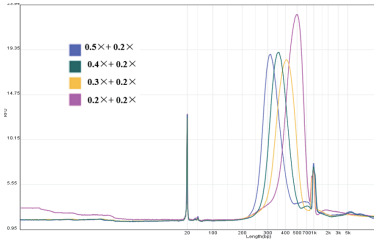
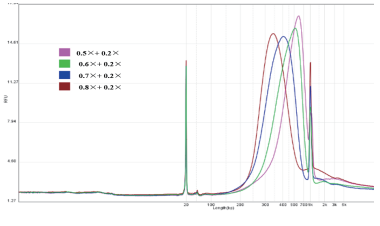
Example of selection of different bead ratios of ligation products/PCR products:

200ng cell genomic DNA template input, fragmentation time was 12 min, ligation products were selected with different magnetic bead ratio(0.5×+0.2×/0.4×+0.2×/0.3×+0.2×/0.2×+0.2×), PCR amplificated for 7 cycles, library preparation products.



50 ng cell genome DNA template was put in, fragmentation time was 12 min, ligation products were completely recovered, PCR amplificated for 9 cycles, PCR products were selected with different magnetic bead ratios (0.5×+0.2×/0.6×+0.2×/0.7×+0.2×/0.8×+0.2×).



Appendix 3: About library amplification

1. The library amplification step requires tight control of the number of amplification cycles. Insufficient number of cycles will lead to low library yield, and excessive number of cycles will lead to various adverse consequences such as increased library preference, increased repeatability, and increased chimeric products. It is recommended to amplify according to the recommended number of cycles.
2. The use of primers for library amplification needs to be selected according to the type of adapter used. If the complete adapter is used, it does not have to perform amplification or can be amplified with universal primers. If an incomplete adapter is used, a matching primer must be used for amplification of at least 3 cycles
3. Differences in the structure of the Illumina and MGI adapters will lead to a slight difference in the ligation efficiency and a slight difference in the concentration of the ligation product and PCR product.

Version: 11/2023

CWseq Universal DirectFast DNA Library Prep Kit (Illumina & MGI)

Cat. No. : CW3048S（24 rxns）
CW3048M（96 rxns）

Storage Condition: Box 1 should be stored at -20 °C and transported on dry ice.
Box 2 should be stored at 2-8 °C and transported with ice pack.

Components

Box 1

Component	CW3048S (24 rxns)	CW3048M (96 rxns)
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

Box 2

Component	CW3048S (24 rxns)	CW3048M (96 rxns)
CMPure Beads	1.5 mL×2	4 mL×3

Introduction

CWseq Universal DirectFast DNA Library Prep Kit is a next-generation sequencing enzyme-based library kit developed for Illumina and MGI sequencing platforms. It contains three major blocks of premixed enzymes required for DNA fragmentation/end repair/A-tailing, Adapter ligation and library enrichment in library preparation. Controlled reaction time allows sample DNA from different sources, such as 0.1 ng-1 μg genomic DNA, PCR amplification products, FFPE, etc., to be made into small fragments, avoiding cumbersome ultrasound processes and instrument dependence. This kit simplifies the purification step, shortens the setup time, uses high-fidelity DNA polymerase for library enrichment, and performs PCR amplification without preference to ensure the accuracy of sequencing results.

