Tinzyme Co., Limited

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

NGS DNA Library Kit for Illumina (50 ng)

Product Number:PCK452

Shipping and Storage

Storage at -20°C and transport on dry ice.

Components

Components	PCK452 24 rxns	PCK452 96rxns
TPS V50	144 µL	576 µL
5×FA Reaction Buffer	144 µL	576 µL
2×HiFidelity PCR Mix	600 µL	2×1.2 mL
PPM	48 µL	192 µL

Description

This kit is specially developed for the Illumina high-throughput sequencing platform. It provides the enzyme premix system and reaction buffer required for genomic DNA library construction, including all components except PCR primers. Compared with traditional library construction kits, this kit uses a novel transposase method for library construction, which can complete DNA fragmentation, end repair and adapter ligation in one simple enzymatic reaction, significantly reducing the amount of template used. reducing the experimental operation steps and shortening the library construction time; using high-fidelity DNA polymerase for library enrichment, unbiased PCR amplification, expanding the coverage area of the sequence, and efficiently preparing DNA for the Illumina next-generation sequencing platform library. This kit is suitable for starting template DNA input of 50 ng. All reagents in the kit have undergone strict quality control and functional verification to ensure the stability and reproducibility of library construction to the greatest extent.

Reagents to Be Supplied by User

1. Magnetic stand.
2. DNA purification and recovery kit
3. Library PCR primer kit and PCR thermal cycler
4. Absolute ethanol, 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 kits and library samples.

Important Points Before Starting

1. Avoid repeated freezing and thawing of reagents.
2. The PCR products are easily contaminated 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 special pipette to regularly clean each experimental area.
3. Magnetic bead purification: The magnetic beads should be equilibrated to room temperature before use. All operations on the magnetic beads should be carried out at room temperature. 80% ethanol should be prepared and used immediately. After rinsing, the magnetic beads should be dried until the surface has no liquid reflection and is frosted. , Insufficient drying of magnetic beads will cause ethanol residue to affect subsequent experiments, and excessive drying of magnetic beads will affect DNA recovery efficiency.
4. This kit is suitable for the construction of human genomic DNA library. If the DNA sample is a PCR product, its length should be more than 500 bp. Since the transposase cannot act on the DNA end, it is recommended to extend the PCR product at both

ends when preparing the PCR product. 50-100 bp to avoid low end sequencing coverage.

Sample preparation

1. DNA purity requirements: A260/A280 = 1.8-2.0.
2. Sample DNA: Dissolve in ultrapure water.
3. DNA quantification: Too much or too little DNA input will affect the quality of the library. It is recommended to use Nano to detect the purity of genomic DNA and then to use Qubit to detect the concentration of the genome. Each sample is measured 3 times and the average value is obtained (do not use Template quantification based on any absorbance measurement-based assay).

Protocol

DNA fragmentation, adapter ligation reaction

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

Component	Volume
50 ng DNA	X μL
TPS V50	6 μL
5×FA Reaction Buffer	6 μL
ddH_2O	To 30 μL

2. Mix by pipetting gently and centrifuge briefly to collect all components to the bottom of the tube.
3. Put the above PCR tube in the thermal cycler, open the hot cover, and the reaction procedure is as follows:

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

After the DNA fragmentation reaction is completed, the transposase is still in a high activity state and should be purified immediately to prevent the library fragment from becoming smaller due to excessive DNA fragmentation.

Fragmentation product purification

1. Magnetic Bead should be shaken and mixed before use, and then equilibrated at room temperature for 30 minutes.
2. Add 50 μl of magn etic beads equilibrated to room temperature to the fragmented product, vortex for 5 seconds, and then stand at room temperature for 5 minutes.
3. Centrifuge briefly, place the centrifuge tube on a magnetic stand, and separate the magnetic beads from the supernatant solution until the solution is clear (about 3-5 minutes), carefully aspirate the supernatant and discard, avoiding contact with the bound target during this time. DNA magnetic beads.

   Note: Do not discard the magnetic beads.
4. Continue to keep the centrifuge tube fixed on the magnetic stand, add 200 μL of freshly prepared 80% ethanol to the centrifuge tube, leave at room temperature for 30 seconds, and carefully discard the supernatant.

   Note: When adding ethanol, the liquid should not be pipetted directly onto the magnetic beads.
5. Repeat step 4.
6. Keep the centrifuge tube fixed on the magnetic stand, let it dry at room temperature until the surface of the magnetic beads is slightly cracked, and add 23 μL ddH_2O to dissolve.

   Note: Do not over-dry the magnetic beads to avoid affecting elution efficiency.
7. Remove the centrifuge tube from the magnetic stand, vortex to completely resuspend the magnetic beads, and let stand at room temperature for 5 minutes. Briefly centrifuge, place the tube on a magnetic rack until the solution is clear, transfer 21μL of the supernatant to a new 200μL PCR tube.

PCR amplification

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

Component	Volume
Fragmentation product	21 μL
PPM	2 μL
Primer N5	1 μL
Primer N7	1 μL
2×HiFidelity PCR Mix	25 μL

2. Mix by pipetting gently and centrifuge briefly to collect all components to the bottom of the tube.
3. Put the above PCR tube in the thermal cycler, open the hot cover, and the reaction procedure is as follows:

Step	Temperature	Time	Cycles
Extend	72°C	3 min	
Predenaturation	98°C	30 s	
Transsexual	98°C	15 s	5-10
Annealing	60°C	30 s	
Extend	72°C	3 min	
Final Extension	72°C	5 min	
Save	4°C	Hold	

Selective recovery of library DNA fragments

Note: Amplification products can also be subjected to fragment length sorting and purification using a gel recovery kit. If there is no special requirement for the length distribution of the library, the amplified product may not be subjected to selective recovery of DNA fragments.

