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# Universal DNA Library Kit (Illumina&MGI)

**Product Number: PCK48**

## Shipping and Storage

Box 1 -20℃ storage, dry ice transportation.

Box 2 2-8℃, transported in ice bags

## Components

1. Box 1

| Component | PCK48 24rxns | PCK48 96rxns |
|---|---|---|
| FER Buffer | 240 μL | 960 μL |
| FER Enzyme Mix | 120 μL | 480 μL |
| T4 DNA Ligase | 72 μL | 288 μL |
| T4 DNA Ligase Buffer | 336 μL | 672 μL×2 |
| 2×Super HiFi PCR Mix | 600 μL | 1.2 mL×2 |
| Neutralization Reagent | 120 μL | 480 μL |

2. Box 2

| Component | PCK48 24rxns | PCK48 96rxns |
|---|---|---|
| Magnetic Bead | 1.5 mL×2 | 4 mL×3 |

## Description

The Universal DNA Library Kit (Illumina&MGI) is a second-generation sequencing enzyme digestion library kit developed for Illumina and MGI sequencing platforms. It includes three modules: DNA fragmentation/end repair/addition of A, adapter connection, and pre mixed enzymes required for library enrichment in library construction.By controlling the reaction time, sample DNA fragments from different sources such as 0.1ng-1μg genomic DNA, PCR amplification products, and FFPE can be reduced to small fragments, avoiding tedious ultrasound processes and instrument dependence.This kit simplifies the purification steps and shortens the library construction time. In the amplification module, high fidelity DNA polymerase is used for library enrichment, and PCR amplification is performed without preference, ensuring the accuracy of sequencing results.

## Features

1. Accurate enzyme digestion, fragment end repair and A tube completion, without the need for purification and direct connection to the connector.
2. After connecting the joint, use matching magnetic beads for direct sorting (optional).
3. High fidelity enzymes for PCR enrichment and amplification minimize amplification preference.
4. Suitable for building libraries for different species such as humans, animals, plants, microorganisms, etc. The resulting library is applicable to various platforms such as Illumina and MGI.

## Self provided instruments, reagents, and consumables

1. Joint Primer Kit

   MGI platform: Single ended short connector primer kit I/II/III;

   Illumina platform: single ended short connector primer kit I/II, double ended short connector primer kit.
2. DNA quality control: Agilent Technologies 2100 Bioanalyzer or other equivalent products.
3. DNA purification magnetic beads: use the kit's built-in matching magnetic beads.

4. Other materials: low adsorption PCR tube, 1.5 mL centrifuge tube, filter gun head, magnetic rack, anhydrous ethanol (100% ethanol, analytically pure), deionized water (pH 7.0-8.0), PCR instrument, etc.

## Preparation and precautions before the experiment

**1. Sample Fragmentation**

1.1. Sample eluent: It is recommended to elute DNA samples with pure water. To determine the sample concentration and quality, it is recommended that sample A260/280=1.8-2.0, and the sample loading amount should be between 0.1ng-μg.

1.2. If there are many impurities in the sample that will affect downstream experiments, it is necessary to purify the sample DNA through magnetic beads, screen out the impurities, and wash it off with pure water.

1.3. This reagent kit is compatible with different species and can be recommended for use according to the instructions. For FFPE samples, different interruption times are selected based on the degree of degradation. For specific conditions, please refer to Appendix 1 for examples.

1.4. This reagent kit has a certain degree of resistance to metal chelating agents. It is recommended to control the final concentration of EDTA in the system within 0.2mM. If the final concentration of the system is ≥0.2mM, it is recommended to add a neutralization buffer.

**2. Joint**

2.1. This reagent kit does not come with sequencing platform connector primers, and requires self matching of corresponding platform connector primers to complete library construction. This reagent kit is compatible with both single and double end connectors of conventional reagent kits.

2.2. The usage of connectors directly affects the quality of the library. If the input is too high, there will be residual dimers. If the input is too low, it will affect the connection between connectors and inserted fragments. The usage of connectors can be selected according to Table 5.

**3. Magnetic bead**

3.1. This reagent kit comes with magnetic beads, which are preferred for library purification or screening.

3.2. When customers match other purified magnetic beads, they need to re explore the proportion of magnetic bead sorting and purification.

**4. Library amplification**

4.1. This kit does not provide connector amplification primers and requires self matching of connector primers from relevant platforms for amplification.

4.2. The number of cycles for library amplification is set based on the input amount. Problems such as low outbound concentration, high amplification preference, and accumulation of amplification mutations have increased. Please refer to Table 10 for specific cycle numbers.