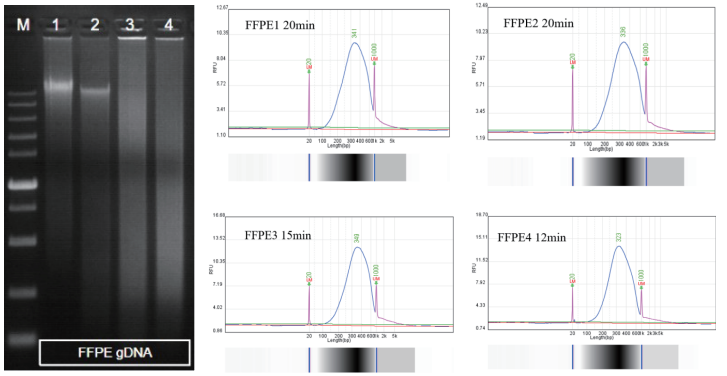
Precautions

1. Precise digestion. Fragmentation, end repair and A-tailing can be completed in one tube. No purification is required to directly ligate the adapter.
2. After ligating the adapter, the matching magnetic beads can be used for direct selection (optional).
3. Using high-fidelity enzymes for PCR enrichment and amplification, minimizing amplification preference.
4. Suitable for preparing libraries of different species such as humans, animals, plants, and microorganisms, and the prepared library is suitable for Illumina and MGI platforms.

Instruments, Reagents and Consumables that not Included

1. Adapter primer kit
   MGI: NGS Multiplex Oligos for MGI (Index primer mix Set I/II/III)
   (Cat.No. CW3014/CW3015/CW3016)
   Illumina: NGS Multiplex Oligos for Illumina (Index Primers Set I/II) (Cat.No. CW2586/CW2587), NGS Combinatorial Dual Index Primers Kit for Illumina (Cat.No. CW3042).
2. DNA quality control: Agilent Technologies 2100 Bioanalyzer or other equivalent.
3. DNA magnetic beads: Use the magnetic beads included with the kit.
4. Other materials: PCR tubes with low adsorption, 1.5 mL centrifuge tubes, filter tips, magnetic rack (DynaMagTM-2 Cat.No. 12321D recommended), absolute ethanol (100% ethanol, analytically pure), deionized water (pH between 7.0-8.0), PCR instruments, etc.

2. FFPE samples with different degrees of degradation, input 100 ng, different fragmentation time, direct recovery of library preparation products, PCR amplificated for 7 Cycles, library preparation products.



Sample Type	Quality Grade	Starting Input	Fragmentation Time	Cycles	Product Concentration	Product 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

Appendix 2: About bead-based size selection

1. Magnetic beads vary greatly from manufacturer to manufacturer. It is recommended to use self-contained magnetic beads in the kit for selection.
2. The amount of beads used is often identified by a multiplier "×" that indicates how many times the volume of beads is used relative to the original sample volume. If the original volume of the sample is 100 μL, the volume of the first round of beads should be 0.7 × 100 μL = 70 μL, and the volume of the second round of beads should be 0.2 × 100 μL = 20 μL.
3. The amount of magnetic beads used directly affects the lower limit of purifiable DNA length. The higher the number of ×, the shorter the lower limit of purifiable DNA length, and vice versa. The principle of bead-based size selection is to determine the size of the fragment size of the final selected target DNA by adjusting the amount of magnetic beads in different proportions in two steps. The first round of magnetic beads binds larger molecular weight DNA and removes this part of the product by discarding the magnetic beads. The second round of magnetic beads combine the larger molecular weight DNA in the remaining product and removes the smaller molecular weight DNA by discarding the supernatant.
4. The volume for bead-based size selection should be ≥ 50 μL. The effect will be unstable if the volume is too small.

Library Quality Control

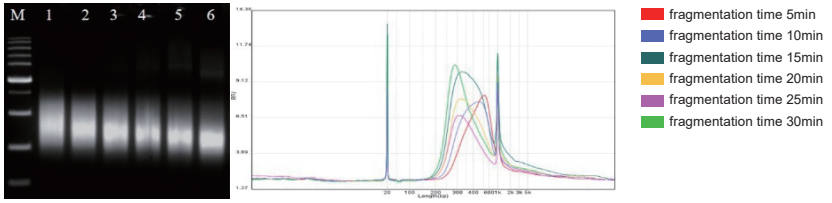
1. There are generally two methods for library concentration detection: one is based on double-stranded DNA fluorescent dyes, such as Qubit, PicoGreen, etc. The other is based on qPCR absolute quantification. Spectroscopic detection-based methods such as NanoDrop are not recommended.
2. Library length distribution detection can be performed with capillary electrophoresis -based or microcontrolled flow-based equipment such as the Agilent Bioanalyzer 2100.