1. Magnetic Bead should be shaken and mixed before use, and then equilibrated at room temperature for 30 minutes.
2. Transfer the PCR product to a 1.5 ml centrifuge tube, add water to 100 μl, add a certain volume of magnetic beads equilibrated to room temperature, vortex for 5 seconds, and let stand at room temperature for 5 minutes.
3. Centrifuge briefly, place the centrifuge tube on a magnetic stand, and separate the magnetic beads from the supernatant until the solution is clear. Carefully transfer the supernatant to a new 1.5 ml centrifuge tube.

   Note: Do not discard the supernatant.
4. Add several volumes of magnetic beads to the supernatant, vortex for 5 seconds, and let stand at room temperature for 5 minutes.
5. Briefly centrifuge, place the centrifuge tube on a magnetic stand, and separate the magnetic beads from the supernatant until the solution is clear. Carefully aspirate and discard the supernatant, avoiding contact with the magnetic beads bound to the target DNA.

   Note: Do not discard the magnetic beads.
6. Continue to keep the centrifuge tube fixed on the magnetic stand, add 200 μl of freshly prepared 80% ethanol to the centrifuge tube, leave at room temperature for 30 seconds, and carefully discard the supernatant.

   Note: When adding ethanol, the liquid should not be pipetted directly onto the magnetic beads.
7. Repeat step 6 once.
8. Keep the centrifuge tube fixed on the magnetic stand, let it dry at room temperature until the surface of the magnetic beads is slightly cracked, and add 22μl ddH_2O to dissolve.

   Note: Do not over-dry the magnetic beads to avoid affecting elution efficiency.
9. Remove the centrifuge tube from the magnetic stand, vortex to completely resuspend the magnetic beads, and let stand at room temperature for 5 minutes. Centrifuge briefly, place the tube on the magnetic stand until the solution is clear, and transfer the supernatant solution to a new centrifuge tube.

Attached table: Recommended dosage of magnetic beads when selecting and recycling different fragments

DNA library size	insert	230 bp	330 bp	430 bp
	(insert+adaptor+primer)	350 bp	450 bp	550 bp

Magnetic bead dosage	first choice	65 μL	55 μL	45 μL
	first choice	50 μL	30 μL	30 μL

Library DNA Fragment Purification

1. Magnetic Bead should be shaken and mixed before use, and then equilibrated at room temperature for 30 minutes.
2. Add 50 μl of magnetic beads equilibrated to room temperature to the PCR product, vortex for 5 seconds, and then stand at room temperature for 5 minutes.
3. Centrifuge briefly, place the centrifuge tube on a magnetic stand, and separate the magnetic beads from the supernatant solution until the solution is clear (about 3-5 minutes). Carefully aspirate the supernatant and discard it, avoiding contact with the bound target DNA during this time. magnetic beads.

   Note: Do not discard the magnetic beads.
4. Continue to keep the centrifuge tube fixed on the magnetic stand, add 200 μl of freshly prepared 80% ethanol to the centrifuge tube, leave at room temperature for 30 seconds, and carefully discard the supernatant.

   Note: When adding ethanol, the liquid should not be pipetted directly onto the magnetic beads.
5. Repeat step 4.
6. Keep the centrifuge tube fixed on the magnetic stand, let it dry at room temperature until the surface of the magnetic beads is slightly cracked, and add 22 μl ddH_2O to dissolve.

   Note: Do not over-dry the magnetic beads to avoid affecting elution efficiency.
7. Remove the centrifuge tube from the magnetic stand, vortex to completely resuspend the magnetic beads, and let stand at room temperature for 5 minutes. Centrifuge briefly, place the tube on the magnetic stand until the solution is clear, and transfer the supernatant solution to a new centrifuge tube.

Library Quality Assurance

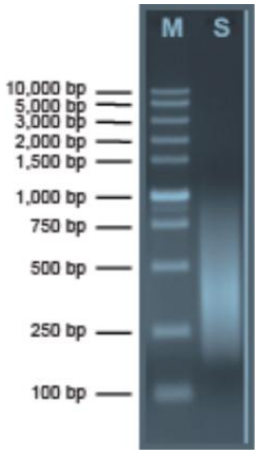
1. Library concentration determination

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

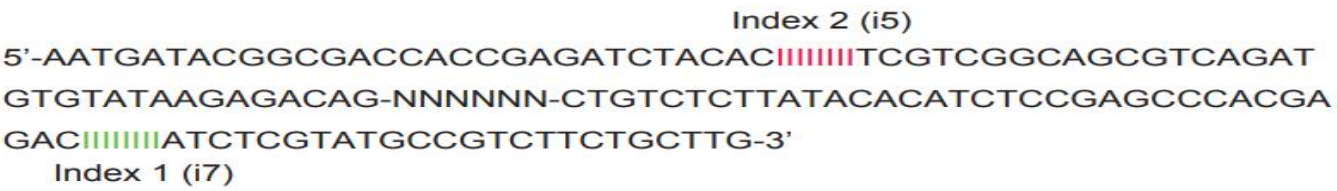
Average total library length	Approximate conversion formula
300 bp	1 ng/μl=5.0 nM
400 bp	1 ng/μl=3.8 nM
500 bp	1 ng/μl=3.0 nM

2. Library Fragment Distribution

The prepared DNA library can be detected by agarose gel electrophoresis or the automatic nucleic acid and protein analysis system Qsep100 to detect the fragment length distribution in the DNA library.



3. Library structure



IIIIIIII: Index 2 (i5), 8 bases; IIIIIIII: Index 1 (i7), 8 bases; NNNNNN: insertion sequence