**5. Library length sorting**

5.1. During the library construction process, length sorting can result in significant DNA loss, leading to a decrease in library complexity and output. When the input DNA is less than 50ng, library screening or screening after PCR amplification is not recommended.

5.2. The position for length sorting can be selected after connector connection or PCR amplification for sorting. The specific sorting steps can refer to the operation content.

**6. Other precautions**

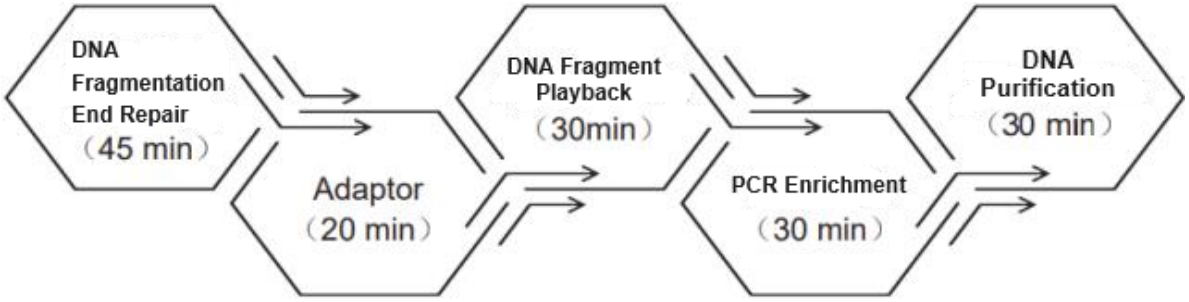
6.1. Due to the enzymatic reaction involved in the fragmentation process using this product, the fragmentation process is sensitive to factors such as reaction temperature, reaction time, system preparation, and DNA sample loading.

6.2. To avoid the impact of repeated freeze-thaw of reagents on library production, it is recommended to pack and store them for the first time in use.

6.3. PCR products are prone to contamination due to improper operation, resulting in inaccurate experimental results. It is recommended to isolate the PCR reaction system preparation area from the PCR product purification area, and use a dedicated pipette to regularly clean each experimental area.

6.4. Take out the corresponding reagent from the kit, centrifuge briefly, and place the enzyme mixture on ice for use; The buffer solution needs to be dissolved at room temperature before use, shaken and centrifuged, placed on ice for use, and deionized water placed at room temperature for use; Please prepare the mixture on ice. After the buffer solution in the reagent kit freezes and dissolves, precipitation may occur. Precipitation does not affect the function of the reagent. Please shake and mix thoroughly until the precipitation disappears before use.

## Schematic diagram of DNA database construction process




## DNA library construction process

Please read this operating instruction carefully before the experiment and choose the operating plan based on the type of sequencing platform used. Before the experiment starts, clarify the nucleic acid concentration, and the input amount of this reagent kit is 0.1ng-1μg. The recommended sample size is 1ng-500ng DNA. DNA solution does not contain chelating agents, if DNA dissolves in 1×TE or EDTA containing solutions, it is recommended to use magnetic beads for purification or add Neutralization Reagent.

## Protocol

**1. DNA Fragmentation and End Repair Plus A**

1.1. After the FER Buffer melts, shake and mix well. FER Enzyme Mix lightly flicks its fingers and mixes well. Centrifuge briefly to collect and place on ice.

1.2. Add the following reagents to the 200μL PCR tube:

Table 1 Preparation of Fragmentation and End Repair Reaction Systems

| Component | Volume |
|---|---|
| Double-stranded DNA | 1ng-500ng |
| FER Buffer | 10μL |
| FER Enzyme Mix | 5μL |
| Neutralization Reagent | Optional |
| NF Water | Up to 50μL |

**Note: Before starting the experiment, please confirm whether the template DNA contains ≥0.2mM EDTA. If it does, add a corresponding volume of Neutralization Reagent to neutralize EDTA according to the final concentration of EDTA in the fragmented system. The added volume is based on the final concentration of EDTA in a 50μL reaction system, 0.375μL Neutralization Agent is added every 0.1 mM. For example, if the DNA template contains 1mM EDTA and 20μL of the template is added to a 50μL fragment end repair reaction system, the final concentration of EDTA in the system is 0.4mM, and the amount of Neutralization Reagent added is 1.5μL.**