Appendix 1: About fragmentation time

1. The fragmentation reaction is a time-dependent enzymatic reaction. The size of the fragmentation product depends on the reaction time, so the control of fragmentation time should be very precise. The fragmentation reaction is recommended to be performed on ice. The PCR instrument program should be set in advance, and the temperature is pre-cooled to 4 °C.
2. In fragmentation enzymatic reactions, metal ion chelators such as metal ions/EDTA have a great influence on the reaction. Templates containing higher concentrations of EDTA (final concentration ≥ 0.2 mM) are recommended for purification or addition of Neutralization Reagent.
3. The distribution range of time-fragmented products with the same amount of different inputs is basically the same, and the main peak is slightly different.

Examples of library preparation based on different sample fragmentation:

1. 100 ng cell genome DNA template input, different fragmentation time (5min/10min/ from 15min/20min/25min/30min), ligation products are directly recovered, PCR amplificated for 7 cycles, library preparation products.



Protocol

1. qPCR reaction system

1.1 Sample eluent: DNA samples are recommended to elute with pure water. To determine the sample concentration and sample quality, it is recommended that A260/280 of the sample should be between 1.8-2.0, and the sample loading amount should be between 0.1 ng-1 μg.

1.2 If there are many impurities in the sample that will affect the downstream experiment, the sample DNA needs to be purified by magnetic beads, screened out the impurities, and eluted with pure water.

1.3 This kit is compatible with different species, and can be recommended according to the instructions. For FFPE samples, choose different fragmentation time according to the degree of degradation. Please refer to Appendix 1 for specific conditions.

1.4 This kit has a certain degree of resistance to metal chelating agent to a certain extent. It is recommended that the final concentration of EDTA in the system should be controlled within 0.2 mM. If the final concentration of the system is ≥ 0.2 mM, it is recommended to add a neutralizing buffer.

2. Adapter

2.1 This kit does not include adapter primers for sequencing platforms. It is necessary to match the adapter primers of the corresponding platform to complete the library preparation. This kit is compatible with both conventional single- and dual-ended kits.

2.2 The amount of adapter used will directly affect the quality of the library. High input will cause dimer residue, and low input will affect the ligation between the adapter and the inserted fragment. The amount of adapter used can be selected according to Table 5.

3. Magnetic beads

3.1 This kit includes magnetic beads. The magnetic beads provided with the kit are preferred for library purification or screening.

3.2 When matching other purified magnetic beads, it is necessary to re-explore the proportion of magnetic bead selection and purification.

4. Library amplification

4.1 This kit does not provide adapter amplification primers. It is necessary to self-match the adapter primers of the relevant platform for amplification.

4.2 The number of cycles of library amplification is set according to the input amount. Low input will cause low outbound concentration, and high input will cause amplification preference and accumulation of amplification mutations. The specific number of cycles should be referred to Table 10.

5. Library amplification

5.1 If size selection is carried out during the library preparation process, the amount of DNA loss will be large, which will lead to a decrease in library complexity and output. Size selection or selection after PCR amplification is not recommended when the input DNA is less than 50 ng.

5.2 Size selection can be performed after the adapter is ligated or after PCR amplification. The specific selection steps can refer to the operation procedure.

6. Additional Precautions

6.1 Since the fragmentation process performed by using this product is an enzymatic reaction, the fragmentation process is sensitive to factors such as reaction temperature, reaction time, system preparation, and DNA loading amount.

6.2 In order to avoid repeated freeze-thaw of reagents affecting library yield, it is recommended to save in separate packages when first used

6.3 PCR products are easy to produce 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, use a special pipette, and clean each experimental area regularly. It is recommended to use Cowin's RNase and DNA Remover (CW3141).

6.4 When taking out the corresponding reagent in the kit, centrifuge briefly and place the enzyme mixture on ice for use. Before the buffer is used, it should be dissolved at room temperature and then shake and centrifuge. Place the buffer on ice for use, and place the deionized water at room temperature for use. Please prepare the mixture on ice. The buffer in the kit may precipitate after freezing and dissolving. Precipitation does not affect the function of the reagent, please shake well until the precipitate disappears before use.

