Version: 03/2024

NGS Fast DNA Library Prep Set for Illumina

Cat. No. : CW2585S (24 rxns)
CW2585M (96 rxns)

Storage Condition: -20 °C, dry ice transport.

Components

Component	CW2585S 24 rxns	CW2585M 96 rxns
End Prep Enzyme Mix	48 μL	192 μL
10×End Repair Reaction Buffer	200 μL	800 μL
T4 DNA Ligase	48 μL	192 μL
T4 DNA Ligase Buffer	400 μL	2×800 μL
2×HiFidelity PCR Mix	600 μL	2×1.2 mL

Introduction

This kit provides the enzyme premix system and reaction buffer required for DNA library preparation, including all components except adapters and PCR primers for Illumina NGS platform. Compared with the conventional library preparation method, the kit is simple and convenient, and greatly reduces the library preparation time. In addition, this kit uses high fidelity DNA polymerase for library enrichment and unbiased PCR amplification, which expands the coverage area of the sequence and can effectively prepare DNA libraries for Illumina NGS platform. All reagents provided in the kit have undergone strict quality control and functional verification to ensure maximum stability in library preparation.

Features

- End repair, phosphorylation and A-tailing can be completed in one step.
- No cleanup needed before adapter ligation.
- Ultra - fidelity amplification to minimize amplification preference.
- Support multiple samples and the library can be sequenced by Illumina GAIIx, HiSacnSQ, HiSeq 2500/2000/1000 and MiSeq sequencing platforms.

Library Fragment Distribution

The length distribution of fragments in the DNA library can be detected by agarose gel electrophoresis or Agilent 2100 Bioanalyzer.

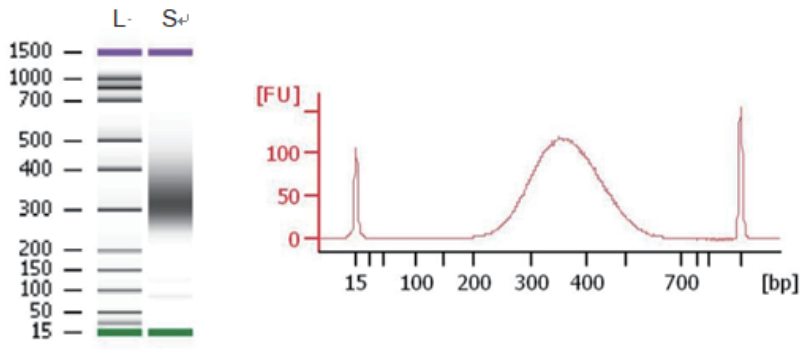


Figure 1: Agilent 2100 Bioanalyzer library analysis results
L: DNA Ladder;
S: The library was prepared using 200 ng human genome DNA, and the results were selected and recovered by magnetic beads.

Library structure

5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCC
GATCT [Target Sequence] AGATCGGAAGAGCACACGTCTGAACTCCAGTCACNNN
NNNATCTCGTATGCCGTCTTCTGCTTG-3'
NNNNN: index, 6bases

Self-Provided Instruments, Reagents and Consumables

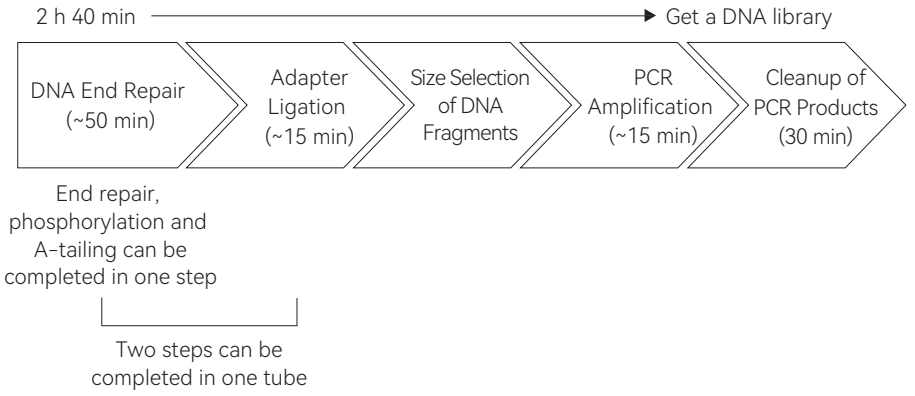
1. Magnetic rack: It is recommended to use DynaMagTM-2 (Cat. No. 12321D).
2. DNA purification and recovery kit: It is recommended to use CoWin's MagBead DNA Purification Kit (for NGS Size Selection) (Cat. No. CW2508).
3. Adapter and primer kit: It is recommended to use CoWin's NGS Multiplex Oligos for Illumina (Index Primers Set I/II) (Cat. No. CW2586/CW2587).
4. Absolute ethanol, EB (10 mM Tris-HCl, pH 8.0) 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.

