipette and discard the supernatant;

2.6.3. Keep the centrifuge tube fixed on the magnetic stand,add 250μL of freshly prepared 80% ethanol,and completely discard the ethanol (about 1 min) after the suspended magnetic beads are completely adsorbed;

2.6.4. Repeat step 4 once,and try to suck the liquid at the bottom of the tube as much as possible for the last time. If a small amount remains on the wall of the tube,you can centrifuge the centrifuge tube momentarily. After separating on the magnetic stand,use a small-scale pipette to suck up the liquid at the bottom of the tube;

Note:Do not aspirate the magnetic beads,so as not to affect the yield.

2.6.5. Keep the centrifuge tube fixed on the magnetic stand,open the cap of the centrifuge tube,and dry at room temperature for 5-6 min,until the magnetic beads have no reflection or cracks;

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

2.6.7. Centrifuge briefly,place the centrifuge tube on a magnetic rack,and let it stand for 5 minutes until the liquid is clear. Transfer 30μl of the supernatant to a new 1.5mL centrifuge tube for on-machine testing or store at -20°C.

2.7. Quality Control of Libraries

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 quantification method for library concentration determination. Length distribution detection of library:Length distribution detection was performed by Agilent 2100 Bioanalyzer; LabChip GXll Touch microfluidic capillary electrophoresis and other equipment.

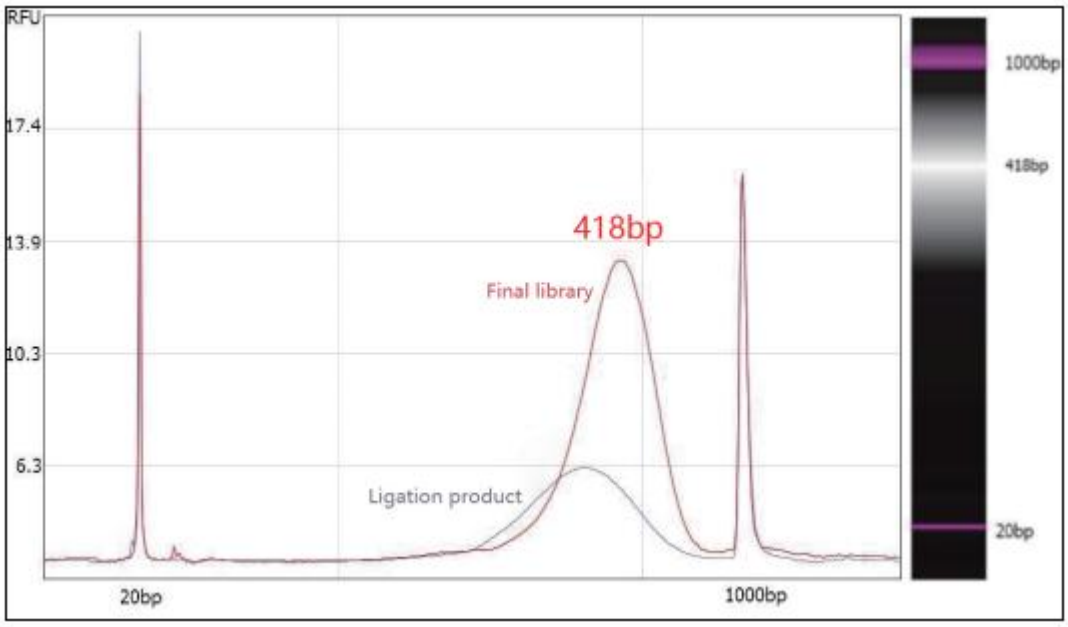


Figure:The DNA library rapid preparation kit constructs the DNA library of the Illumina sequencing platform,and detects the length distribution of the products in each step of the library preparation process. Illumina platform DNA library preparation protocol.

MGI platform DNA library preparation protocol

* Please read the instructions carefully before the experiment and select the operation plan according to the type of sequencing platform to be used.

1. Sample processing

1.1. Sample Requirements

This kit is suitable for library preparation from genomic DNA extracted from all animal,plant,bacterial and other species samples. It is recommended to use high-quality genomic DNA with good integrity and A260/A280=1.8~2.0 for interruption. If the DNA distribution of the samples after physical interruption is concentrated and the purity is high,end repair can be carried out directly,and the complete recovery scheme of the adapter ligation products is used. If the interrupted product is more diffuse,fragment screening needs to be performed. For specific protocols,see 1.2.

