Library Quality Testing

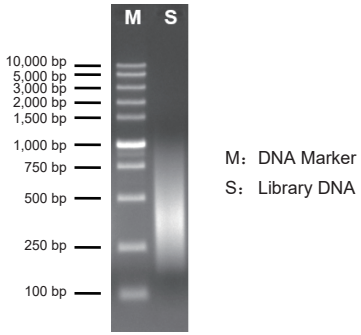
Library Concentration Determination

In order to obtain high-quality sequencing results, accurate quantification of DNA libraries is required. The use of real-time PCR methods is recommended for absolute quantification of DNA libraries. Besides, fluorescent dye methods such as the Qubit method or the picogreen fluorescent dye method can be used. Quantitative methods based on absorbance measurements should not be used here. The molarity of the DNA library can be converted using the following approximate formula.

The Average Total Length of the Library	Approximate Conversion Formulas
300 bp	1 ng/μL=5.0 nM
400 bp	1 ng/μL=3.8 nM
500 bp	1 ng/μL=3.0 nM

Library Fragment Distribution

The length distribution of fragments in the DNA library can be detected by agarose gel electrophoresis or the Qsep100 automated nucleic acid and protein analysis system.



M：DNA Marker
S：Library DNA

Version: 02/2024

NGS TPH DNA Library Prep Set for Illumina (1 ng)

Cat. No. : CW2847S (24 rxns)
CW2847M（96 rxns）
Storage Condition: -20°C storage, dry ice transportation.

Components

Component	CW2847S 24 rxns	CW2847M 96 rxns
TPS V5	120 μL	480 μL
5×FA Reaction Buffer	96 μL	384 μL
2×HiFidelity PCR Mix	600 μL	2×1.2 ml
TS Buffer	72 μL	288 μL

* This kit is suitable for the preparation of human genomic DNA libraries, and the starting template DNA input is 1 ng. Cowin also have transposase-based library preparation kits for 50 ng (CW2845) and 5 ng (CW2846) of human genomic DNA input, and different kits are recommended for different amounts of DNA input to obtain higher quality libraries.

* The components in the table correspond in order as follows:

TPS V1, transposase;

5×FA Reaction Buffer, transposase reaction buffer;

2× HiFidelity PCR Mix, amplification buffer;

TS Buffer, termination buffer

Introduction

The kit is a specially developed kit for the Illumina high-throughput sequencing platform, which provides the enzyme master mix and reaction buffer required for genomic DNA library preparation, and contains all components except PCR primers. Compared with the traditional library preparation kit, this kit uses a new transposase method for library preparation. It can complete the DNA fragmentation, end repair and adapter ligation reaction through a simple enzymatic reaction, which significantly reduces the amount of template used, reduces the experimental operation steps, and shortens the library preparation time. This kit uses high-fidelity DNA polymerase for library enrichment, performs PCR amplification without preference, and expands the coverage area of the sequence, which can effectively prepare DNA libraries for Illumina second-generation sequencing platform.The kit is suitable for starting template DNA inputs of 1 ng. All reagents in the kit have undergone strict quality control and functional validation to ensure maximum stability and reproducibility of library preparation.

Features

- DNA fragmentation and adapter ligation are completed in one step.
- Ultra-fidelity amplification, which minimizes amplification preference.

Equipment and Reagents to be Supplied by user

1. Magnetic Rack: DynaMagTM-2 (Cat. No. 12321D) is recommended.
2. DNA Purification and Recovery Kit: It is recommended to use Cowin's MagBead DNA Purification Kit (for NGS Size Selection) (Cat. No. CW2508).
3. Library PCR Primer Kit and PCR Thermal Cycler: It is recommended to use Cowin's NGS TP Index Kit for Illumina (Cat. No. CW2958/CW2959/CW2960/CW2961/CW2962/CW2963).
4. Absolute ethanol and deionized water (pH between 7.0-8.0).
5. Reaction tubes: It is recommended to use low-adsorption PCR tubes and 1.5 ml centrifuge tubes.
6. Pipette tips: It is recommended to use high-quality filter tips to prevent contamination of kit and library samples.