   Pipette tips: It is recommended to use high-quality filter tips to prevent contamination of kit and library samples.

Preparation And Important Notes Before the Experiment

1. Avoid repeated freeze-thaw of reagents. It is recommended to separate the remaining reagents into small packages after the first use of the kit.
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.

Schematic Diagram of DNA Library Building Process



Cleanup of PCR Products

1. Mix CMPure well with shaking for 20 s before use.
2. Transfer the ligation product to a new 1.5 mL centrifuge tube.
3. Pipette 1 time the sample volume of CMPure to tube, mix well with shaking for 5 s, 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 and discard, avoiding contact with magnetic beads binding to the target DNA.

   Note: Do not discard the magnetic beads.
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 carefully discard the supernatant.
6. Repeat Step 5.
7. Keep the centrifuge tube fixed on the magnetic rack and open the lid, then let stand at room temperature for 10 min to dry the magnetic beads in air.
8. Remove the centrifuge tube from the magnetic rack, add 30 μL of EB or deionized water to elute. Pipette blow or shake thoroughly to mix and dissolve at room temperature for 5 min.
9. Centrifuge briefly. Place the centrifuge tube on the magnetic rack until the solution is clear (takes about 5 min), and transfer 25 μL of the eluate to a new PCR tube. The DNA library should be stored at -20 ℃.

Library Quality Control

Library concentration

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 molar concentration of DNA library was converted.

The Average Total Length of the Library	Approximate Conversion Formulas	Cluster Reaction DNA Library Concentration
200 bp	1 ng/μL= 7.5 nM	6-12 pM
300 bp	1 ng/μL=5.0 nM	6-12 pM
400 bp	1 ng/μL=3.8 nM	6-12 pM
500 bp	1 ng/μL=3.0 nM	6-12 pM

9. Centrifuge briefly. Place the centrifuge tube on the magnetic rack until the solution is clear (takes about 5 min), and transfer 23 μL of the eluate to a new PCR tube.

Attached Table 1: Recommended amount of magnetic beads for size selection

DNA Fragment Size	Insert fragments	150 bp	200 bp	250 bp	300-400 bp	400-500 bp	500-700 bp
	(Insert fragment +adapter+primer)	270 bp	320 bp	400 bp	400-500 bp	500-600 bp	600-800 bp
Amount of Magnetic Beads	First selection	85	70	55	50	45	35
	Second selection	25	25	20	20	20	15

PCR amplification

1. Add the following reagents into the PCR tube and mix.

Reagent	Volume
DNA fragment after adapter ligation	23 μL
2×HiFidelity PCR Mix	25 μL
Univesial primer	1 μL
Index primer	1 μL
Total volume	50 μL

2. PCR reaction conditions.

Step	Temperature	Time	
Pre-denaturation	98 °C	30 s	
Denaturation	98 °C	10 s	} 6 to 16 cycles
Annealing	65 °C	30 s	
Extension	72 °C	30 s	
Terminal Extension	72 °C	5 min	

Note: It is recommended that the number of PCR cycles should be 6 for 1 μg initial sample, 10 for 50 ng sample, and 14-15 for 5 ng sample. The number of PCR cycles can also be optimized according to experimental needs.

Protocol

Sample requirements: 5 ng-1 μg fragmented double-stranded DNA, dissolved in EB (10 mM Tris-HCl pH 8.0) or deionized water.
DNA purity requirements: OD_{260}/OD_{280}=1.8~2.0.