1.2. DNA fragmentation method and fragment screening

1.2.1. Interruption

Please interrupt the genomic DNA to the desired main band range,and set the corresponding interrupt parameters according to different Covairs models.

1.2.2. Fragment screening

After fragmentation,the DNA distribution range is wide,and fragment screening is usually required to control the fragment concentration of the final library. The magnetic bead fragment screening protocol is recommended (Table 1),and fragment screening can also be performed by gel-cutting purification.

Table 1 Recommended dosage of magnetic beads for obtaining different DNA main bands (100μL reaction system)

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

Note:Selective recovery of DNA fragments is an optional step,and the amount of DNA loss is approximately 60-95% for magnetic bead fragment screening. If the fragments of the interrupted products are concentrated,the library can be built directly. The amount of starting sample is less than 50ng,and selective recovery of DNA fragments is not recommended. In addition,when constructing DNA libraries of different sizes,the amount of magnetic beads used for selective recovery of DNA fragments is different. For the specific amount of magnetic beads,please refer to Table 1.

In the following steps,the peak length of recovered DNA fragments can be selected to be 210bp,and the initial volume of the reaction is 100μL.

1.2.2.1. Before using the Magnetic Bead,let it stand at room temperature for 30 min,and vortex the Magnetic Bead for 20s to thoroughly mix it into a homogeneous solution;

1.2.2.2. Add deionized water to the interrupted system to make the reaction volume up to 100μL;

1.2.2.3. Transfer the above reaction system to a new 1.5mL centrifuge tube;

1.2.2.4. Add 100μL of well-mixed Magnetic Bead,vortex for 5s,and let stand for 5min at room temperature;

1.2.2.5. Briefly centrifuge,place the centrifuge tube on a magnetic stand to separate the magnetic beads and the supernatant solution until the solution is clear (about 5 min),carefully transfer the supernatant solution to a new 1.5mL centrifuge tube,and discard magnetic beads;

Note:Do not discard the supernatant.

1.2.2.6. Add 50μL of mixed Magnetic Bead to the supernatant,vortex for 5s,and place at room temperature for 5min;

1.2.2.7. Briefly centrifuge,put the centrifuge tube on the magnetic stand,and separate the magnetic beads from the supernatant solution until the solution is clear (about 5minutes),carefully aspirate the supernatant and discard,avoiding contact with the magnetic beads that have bound the target DNA during this period;

Note:Do not discard the magnetic beads.

1.2.2.8. Continue to keep the centrifuge tube fixed on the magnetic stand,add 250μL of freshly prepared 80% ethanol to the centrifuge tube,and place at room temperature for 30 s. After the suspended magnetic beads are completely adsorbed,carefully discard the supernatant;

1.2.2.9. Repeat step 8;

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

1.2.2.11. Remove the centrifuge tube from the magnetic stand,add 46μL of deionized water (self-provided),vortex to completely resuspend the magnetic beads in the eluent,and let stand at room temperature for 5min;

1.2.2.12. Centrifuge briefly,place the tube on a magnetic stand until the solution is clear (about 5min),and transfer 43μL of the eluate to a new PCR tube for use in downstream end repair.

Note:Be sure not to transfer the magnetic beads. Contamination of a small amount of magnetic beads can affect the normal progress of subsequent DNA library preparation.

1.3. Quantification and quality control of DNA from library samples

The DNA of the library building sample refers to the DNA in the end-repair step. The amount of sample DNA compatible with this kit is 0.1-1000ng,and the volume is ≤ 43μL. It should be ensured that the DNA fragments of the library building samples are concentrated as much as possible. The more concentrated the fragments,the better the sequencing quality; otherwise,the sequencing quality will decrease.

2. Library preparation process

Regarding the sample DNA for library preparation:If the DNA of the sample after physical interruption is concentrated and high in purity,end repair can be performed directly,and the adapter ligation product can be completely recovered. Length sorting is usually required if the DNA of the physically disrupted sample has a wide distribution. It is recommended to use the magnetic bead double-selection scheme,and it can also be sorted by gel-cutting purification. Refer to 1.2 for the magnetic bead dual selection scheme.