1.3. Mix well with light bullets, centrifuge briefly and place on ice for immediate PCR reaction.

1.4. Fragmentation and end repair procedures are shown in the table below (PCR instrument hot cover temperature 70°C)

Table 2 Fragmentation and End Repair Reaction Procedure

| Step | Temperature | Time |
|---|---|---|
| 1 | 4°C | 1 min |

| | | |
|---|---|---|
| 2 | 32℃ | 20 min（Optional） |
| 3 | 65℃ | 30 min |
| 4 | 4℃ | Hold |

1.5. The fragmentation time is adjusted based on the target fragment size, as shown in Table 3.

Table 3 Relationship between Fragmentation Time and Target Fragment Size

| | 32℃ incubation time（min） | | | |
|---|---|---|---|---|
| Insert Size | 200 bp | 250 bp | 350 bp | 450 bp |
| 100 ng DNA | 20-30 min | 15-20 min | 10-15 min | 5-10 min |

**Note:1)Select the incubation time at 32℃ based on the expected insertion fragment size, and the fragment size decreases with the extension of reaction time**

**2)If there is a slight deviation between the results and the expected size, the reaction time can be adjusted as appropriate. 3-5 minutes can be added or subtracted from the recommended reaction time.**

**3)Reduce fragmentation time of FFPE samples according to their quality as appropriate.**

1.6. Immediately conduct joint connection reaction after the reaction is completed.

**2. Joint connection reaction**

2.1. Directly add the following reagents to the reaction solution of adding A to the completed DNA fragment end repair:

Table 4 Joint Connection Reaction Procedure

| Component | Volume |
|---|---|
| T4 DNA ligase buffer | 14μL |
| T4 DNA ligase | 3μL |
| Adaptor for Illumina/MGI | 5μL |
| NF Water | 8μL |
| Total | 30μL |

2.2. This step involves adding adapters that match different platforms, and the adapter concentration needs to be matched with different concentrations depending on the input amount. The specific content is shown in Table 5.

Table 51ng-500ng gDNA Recommended Adaptor Concentration for Use

| gDNA | Adapto working concentration | Adaptor Pre dilution concentration |
|---|---|---|
| 100 ng-500 ng | 10 μM | Undiluted |
| 25 ng-100 ng | 5 μM | 1:2 |
| 5 ng-25 ng | 1 μM | 1:10 |
| 1 ng-5 ng | 0.1-0.2 μM | 1:10-1:100 |

2.3. Shake and mix well, briefly centrifuge to collect the solution to the bottom of the tube.

2.4. Place the PCR tube in the PCR instrument and run the following program.

Table 6 Connection Reaction Procedure

| Step | Temperature | Time |
|---|---|---|
| 1 | Heated lid | 25℃ |
| 2 | 23℃ | 20 min |
| 3 | 4℃ | Hold |

2.5. Shake well and centrifuge briefly to collect the solution to the bottom of the tube.

2.6. 23℃ warm bath for 20 minutes.

**Note: If using a PCR instrument for this operation, please set the heat cap to 25℃.**

**3. Purification of connecting products**

Use a reagent kit with magnetic beads for purification and recovery of DNA fragments. There are two options for purification of connecting products, selective recovery and complete recovery. If the initial sample size is less than 50ng, it is recommended to choose option one (complete recovery of DNA fragments); If the input amount is greater than 50ng, choose Scheme 2 (DNA fragment selection and recovery).

3.1. Option 1: Complete DNA recovery

3.1.1. Take out the Magnetic Bead 30 minutes in advance and place it at room temperature. Shake thoroughly and mix well before use;

3.1.2. Transfer the connecting product to a new 1.5mL centrifuge tube and add water to the reaction system to 100μl.

3.1.3. Take 80μl of Magnetic Bead into 100μl of the product, shake thoroughly and mix well, and incubate at room temperature for 5 minutes;

3.1.4. Instantaneous centrifugation, place the centrifuge tube on a magnetic rack and let it stand for 5 minutes until the liquid clears. The pipette absorbs and discards the supernatant;

3.1.5. Keep the centrifuge tube fixed on the magnetic rack, add 250μl of freshly prepared 80% ethanol, let it stand at room temperature for 30 seconds, and discard the supernatant;

**Note:It is necessary to use freshly prepared ethanol, otherwise it will affect the experimental results.**

3.1.6. Repeat step 5 once, and try to dry the liquid at the bottom of the tube as much as possible for the last time. If there is a small amount of residue on the tube wall, the centrifuge tube can be temporarily centrifuged. After separation on a magnetic rack, use a small range pipette to suck the liquid at the bottom of the tube dry.