1. Take out CMPure 30 min in advance and place it at room temperature, mix well with shaking before use.
2. Transfer the PCR reaction solution to a new 1.5 mL centrifuge tube and replenish the reaction system to 100 μL with water.
3. Pipette 70 μL of CMPure to the PCR product, mix well with shaking, and let stand at room temperature for 5 min.
4. Centrifuge briefly. Place the centrifuge tube on a magnetic rack to separate the magnetic beads from the supernatant until the solution is clear (takes about 5 min). Carefully pipette the supernatant, avoiding contact with magnetic beads binding to the target DNA.
   Note: Do not discard the supernatant.
5. Add 20 μL of well-mixed CMPure to the supernatant, vortex for 5 s and leave at room temperature for 5 min.
6. Centrifuge briefly. Place the centrifuge tube on a magnetic rack to separate the magnetic beads from the supernatant until the solution is clear (takes about 5 min). Carefully pipette the supernatant and discard, avoiding contact with magnetic beads binding to the target DNA.
   Note: Do not discard the magnetic beads.
7. Keep the centrifuge tube fixed on the magnetic rack, and add 250 μL of freshly configured 80% ethanol to the centrifuge tube. Leave it at room temperature for 30 seconds, and carefully discard the supernatant.
   Note: Be sure to use freshly configured ethanol, otherwise it will affect the experimental results.
8. Repeat step 7 once, and drain the liquid at the bottom of the tube as much as possible. Centrifuge the tube instantaneously when there is a small amount remaining on the tube wall. After separating on the magnetic rack, use a pipette of small capacity to pipette the liquid at the bottom of the tube.
   Note: Do not pipette the beads to avoid affecting the yield.
9. Keep the centrifuge tube fixed on the magnetic rack and open the lid, then let stand at room temperature for 3-5 min to dry the magnetic beads in air. The magnetic beads should have no reflection or cracking.
   Note: Do not heat to dry or over-dry the magnetic beads, otherwise it will affect the yield.
10. Remove the centrifuge tube from the magnetic rack, add 22 μL of NF water. Vortex and shake to completely resuspend the magnetic beads in the NF water and let stand for 5 min at room temperature.
11. Centrifuge briefly. Place the centrifuge tube on the magnetic rack until the solution is clear (takes about 5 min), and transfer 20 μL of the eluate to a new PCR tube.

4. Keep the centrifuge tube fixed on the magnetic rack, and add 250 μl of freshly configured 80% ethanol to the centrifuge tube. Leave it at room temperature for 30 seconds, and carefully discard the supernatant.

   Note: Be sure to use freshly configured ethanol, otherwise it will affect the experimental results.

5. Repeat step 4 once, and drain the liquid at the bottom of the tube as much as possible. Centrifuge the tube instantaneously when there is a small amount remaining on the tube wall. After separating on the magnetic rack, use a pipette of small capacity to pipette the liquid at the bottom of the tube.

   Note: Do not pipette the beads to avoid affecting the yield.

6. Keep the centrifuge tube fixed on the magnetic rack and open the lid, then let stand at room temperature for 3-5 min to dry the magnetic beads in air. The magnetic beads should have no reflection or cracking.

   Note: Do not heat to dry or over-dry the magnetic beads, otherwise it will affect the yield.

7. Remove the centrifuge tube from the magnetic rack, add 22 μL of NF water. Vortex and shake to completely resuspend the magnetic beads in the NF water and let stand for 5 min at room temperature.
8. Centrifuge instantaneously. Place the centrifuge tube on the magnetic rack for 2 min until the solution is clear, and transfer 22 μL of the supernatant to a new PCR tube. Proceed to the next step or store the product at -20 °C.