2.1. End Repair

2.1.1. Depending on the sample concentration,take an appropriate amount of sample (100ng recommended) into a new PCR tube and add deionized water to a total volume of 43μL (see Table 2). DNA Control can be added to each batch of library preparation as a quality control substance for library Preparation The sampling volume of DNA Control is 2μL,and 41μL of deionized water is added to make the total system 43μL. The following end repair reaction mixture is prepared in a PCR tube:

Table 2 Preparation of end repair reaction solution

Component	Volume
Fragmented DNA	X
ERAT Mix	2μL
10×ERAT Buffer	5μL
Deionized water	43-X
Total	50μL

2.1.2. Shake and mix for 5 s,and centrifuge briefly to collect the reaction solution to the bottom of the tube to ensure that there are no air bubbles in the reaction solution;

2.1.3. Place the PCR tube containing the reaction mixture in the previous step on the PCR machine and react according to the following conditions:

Table 3 End Repair Reaction Conditions

Temperature	Time
Hot Lid (85℃)	
37℃	15min
65℃	15min
4℃	Hold

2.2. Adaptor connection

To construct the DNA library of Illumina sequencing platform.

2.2.1. Add the following reaction mixture directly to the above reaction solution that has completed DNA end repair

Table 4 Preparation of adaptor ligation reaction mixture

Component	Volume
T4 DNA Ligase Buffer	14μL
T4 DNA Ligase	3μL
Adaptor for MGI	5μL
Deionized water	8μL

Total	30μL

Note:1）If the input amount of the library sample is less than 50ng,please dilute the Adaptor for Illumina 10 times with deionized water before use.

2）Shake and mix for 5 s,please ensure that the mixing is sufficient,and centrifuge briefly to collect the reaction solution to the bottom of the tube;

3）Place the PCR tube containing the reaction solution in the previous step on the PCR machine,and perform the reaction according to the conditions in the following table:

Table 5. Adaptor ligation reaction procedure

Temperature	Time
Hot Lid (30℃)	
23℃	20min
4℃	Hold

2.3. Ligation product purification

2.3.1. Take out the Magnetic Bead magnetic beads 30 minutes in advance and place them at room temperature. Shake and mix well before use;

2.3.2. Pipette 1 volume (80μL) of Magnetic Bead into 80μL ligation product,shake and mix for 5 s,and incubate at room temperature for 5 min;

2.3.3. Centrifuge briefly,place the centrifuge tube on a magnetic stand,let it stand for 5 minutes until the liquid is clear,pipette and discard the supernatant;

2.3.4. Keep the centrifuge tube fixed on the magnetic stand,add 250μL of freshly prepared 80% ethanol,and completely discard the ethanol after the suspended magnetic beads are completely adsorbed (about 1 min).

2.3.5. Repeat step 4 once,and try to suck the liquid at the bottom of the tube as much as possible for the last time. If a small amount remains on the tube wall,you can centrifuge the centrifuge tube momentarily. After separation on the magnetic stand,use a small-scale pipette to suck up the liquid at the bottom of the tube.

Note:Do not aspirate the magnetic beads,so as not to affect the yield.

2.3.6. Keep the centrifuge tube fixed on the magnetic stand,open the cap of the centrifuge tube,and dry at room temperature for 5-6 minutes,until the magnetic beads have no reflections or cracks;

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

2.3.8. Centrifuge briefly,place the centrifuge tube on a magnetic stand,and let it stand for 5 minutes until the liquid is clear. Transfer all 44μL of the supernatant to a new PCR tube for the next reaction or store at -20°C.

2.4. PCR amplification

2.4.1. Prepare PCR reaction mixture (Table 6):add 6μL Index Primer Mix to the PCR tube in the previous step,add an Index Primer Mix or a set of Index to each sample Primer Mix followed by 50μL of 2x PCR Mix.

Table 6 Preparation of PCR reaction mixture

Component	Volume
2×PCR Mix	50μL
Index primer Mix	6μL
Purification of recovered linker ligation products	44μL
Total	100μL

Note:Index Primer Mix is made up of Universal primer and Index primer,of which there are 128 Index primers. According to different mixed sequencing strategies,each sample can be added with a different Index,or a sample can be added with a set of Index; the final mixed library is required to ensure that all indexes are assembled in sets. In Index1-16,there are 4 sets,4 sets in total,namely 1-4,5-8,9-12,13-16. In Index 17-128,8 are a set,a total of 14 sets,namely 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.