**Note:Do not absorb magnetic beads to avoid affecting production.**

3.1.7. Keep the centrifuge tube fixed on the magnetic frame, open the centrifuge tube cover, and dry at room temperature for 3-5 minutes until the magnetic beads have no reflection or cracking;

**Note:Do not heat and air dry, do not excessively dry the magnetic beads, otherwise it will affect the yield.**

3.1.8. Remove the centrifuge tube from the magnetic rack, add 22μl of NF water for DNA elution, blow with a pipette or shake thoroughly, mix well, and dissolve at room temperature for 5 minutes;

3.1.9. Instantaneous centrifugation, place the centrifuge tube on a magnetic rack and let it stand for 5 minutes until the liquid clears. Transfer all 20μl of the supernatant to a new PCR tube for the next reaction or store at -20℃.

3.2. Option 2: Selective recovery of DNA fragments

When conducting DNA selective recovery, target fragments of different sizes are selected as needed. Table 7 shows the required amount of magnetic beads for the screening process of different fragment sizes.

Table 7 Recommended dosage of magnetic beads for obtaining DNA main bands (100μL reaction system)

| DNA Fragment Size | Insert fragment+Adaptor | 300 bp | 350 bp | 400 bp | 450 bp |
|---|---|---|---|---|---|
| Amount of magnetic beads used | First Choice | 50μL | 40μL | 30μL | 20μL |
| | Second Choice | 20μL | 20μL | 20μL | 20μL |

The main peak of the target fragment screened in the following process is around 350bp.

3.2.1. Take out the Magnetic Beads 30 minutes in advance and place them at room temperature. Shake thoroughly and mix well before use.

3.2.2. Transfer the connecting product to a new 1.5mL centrifuge tube and replenish the reaction system to 100μL.

3.2.3. Suck 40μL of Magnetic Bean into the connecting product, mix thoroughly with vortex shaking, and let stand at room temperature for 5 minutes.

3.2.4. Short centrifugation, place the centrifuge tube on a magnetic rack to separate the magnetic beads from the supernatant solution until the solution is clear (about 5 minutes), carefully suck the supernatant, and avoid contact with the magnetic beads that have already bound to the target DNA during this period.

**Note: Do not discard the upper clean.**

3.2.5. Add 20μL of evenly mixed Magnetic Beads to the supernatant, vortex shake for 5 seconds, and let it sit at room temperature for 5 minutes.

3.2.6. Short centrifugation, place the centrifuge tube on a magnetic rack to separate the magnetic beads from the supernatant solution until the solution is clear (about 5 minutes), carefully suck the supernatant, and avoid contact with the magnetic beads that have already bound to the target DNA during this period.

**Note: Do not discard the magnetic beads.**

3.2.7. Keep the centrifuge tube fixed on the magnetic rack, add 250μL of freshly prepared 80% ethanol, let it stand at room temperature for 30 seconds, and discard the supernatant;

**Note: It is necessary to use freshly prepared ethanol, otherwise it will affect the experimental results.**

3.2.8. Repeat step 7 once, and try to dry the liquid at the bottom of the tube as much as possible for the last time. If there is a small amount of residue on the tube wall, the centrifuge tube can be temporarily centrifuged. After separation on a magnetic rack, use a small range pipette to suck the liquid at the bottom of the tube dry.

**Note: Do not absorb magnetic beads to avoid affecting production.**

3.2.9. Keep the centrifuge tube fixed on the magnetic frame, open the non stick tube cover, and dry at room temperature for 3-5 minutes until the magnetic beads have no reflection or cracking;

**Note: Do not heat and air dry, do not excessively dry the magnetic beads, otherwise it will affect the yield.**

3.2.10. Remove the centrifuge tube from the magnetic holder, add 22μL of NF Water, vortex oscillate to completely resuspend the magnetic beads in ionized water, and let stand at room temperature for 5 minutes;

3.2.11. Centrifuge briefly, place the centrifuge tube on a magnetic rack until the solution clears (approximately 5 minutes), and transfer 20μL of the clarified solution to a new PCR tube.

## 4. PCR Amplification

4.1. Prepare PCR reaction mixture according to Table 8

Table 8 Preparation of PCR reaction mixture

| Component | Volume |
|---|---|
| 2×Super HiFi PCR Mix | 25 μL |
| Index primer Mix | 5 μL |
| Purified and recycled connector connection products | 20 μL |
| Total | 50 μL |

**Note: Index primer Mix can be selected based on the use of different connector primer kits on different platforms. If you choose Kangwei reagent connector primer kits, you can refer to the corresponding instructions for use.**

4.2. Shake and mix for 5 seconds, and collect the reaction solution to the bottom of the tube through instantaneous centrifugation.