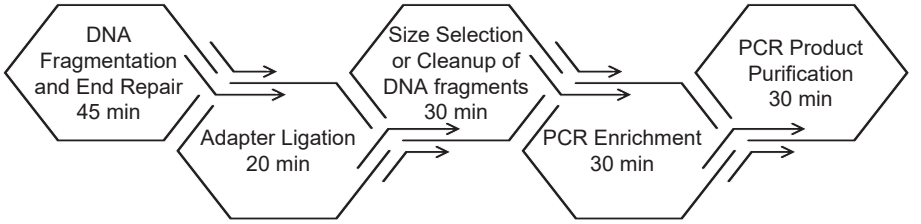
Option 2: Size selection of DNA fragments

When the input amount is too low and the distribution of fragment is required, this option can be selected for size selection, and the ratio of magnetic beads is referred to Table 11

Table 11 Recommended amount of magnetic bead for acquiring the main band of DNA (100 μL reaction system)

DNA Fragment Size	Insert fragment+Adapter	300 bp	350 bp	400 bp	450 bp
Amount of Magnetic Bead	1st Bead Addition	80 μL	70 μL	65 μL	60 μL
	2nd Bead Addition	20 μL	20 μL	20 μL	20 μL

Schematic Diagram of the DNA Library Preparation Process



DNA Library Preparation Process

* * Please read this procedure carefully before experiment and select the protocol according to the type of sequencing platform used. Before starting the experiment, the nucleic acid concentration should be clarified. The input amount of this kit is 0.1 ng-1 μg, and the recommended sample loading amount is 1 ng-500 ng DNA. DNA solutions do not contain chelating agents. If DNA is dissolved in 1×TE or EDTA-containing solutions, it is recommended to use magnetic beads for purification or add Neutralization Reagent.

Procedure

DNA Fragmentation, End Repair and A-Tailing

1. Shake to mix the FER Buffer after melted. Flick to mix the FER Enzyme Mix, briefly centrifuge to collect and place it on ice.
2. Add the following reagents to the 200 μL PCR tube:

Table 1 Preparation of fragmentation and end-repair reaction systems

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

Note: Before the experiment begins, confirm that whether the template DNA contains ≥0.2 mM EDTA. If so, add 0.375 μL of Neutralization Reagent per 0.1 mM of the final concentration of EDTA in the 50 μL fragmentation system. For example, if a DNA template contains 1 mM EDTA, and 20 μL template is added to a 50 μL fragmentation end repair reaction system. So, the final concentration of EDTA in the system is 0.4 mM, and the amount of Neutralization Reagent should be 1.5 μL.

3. Flick to mix. Briefly centrifuge the collection and place on ice, and immediately proceed to PCR reaction.
4. Fragmentation and end repair procedures are shown in the table below (thermal cycler lid temperature is 70°C).

Table 2 Fragmentation and end repair reaction procedures

Steps	Temperature	Time
1	4 °C	1 min
2	32 °C	20 min（adjustable）
3	65 °C	30 min
4	4 °C	Hold

5. The fragmentation time is adjusted according to the target fragment size, as shown in Table 3.

Table 3 Relationship between fragmentation time and target fragment size

	32 °C Incubation time（min）			
Insert Fragment 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 according to the expected insert size. The fragment size decreases with the extension of reaction time.

2) If there is a small deviation between the result and the expected size, the recommended reaction time can be increased or decreased by 3-5 min as appropriate.

3) For FFPE samples, fragmentation time can be reduced according to their quality.

6. **Immediately** proceed to the adapter ligating reaction after the end of the reaction.

Adapter Ligation

1. Add the following reagents directly to the reaction solution that has completed DNA fragmentation, end repair and A-tailing:

Table 4 Adapter ligating reaction procedure

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

4. The number of cycles required for the reaction should be adjusted according to the amount of DNA input, and the specific number of cycles should to be referred to Table 10

Table 10 Recommended amplification cycles to obtain 100 ng and 1 μg libraries

Sample DNA	The number of cycles required for the product	
	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) If 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 size is big, the number of cycles can be appropriately increased to obtain a sufficient amount of library.

3) When performing bead-based size selection, it is recommended to amplify the library with high cycle number to obtain a sufficient amount of the library.