DNA end repair

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

Reagent	Volume
10×End Repair Reaction Buffer	6.5 μL
End Prep Enzyme Mix	2 μL
Fragmented DNA	X (5 ng-1 μg)
RNase-free Water	to 65 μL

2. Gently pipette to mix and centrifuge briefly to collect all the components 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:
   15 min @ 12°C
   15 min @ 37°C
   20 min @ 72°C
   Hold on 4 °C

Adaptor ligation

It is recommended to use CoWin's adapter. You can also use NEB's or Illumina's adapter. The specific connection method can be referred to the product instruction manual of each company. Here are the steps for ligation with CoWin's adapter:

1. Add the following reagents directly to the reaction solution that has completed DNA end repair:

Reagent	Volume
T4 DNA ligase buffer for Illumina	14 μL
T4 DNA ligase	2 μL
Adapter	2.5 μL

At this time, the total volume of solution in the tube is 83.5 μL.

Note: If the starting sample size is less than 100 ng, please dilute the adaptor with deionized water 10 times to 1.5 μM before use.

2. Blow and mix the solution with a pipette and then centrifuged briefly to collect the solution to the bottom of the tube.
3. Bathe at 20 ℃ for 15 minutes.

Note: If using a thermal cycler for this operation, please close the hot lid.

Size selection of DNA fragments

It is recommended to use CoWin's MagBead DNA Purification Kit (for NGS Size Selection) (Cat. No. CW2508) for this step.

Note: The size selection of DNA fragments is an optional step. If the starting sample size is less than 50 ng, it is not recommended to perform the size selection. Please refer to page 5 of the instruction manual for direct cleanup of DNA fragments. In addition, when preparing DNA libraries of different sizes, the amount of magnetic beads used for the size selection of DNA fragments 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.
In the following steps, the maximum length of the recovered DNA fragment is 320 bp (the length of the inserted fragment is 200 bp), and the initial volume of the reaction is 100 μL.

1. 1.Mix CMPure well with shaking for 20 s before use.
2. Add 16.5 μL of deionized water to the adapter ligation reaction solution, so the adapter ligation reaction buffer system is replenished to 100 μL.

   Note: If using NEB's adapter, only 13.5 μL deionized water is needed.
3. Transfer the ligation product to a new 1.5 mL centrifuge tube.
4. Pipette 70 μL of CMPure to the product, mix well with shaking 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 (takes about 5 min). Carefully pipette the supernatant to a new 1.5 mL tube and discard the magnetic beads.

   Note: Do not discard the supernatant.
6. Add 25 μL of well-mixed CMPure to the supernatant, vortex for 5 s and leave at room temperature for 5 min.
7. 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.

8. 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.
9. Repeat Step 8.
10. Keep the centrifuge tube fixed on the magnetic rack and open the lid, then let stand at room temperature for 10 min to dry the magnetic beads in air.
11. Remove the centrifuge tube from the magnetic rack, add 28 μL of 10 mM Tris-HCl (pH 8.0) or deionized water. Vortex and shake to completely resuspend the magnetic beads in the solution and let stand for 5 min at room temperature.
12. Centrifuge briefly. Place the centrifuge tube on the magnetic rack until the solution is clear (takes about 5 min), and transfer 23 μL of the eluate to a new PCR tube.

    Note: Do not transfer magnetic beads. Trace contamination of magnetic beads can affect the normal process of subsequent PCR reaction.

Another option: cleanup of DNA fragments

1. Mix CMPure well with shaking for 20 s before use.
2. Transfer the ligation product to a new 1.5 mL centrifuge tube.
3. Pipette 1 time the sample volume of CMPure to tube, mix well with shaking for 5 s, 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 and discard, avoiding contact with magnetic beads binding to the target DNA.

   Note: Do not discard the magnetic beads.
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 carefully discard the supernatant.
6. Repeat Step 5.
7. Keep the centrifuge tube fixed on the magnetic rack and open the lid, then let stand at room temperature for 10 min to dry the magnetic beads in air.
8. Remove the centrifuge tube from the magnetic rack, add 28 μL of EB (self-prepared) or deionized water to elute. Pipette blow or shake thoroughly to mix and dissolve at room temperature for 5 min.