9. Remove the centrifuge tube from the magnetic rack, vortex and shake to completely resuspend the magnetic beads in the eluate and let stand for 5 min at room temperature. Centrifuge briefly. Place the centrifuge tube on the magnetic rack until the solution is clear, and transfer the supernatant to a new PCR tube.

Appendix: Recommended amount of magnetic bead for different sizes of fragment selected

DNA library size	Inserts	230 bp	330 bp	430 bp
	(Insert+Adapter+Primer)	350 bp	450 bp	550 bp
Amount of magnetic bead	1st Bead Addition	65 μL	55 μL	45 μL
	2nd Bead Addition	50 μL	30 μL	30 μL

Cleanup of DNA Fragments

It is recommended to use Cowin's MagBead DNA Purification Kit (for NGS Size Selection) (CMPure, CW2508).

1. Take out CMPure 30 min in advance and place it at room temperature, mix well with shaking before use.
2. Pipette 50 μL of magnetic beads balanced to the room temperature to the PCR product. Vortex and shake thoroughly for 5 sec and let stand 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 3-5 min). Carefully pipette the supernatant and discard, avoiding contact with magnetic beads binding to the target DNA.
   Note: Do not discard the magnetic beads.
4. Keep the centrifuge tube fixed on the magnetic rack, and add 200 μL of freshly configured 80% ethanol to the centrifuge tube. Leave it at room temperature for 30 seconds, and carefully discard the supernatant.
   Note: When adding ethanol, the liquid should not be blown directly onto the magnetic bead.
5. Repeat step 4.
6. Keep the tubes fixed on the magnetic rack, let stand and dry at room temperature until the surface of the beads is slightly cracked, and add 22 μL of ddH_2O to dissolve.
   Note: Do not over-dry the beads, otherwise it will affect the elution efficiency.
7. Remove the centrifuge tube from the magnetic rack, vortex and shake to completely resuspend the magnetic beads in the eluate and let stand for 5 min at room temperature. Centrifuge briefly. Place the centrifuge tube on the magnetic rack until the solution is clear, and transfer the supernatant to a new PCR tube.

Size Selection of DNA Fragments

It is recommended to use Cowin's MagBead DNA Purification Kit (for NGS Size Selection) (CMPure, CW2508) for size selection of DNA fragments. When different sizes of DNA fragments are needed, the amount of magnetic beads used is different. For the specific amount of magnetic beads used, please refer to the attached table. If you use magnetic beads from manufacturers other than Cowin, you need to find the optimal bead dosage by yourself.

Note: Amplified products can also be selected and purified using the gel extraction kit. If there are no special requirements for the length distribution range of the library, the amplified products can also be used directly without size selection of DNA fragments. For the purification of DNA fragments, please refer to page 7 of the manual.

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. Pipette magnetic beads to it, mix well with shaking for 5 sec, and let stand 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. Carefully pipette the supernatant to a new 1.5 mL centrifuge tube

   Note: Do not discard the supernatant.
4. Add magnetic beads to the supernatant, vortex for 5 s and leave at room temperature for 5 min.
5. Centrifuge briefly. Place the centrifuge tube on a magnetic rack to separate the magnetic beads from the supernatant until the solution is clear. Carefully pipette the supernatant and discard, avoiding contact with magnetic beads binding to the target DNA.

   Note: Do not discard the magnetic beads.
6. Keep the centrifuge tube fixed on the magnetic rack, and add 200 μL of freshly configured 80% ethanol to the centrifuge tube. Leave it at room temperature for 30 seconds, and carefully discard the supernatant.

   Note: When adding ethanol, the liquid should not be blown directly onto the magnetic bead.

7 Repeat step 6 once.

8. Keep the tubes fixed on the magnetic rack, let stand and dry at room temperature until the surface of the beads is slightly cracked, and add 22 μL of ddH_2O to dissolve.

   Note: Do not over-dry the beads, otherwise it will affect the elution efficiency.