4.3. Place the above PCR tube on the PCR instrument, and refer to Table 9 for the reaction procedure

Table 9 PCR Reaction Procedure

| Temperature | Time | Cycles |
|---|---|---|
| 98℃ | 3 min | |
| 98℃ | 20 s | Refer to Table 10 |
| 60℃ | 20 s | |
| 72℃ | 30 s | |
| 72℃ | 5 min | |
| 4℃ | Hold | |

4.4. The number of cycles required for the reaction should be adjusted based on the amount of DNA input, and the specific number of cycles should refer to Table 10

Table 10 Recommended amplification cycles for obtaining 100 ng and 1μg libraries

| Sample | Number of cycles required for corresponding production | |
|---|---|---|
| DNA | 100 ng | 1 ug |
| 0.1 ng | 13-15 | 16-18 |
| 1 ng | 9-11 | 11-13 |
| 10 ng | 6-8 | 9-11 |
| 100 ng | 3-5 | 6-8 |

| | | |
|---|---|---|
| 500 ng | 0*/1-3 | 3-5 |
| 1000 ng | 0*/1-3 | 2-4 |

**Note: 1) The quality of FFPE samples is poor, and three more cycles can be added to the recommended maximum number of cycles.**

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

**3) When sorting magnetic beads, it is recommended to amplify the library according to a high cycle number to obtain a sufficient library.**

**4) When using a full length adapter when connecting the connector and the library output meets the application requirements, the PCR amplification step can be omitted and the PCR Free library can be directly obtained; If an incomplete connector is used, 1-3rounds of PCR amplification are required to obtain the complete connector sequence required for sequencing.**

5. Purification and recovery of PCR products

PCR product purification can be carried out through two options: selective recovery and complete recovery. If the initial sample size is less than 50 ng and there is a high requirement for fragment distribution concentration, choose option 2 (PCR product recovery). If the input amount is greater than 50 ng, it is recommended to sort and recover the connecting products after the joint is connected (refer to selective recovery of DNA fragments of the connecting products for details).

5.1. Option 1: Complete recovery of PCR products

5.1.1. Take out the Magnetic Bead 30 minutes in advance and place it at room temperature. Shake thoroughly and mix well before use;

5.1.2. Transfer the PCR reaction solution to a new 1.5mL centrifuge tube; Suck 0.9 times the volume of Magnetic Bead into the PCR product, gently blow or shake with a pipette, and mix thoroughly. Incubate at room temperature for 5 minutes;

5.1.3. Short centrifugation, place the centrifuge tube on a magnetic rack to separate the magnetic beads from the supernatant solution until the solution is clear (about 5 minutes), carefully suck the supernatant, and avoid contact with the magnetic beads that have already bound to the target DNA during this period.

Note: Do not discard the magnetic beads.

5.1.4. Keep the centrifuge tube fixed on the magnetic rack, add 250μL of freshly prepared 80% ethanol, let it stand at room temperature for 30 seconds, and discard the supernatant;

Note: It is necessary to use freshly prepared ethanol, otherwise it will affect the experimental results.

5.1.5. Repeat step 4 once, and try to dry the liquid at the bottom of the tube as much as possible for the last time. If there is a small amount of residue on the tube wall, the centrifuge tube can be temporarily centrifuged. After separation on a magnetic rack, use a small range pipette to suck the liquid at the bottom of the tube dry.

Note: Do not absorb magnetic beads to avoid affecting production.

5.1.6. Keep the centrifuge tube fixed on the magnetic frame, open the non stick tube cover, and dry at room temperature for 3-5 minutes until the magnetic beads have no reflection or cracking;

Note: Do not heat and air dry, do not excessively dry the magnetic beads, otherwise it will affect the yield.

5.1.7. Remove the centrifuge tube from the magnetic support and add 22μL of NF Water. Vortex oscillate to completely resuspend the magnetic beads in ionized water and let them stand at room temperature for 5 minutes;

5.1.8. Instantaneous centrifugation, place the centrifuge tube on a magnetic rack and let it stand for 2 minutes until the liquid clears. Transfer all 22μL of the supernatant to a new PCR tube for the next reaction or store at -20 ℃.