2.4.2. Shake and mix for 5s,and centrifuge briefly to collect the reaction solution to the bottom of the tube to ensure that there are no air bubbles in the reaction solution;

2.4.3. Place the PCR tube above on the PCR instrument and react according to the conditions in the table below(Table 7)

Table 7 PCR reaction program

Temperature	Time	Cycles
Hot Lid	On	
98℃	3 min	
98℃	10 s	
60℃	15 s	Refer to Table 8
72℃	30 s	
72℃	5 min	
4℃	Hold	

Note:The number of cycles should be adjusted based on the amount of starting DNA. The specific cycle number can refer to Table 8.

Table 8 Recommended amplification cycles for obtaining 300ng and 1μg libraries

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

Note:1）FFPE samples are of poor quality,3 cycles can be added to the recommended maximum number of cycles.

2）When the DNA quality is poor and the library is long,the cycle number can be appropriately increased to obtain a sufficient library.

3）When using the full-length Adaptor when the adapter is connected,and the library output meets the application requirements,the PCR-Free library can be obtained directly without the PCR amplification step; if the incomplete adapter is used,1-3 rounds of PCR are required Amplify to obtain the complete adaptor sequence required for sequencing.

4）When the sample type is cfDNA,the number of PCR cycles is ≥12.

5）Set the number of DNA Control cycles to 7.

2.5. PCR product purification

2.5.1. Take out the Magnetic Bead 30 minutes in advance and place it at room temperature,and shake and mix well before use;

2.5.2. Add 1 volume of Magnetic Bead to the PCR product,shake and mix for 5 s,and incubate at room temperature for 5 min;

2.5.3. Centrifuge briefly,place the centrifuge tube on a magnetic rack,let stand for 5 min until the liquid is clear,pipette and discard the supernatant;

2.5.4. Keep the centrifuge tube fixed on the magnetic rack,add 250μL of freshly prepared 80% ethanol,wait for suspension After the magnetic beads are completely adsorbed,the ethanol is completely discarded (about 1 min);

2.5.5. Repeat step 4 once,and try to suck the liquid at the bottom of the tube as much as possible for the last time. If a small amount remains on the wall of the tube,you can centrifuge the centrifuge tube momentarily. After separating on the magnetic stand,use a small-scale pipette to suck up the liquid at the bottom of the tube;

Note:Do not aspirate the magnetic beads,so as not to affect the yield.

2.5.6. Keep the centrifuge tube fixed on the magnetic stand,open the cap of the centrifuge tube,and dry at room temperature for 5-6 min,until the magnetic beads have no reflection or cracks;

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

2.5.8. Centrifuge briefly,place the centrifuge tube on a magnetic rack,and let it stand for 5 minutes until the liquid is clear. Transfer 30μL of the supernatant to a new 1.5mL centrifuge tube for on-machine testing or store at -20°C.

2.6. Quality Control of Libraries

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 quantification method for library concentration determination. Length distribution detection of library:Length distribution detection was performed by Agilent 2100 Bioanalyzer; LabChip GXll Touch microfluidic capillary electrophoresis and other equipment.

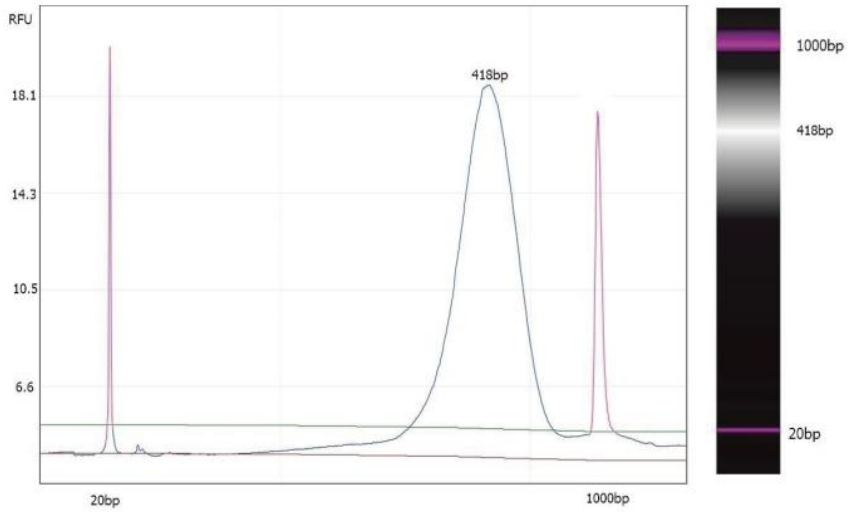


Figure:DNA library rapid preparation kit for preparation of MGI sequencing platform DNA library