Preparation and Important Precautions before the Experiment

1. Avoid repeated freeze-thaw of reagents.
2. PCR products are prone to contamination due to improper operation, resulting in inaccurate experimental results. It is recommended to isolate the preparation area of the PCR reaction system from the purification area of the PCR product, use a special pipette, and clean the experimental area regularly.
3. Magnetic bead purification: magnetic beads should be balanced to room temperature before use, and all operations of magnetic beads should be carried out at room temperature. 80% ethanol should be freshly prepared and used. The magnetic beads should be dried after rinsing until the surface is free of liquid reflections and appears frosted. Insufficient drying of magnetic beads will have ethanol residue and affect subsequent experiments, and excessive drying of magnetic beads will affect DNA recovery efficiency.
4. This kit is suitable for the preparation of human genomic DNA library. If the DNA sample is a PCR product, its length should be guaranteed to be > 500 bp. Since transposase cannot act on DNA ends, it is recommended to extend both ends of PCR products by 50-100 bp to avoid low end-sequencing coverage when preparing PCR products.

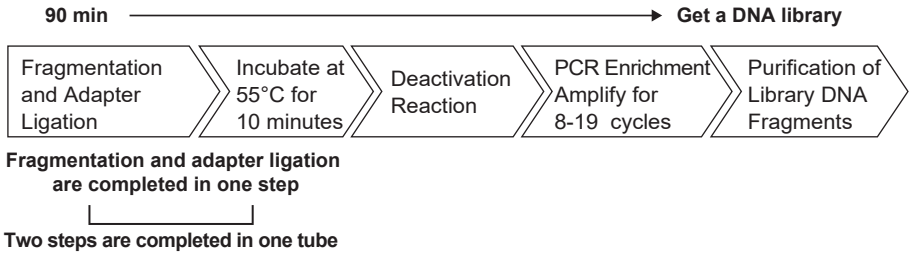
Sample Preparation

DNA purity requirements: A260/A280 = 1.8-2.0.

Sample DNA: Dissolved in ultrapure water.

DNA quantification: Too much or too little DNA input will affect the quality of the library, it is recommended to use Nano to test the purity of genomic DNA and then use Qubit to measure the concentration of the genome, and take the average of 3 measurements per sample (do not use any absorbance-based assay method for template quantification).

Schematic Diagram of the DNA Library Preparation Process



Protocol

DNA fragmentation and adapter ligation reactions

1. Add the following reagents to a 200 µL PCR tube:

Component	Volume
1 ng DNA	X µL
TPS V1	5 µL
5×FA Reaction Buffer	4 µL
ddH_2O	To 20 µL

2. Gently pipette to mix and centrifuge briefly, so that all components are collected to the bottom of the tube.
3. Place the above PCR tube in the thermal cycler and open the hot lid. The reaction procedure is as follows:

Temperature	Time
105 °C	Hot lid
55 °C	10 min
10 °C	Hold

Deactivation Reaction

After the DNA fragmentation reaction is completed, the transposase is still in a highly active state and should be taken away from the thermal cycler and added with termination buffer immediately to prevent the library fragments from becoming smaller due to over-fragmentation of the DNA.

1. Add 3 µL TS Buffer to the PCR tube containing the fragmentation product.
2. Gently pipette to mix and centrifuge briefly, so that all components are collected to the bottom of the tube.
3. Incubate at room temperature for 5 min. If the indoor temperature is too low, it can be placed on thermal cycler to react at 25 °C with the hot lid closed.

PCR Amplification

1. Add the following reagents to a 200 µL PCR tube:

Component	Volume
Fragmentation products	23 µL
Primer N5	1 µL
Primer N7	1 µL
2×HiFidelity PCR Mix	25 µL

2. Gently pipette to mix and centrifuge briefly, so that all components are collected to the bottom of the tube.
3. Place the above PCR tube in the thermal cycler and open the hot lid. The reaction procedure is as follows:

Steps	Temperature	Time	
Extension	72 °C	3 min	
Pre-Denaturation	98 °C	30 s	
Denaturation	98 °C	15 s	8-19 cycles
Anneal	60 °C	30 s	
Extension	72 °C	30 s	
Terminal Extension	72 °C	5 min	
Save	4 °C	Hold	