5.2. Option 2: Selective recovery of DNA fragments

When the investment amount is too low and the requirements for fragment distribution are relatively concentrated, this scheme can be chosen for selective recycling. The proportion of magnetic bead dual selection is shown in Table 11

Table 11 Recommended dosage of magnetic beads for obtaining DNA main bands (100μL reaction system)

| DNA Fragment Size | Insert fragment+Adaptor | 300 bp | 350 bp | 400 bp | 450 bp |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| Amount of magnetic beads used | First Choice | 80μL | 70μL | 65μL | 60μL |
| | Second Choice | 20μL | 20μL | 20μL | 20μL |

5.2.1. Take out the Magnetic Beads 30 minutes in advance and place them at room temperature. Shake thoroughly and mix well before use;

5.2.2. Transfer the PCR reaction solution to a new 1.5 mL centrifuge tube and replenish the reaction system to 100 μL.

5.2.3. Suck 70μL of Magnetic Bead into the PCR product, mix thoroughly with vortex shaking, and let stand at room temperature for 5 minutes.

5.2.4. Short centrifugation, place the centrifuge tube on a magnetic rack to separate the magnetic beads from the supernatant solution until the solution is clear (about 5 minutes), carefully suck the supernatant, and avoid contact with the magnetic beads that have already bound to the target DNA during this period.

Note: Do not discard the upper clean.

5.2.5. Add 20μL of evenly mixed Magnetic Beads to the supernatant, vortex shake for 5 seconds, and let it sit at room temperature for 5 minutes.

5.2.6. Short centrifugation, place the centrifuge tube on a magnetic rack to separate the magnetic beads from the supernatant solution until the solution is clear (about 5 minutes), carefully suck the supernatant, and avoid contact with the magnetic beads that have already bound to the target DNA during this period.

Note: Do not discard the magnetic beads.

5.2.7. Keep the centrifuge tube fixed on the magnetic rack, add 250μL of freshly prepared 80% ethanol, let it stand at room temperature for 30 seconds, and discard the supernatant;

Note: It is necessary to use freshly prepared ethanol, otherwise it will affect the experimental results.

5.2.8. Repeat step 7 once, and try to dry the liquid at the bottom of the tube as much as possible for the last time. If there is a small amount of residue on the tube wall, the centrifuge tube can be temporarily centrifuged. After separation on a magnetic rack, use a small range pipette to suck the liquid at the bottom of the tube dry.

Note: Do not absorb magnetic beads to avoid affecting production.

5.2.9. Keep the centrifuge tube fixed on the magnetic frame, open the non stick tube cover, and dry at room temperature for 3-5 minutes until the magnetic beads have no reflection or cracking;

Note: Do not heat and air dry, do not excessively dry the magnetic beads, otherwise it will affect the yield.

5.2.10. Remove the centrifuge tube from the magnetic support and add 22μL of NF Water. Vortex oscillate to completely resuspend the magnetic beads in ionized water and let them stand at room temperature for 5 minutes;

5.2.11. Centrifuge briefly, place the centrifuge tube on a magnetic rack until the solution clears (approximately 5 minutes), and transfer 20μL of the clarified solution to a new PCR tube.

## Library quality control

1. There are generally two methods for library concentration detection: one is based on double stranded DNA fluorescent dyes such as Qubit, PicoGreen, etc., and the other is based on qPCR absolute quantification. It is not recommended to use spectral detection based methods such as NanoDrop.
2. Detection of library length distribution can be performed using equipment such as Agilent Bioanalyzer 2100 based on capillary electrophoresis or microfluidic principles.

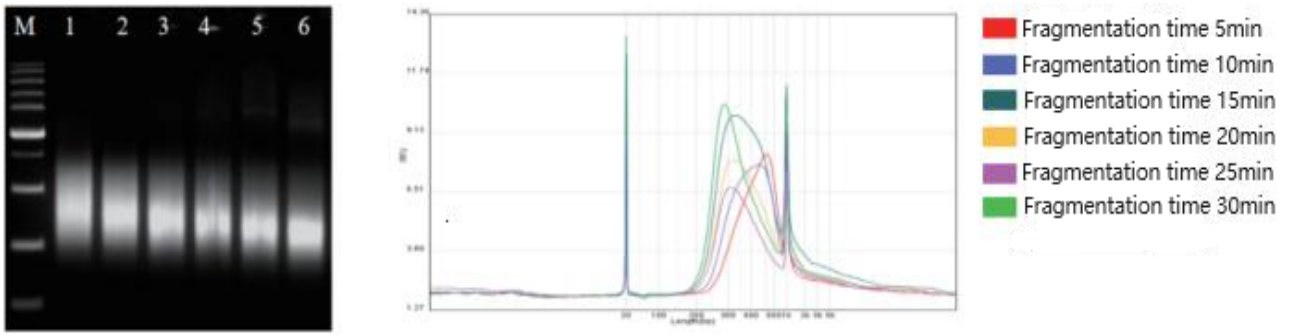
## Attachment 1: Fragmented Printing Time