4) * When the adapter is ligated with a full-length Adapter, and the library output meets the application requirements, the PCR-Free library can be obtained directly without the PCR amplification step. If an incomplete adapter is used, 1-3 rounds of PCR amplification should be performed to obtain the complete adapter sequence required for sequencing.

Purification of PCR products

PCR product purification can be performed with both size selection and cleanup protocols. If the starting sample size is less than 50 ng and the distribution of fragment is required, option 2 (PCR product size selection) should be chosen. If the input amount is more than 50 ng, it is recommended to select and recover the ligation product after the adapter is ligated (refer to the size selection of DNA fragments of the ligation product).

Option 1: Cleanup of PCR products

1. Take out CMPure 30 min in advance and place it at room temperature, mix well with shaking before use.
2. Transfer the PCR reaction solution to a new 1.5 mL centrifuge tube. Pipette 0.9 times the volume of CMPure to the PCR product. Pipette blow or shake thoroughly to mix and incubate at room temperature for 5 min.
3. Centrifuge briefly. Place the centrifuge tube on a magnetic rack to separate the magnetic beads from the supernatant until the solution is clear (takes about 5 min). Carefully pipette the supernatant, avoiding contact with magnetic beads binding to the target DNA.

Note: Do not discard the magnetic beads.

9. Keep the centrifuge tube fixed on the magnetic rack and open the lid, then let stand at room temperature for 3-5 min to dry the magnetic beads in air. The magnetic beads should have no reflection or cracking.

   Note: Do not heat to dry or over-dry the magnetic beads, otherwise it will affect the yield.

10. Remove the centrifuge tube from the magnetic rack, add 22 μL of NF water. Vortex and shake to completely resuspend the magnetic beads in the NF water and let stand for 5 min at room temperature.
11. Centrifuge briefly. Place the centrifuge tube on the magnetic rack until the solution is clear (takes about 5 min), and transfer 20 μL of the eluate to a new PCR tube.

PCR amplification

1. Prepare the PCR reaction mix according to Table 8:

Table 8 Preparation of PCR reaction mixtures

Component	Volume
2×Super HiFi PCR Mix	25 μL
Index primer Mix	5 μL
Recovered ligation product	20 μL
Total	50 μL

Note: Index primer Mix can be selected according to different platforms using different adapter primer kits. If you choose Cowin's adapter primer kit, you can refer to the corresponding instructions.

2. Mix well by vortex for 5 s, centrifuge instantaneously to collect the solution in the bottom of the tube.
3. Place the above PCR tube on the thermal cycler, and the reaction procedure is referred to Table 9.

Table 9 PCR reaction procedure

Component	Time	Cycles
98 °C	3 min	
98 °C	20 s	Refer to Table 10
60 °C	20 s	
72 °C	30 s	
72 °C	5 min	
4 °C	Hold	

2. In this step, add adapter that matches different platforms, and the adapter concentration needs to match according to the different input amounts, as shown in Table 5.

Table 5 Recommended Adapter concentrations for 1 ng-500 ng gDNA

gDNA	Adapter Concentration	Pre-diluted Concentration of Cowin's Adapter
100 ng-500 ng	10 μM	No dilution required
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

3. Mix by shaking, and centrifuge briefly to collect the solution in the bottom of the tube.
4. Place the PCR tube in the PCR instrument and run the following procedure

Table 6 Ligating reaction program

Steps	Volume	Time
1	Hot lid	25 °C
2	23 °C	20 min
3	4 °C	Hold

5. Mix by shaking and centrifuge briefly to collect the solution in the bottom of the tube.
6. Bathe at 23°C for 20 min.

   Note: If using a thermal cycler for this operation, set the hot lid to 25 °C.

Ligation Product Purification

The DNA fragments should be purified with the magnetic beads accompanying the kit. There are two options to purify the ligation products: size selection and cleanup. If the starting sample size is less than 50 ng, it is recommended to choose option 1 (cleanup of DNA fragments). If the input amount is more than 50 ng, option 2 (size selection of DNA fragments) is recommended.