1. Fragmentation reaction is a time-dependent enzymatic reaction, and the size of the fragment product depends on the reaction time. Therefore, the control of fragment time should be very precise. Fragmentation reaction is recommended to be operated on ice, and the PCR instrument should be programmed in advance to cool down to 4 degrees pre cooling;
2. Metal ion/EDTA and other metal ion chelating agents have a significant impact on the fragmented enzymatic reaction. For templates containing higher concentrations of EDTA (system final concentration ≥ 0.2 mM), it is recommended to purify or add Neutralization Agents.

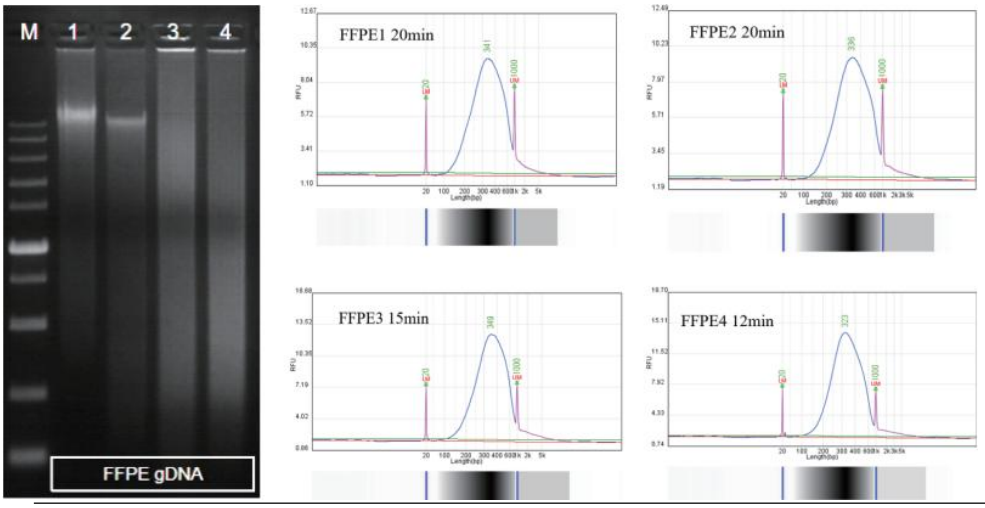
3. The distribution range of fragmented products with different input amounts and the same interruption time is basically consistent, with slight differences in the main peak.

### Example of Fragmented Database Construction for Different Samples

1. 100 ng cell genomic DNA templates were inserted, with different interruption times (5min/10min/15min/20min/25min/30min), and the connecting products were directly recovered. PCR amplification of 7Cycles was performed to establish a library of products.




2. FFPE samples with different degrees of degradation were injected with 100 ng, and the library products were directly recovered at different interruption times. PCR amplification was performed on 7 cycles, and the library products were obtained.




| Type | Grade | initiation mass | Fragmentation time | Amplification cycle | Concentration | volume |
|---|---|---|---|---|---|---|
| FFPE DNA | A | 100 ng | 20 | 6 | 65.2 ng/μL | 30μL |
| FFPE DNA | A | 100 ng | 20 | 7 | 60.7 ng/μL | 30μL |
| FFPE DNA | B | 100 ng | 15 | 7 | 64.1 ng/μL | 30μL |
| FFPE DNA | C | 100 ng | 12 | 7 | 55.6 ng/μL | 30μL |

### Attachment 2: Screening of Magnetic Beads

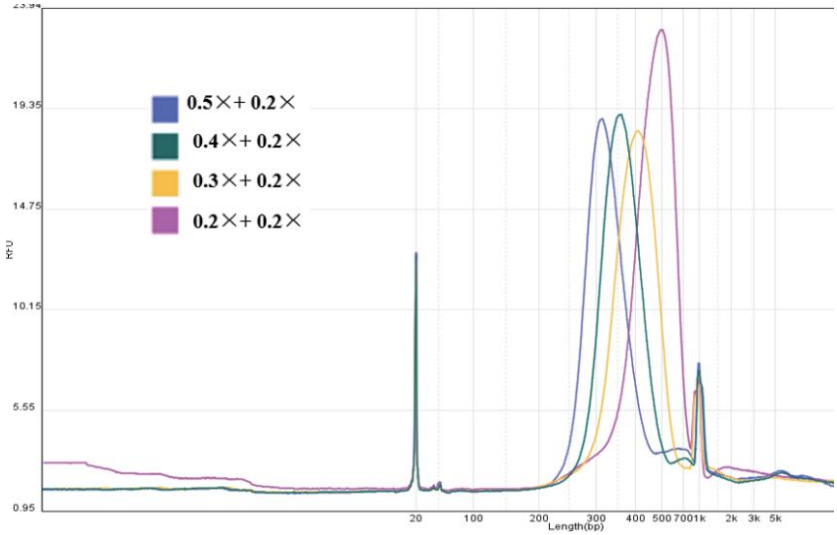
1. There are significant differences in magnetic beads from different manufacturers, so it is recommended to use built-in magnetic beads for double selection in this reagent kit.
2. Commonly used multipliers for magnetic bead usage"×" Identify how many times the volume of the magnetic beads are used relative to the original sample volume. If the original volume of the sample is 100μl. 0.7×/0.2×the amount of magnetic beads used in the first round of sorting is 0.7×100μl=70, the amount of magnetic beads used in the second round is 0.2×100μl=20.
3. The amount of magnetic beads used directly affects the lower limit of DNA length that can be purified, × The higher the number, the shorter the lower limit of the purified DNA length, and vice versa. The principle of magnetic bead sorting is to determine the final size of the target DNA fragment by adjusting the amount of magnetic beads in two different proportions. The first round of magnetic beads combines DNA with larger molecular weights and removes this product by discarding the magnetic beads; The second round of magnetic beads combines with the larger molecular weight DNA in the remaining

product, and removes the smaller molecular weight DNA by discarding the supernatant.

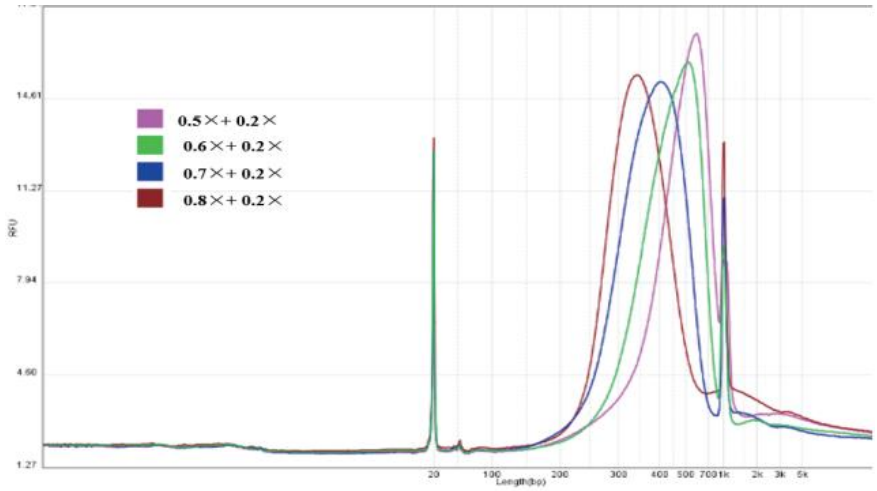
4. The volume for magnetic bead double selection needs to be ≥50μl, and if the volume is too small, magnetic bead screening may result in unstable results.

**Example of screening different magnetic bead ratios for connecting products/PCR products**

1. 200ng cell genome DNA template input, interruption time of 12 minutes, connected products with different magnetic bead ratios for recovery (0.5×+0.2×/0.4×+0.2×/0.3×+0.2×/0.2×+0.2×),PCR amplification of 7 cycles and library building products.




2. 50ng cell genomic DNA template was inserted, with a 12 minute interruption time. The connecting products were fully recovered, and PCR amplification was performed on 9 cycles. The PCR products were recovered in different magnetic bead ratios (0.5×+0.2×/0.6×+0.2×/0.7×+0.2×/0.8×+0.2×).




**Attachment 3: Library Expansion**

1. The amplification step of the library requires strict control of the number of amplification cycles. Insufficient number of cycles will lead to low library production; Excessive number of cycles can lead to increased library preference, increased repeatability, and increased chimeric products. It is recommended to amplify according to the recommended number of cycles.
2. Regarding the use of primers for library amplification, it is necessary to choose according to the type of connector used. If a complete full connector is used, amplification may not be necessary or universal primers may be used for amplification; If incomplete connectors are used, matching primers must be selected for at least 3 cycles of amplification.
3. The difference in the joint structure between Illumina and MGI will result in slight differences in connection efficiency, as well as slight differences in the concentration of connection products and PCR products.