Option 1: Cleanup of DNA fragments

1. Take out CMPure 30 min in advance and place it at room temperature, mix well with shaking before use.
2. Transfer the ligation product to a new 1.5 mL centrifuge tube and replenish the reaction system to 100 μL with water.
3. Pipette 80 μL of CMPure to the 100 μL product, mix well with shaking, and incubate at room temperature for 5 min.
4. Centrifuge briefly. Place the centrifuge tube on a magnetic rack until the solution is clear (takes about 5 min). Pipette the supernatant and discard.
5. Keep the centrifuge tube fixed on the magnetic rack, and add 250 μL of freshly configured 80% ethanol to the centrifuge tube. Leave it at room temperature for 30 seconds, and discard the supernatant.
   Note: Be sure to use freshly configured ethanol, otherwise it will affect the experimental results.
6. Repeat step 5 once, and drain the liquid at the bottom of the tube as much as possible. Centrifuge the tube instantaneously when there is a small amount remaining on the tube wall. After separating on the magnetic rack, use a pipette of small capacity to pipette the liquid at the bottom of the tube.
   Note: Do not pipette the beads to avoid affecting the yield.
7. Keep the centrifuge tube fixed on the magnetic rack and open the lid, then let stand at room temperature for 3-5 min to dry the magnetic beads in air. The magnetic beads should have no reflection or cracking.
   Note: Do not heat to dry or over-dry the magnetic beads, otherwise it will affect the yield.
8. Remove the centrifuge tube from the magnetic rack, add 25 μL of NF water to elute. Pipette blow or shake thoroughly to mix and dissolve at room temperature for 5 min.
9. Centrifuge instantaneously. Place the centrifuge tube on the magnetic rack for 5 min until the solution is clear, and transfer 20 μL of the supernatant to a new PCR tube. Proceed to the next step or store the product at -20 °C.

Option 2: Size selection of DNA fragments

For size selection of DNA fragments, select different sizes of target fragments as needed. Table 7 shows the amount of magnetic beads required during screening for different fragment sizes.

Table 7 Recommended amount of magnetic beads for acquiring the main band of DNA (100 μL reaction system)

DNA Fragment Size	Insert fragment+Adapter	300 bp	350 bp	400 bp	450 bp
Amount of Magnetic Beads	First selection	50 μL	40 μL	30 μL	20 μL
	Second selection	20 μL	20 μL	20 μL	20 μL

The main peak of the target fragment selected by the following process is about 350bp

1. Take out CMPure 30 min in advance and place it at room temperature, mix well with shaking before use.
2. Transfer the ligation product to a new 1.5 mL centrifuge tube and replenish the reaction system to 100 μL with water.
3. Pipette 40 μL of CMPure to the product, mix well with shaking, and incubate at room temperature for 5 min.
4. Centrifuge briefly. Place the centrifuge tube on a magnetic rack to separate the magnetic beads from the supernatant until the solution is clear (takes about 5 min). Carefully pipette the supernatant, avoiding contact with magnetic beads binding to the target DNA.
   Note: Do not discard the supernatant.
5. Add 20 μL of well-mixed CMPure to the supernatant, vortex for 5 s and leave at room temperature for 5 min.
6. Centrifuge briefly. Place the centrifuge tube on a magnetic rack to separate the magnetic beads from the supernatant until the solution is clear (takes about 5 min). Carefully pipette the supernatant and discard, avoiding contact with magnetic beads binding to the target DNA.
   Note: Do not discard the magnetic beads.
7. Keep the centrifuge tube fixed on the magnetic rack, and add 250 μL of freshly configured 80% ethanol to the centrifuge tube. Leave it at room temperature for 30 seconds, and carefully discard the supernatant.
   Note: Be sure to use freshly configured ethanol, otherwise it will affect the experimental results.
8. Repeat step 7 once, and drain the liquid at the bottom of the tube as much as possible. Centrifuge the tube instantaneously when there is a small amount remaining on the tube wall. After separating on the magnetic rack, use a pipette of small capacity to pipette the liquid at the bottom of the tube.
   Note: Do not pipette the beads to avoid affecting the yield.