Tinzyme Co., Limited

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

NGS TPH DNA Library Preps Kit for lllumina (1ng)

Product Number: PCK247

Shipping and Storage

Store at -20℃ and transport on dry ice.

Components

Component	PCK247 24rxns	PCK247 96rxns
TPS V1	36μl	144μl
5×FA Reaction Buffer	96μl	384μl
TS Buffer	72μl	288μl
2× PCR Mix	600μl	2×1.2ml

Note: This kit is suitable for constructing human genomic DNA libraries, with a starting template DNA input of 1ng. Our company also has NGS TPH DNA Library Preps kit for lllumina (5ng) for 5ng (PCK246). To obtain a high-quality library, it is recommended to use different reagent kits for different DNA starting quantities.

Description

This kit is a specialized kit developed specifically for the Illumina high-throughput sequencing platform, providing the enzyme premix system and reaction buffer required for constructing genomic DNA libraries, including all components except PCR primers. Compared with traditional library construction kits, this kit adopts a novel transposase method for library construction, which can complete DNA fragmentation, end repair, and connector connection reactions through a simple enzymatic reaction, significantly reducing the use of templates, reducing experimental steps, and shortening library construction time; The use of high fidelity DNA polymerase for library enrichment and preference free PCR amplification has expanded the coverage area of the sequence, enabling efficient preparation of DNA libraries for the Illumina second-generation sequencing platform. This reagent kit is suitable for a starting template DNA input of 1ng. All reagents in the reagent kit have undergone strict quality control and functional verification, ensuring the stability and repeatability of library construction to the greatest extent possible.

Features

1. DNA fragmentation and splicing are completed in one step.
2. Ultra fidelity amplification minimizes amplification preference to the greatest extent possible.

Self provided instruments, reagents, and consumables

1. Magnetic frame
2. DNA purification and recovery kit: It is recommended to use the Magnetic Bear DNA Purification Kit (for NGS Size Selection) (DNK2508).
3. Library PCR Primer Kit
4. Anhydrous ethanol, deionized water (pH between 7.0-8.0).
5. Reaction tube: It is recommended to use a low adsorption PCR tube and a 1.5 ml centrifuge tube.
6. Gun tip: It is recommended to use high-quality filtering gun tips to prevent contamination of reagent kits and library samples.

Preparation and important precautions before the experiment

1. Avoid repeated freezing and thawing of reagents.
2. 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

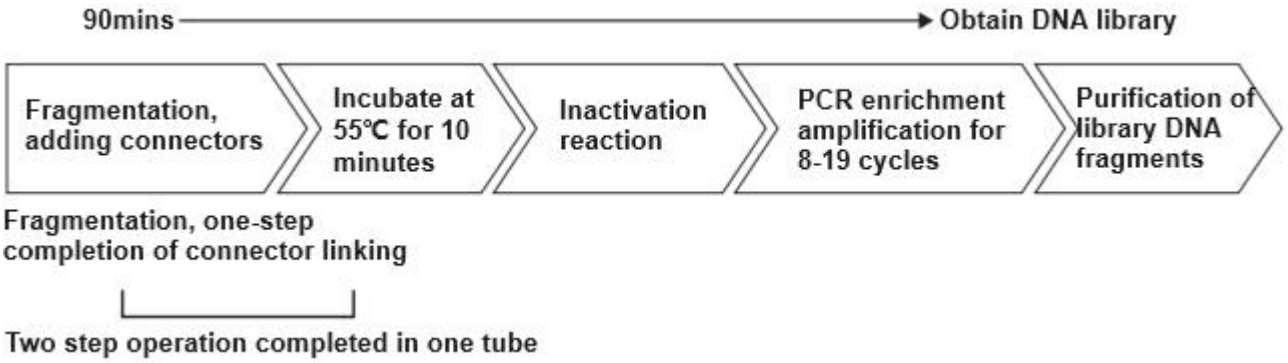
pipette to regularly clean each experimental area.

3. Magnetic bead purification: Before use, the magnetic beads should be balanced to room temperature. All operations of 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 in a frosted state. Insufficient drying of the magnetic beads will have residual ethanol, which will affect subsequent experiments. Excessive drying of the magnetic beads will affect the efficiency of DNA recovery.
4. This kit is suitable for constructing human genomic DNA libraries. If the DNA sample is a PCR product, its length should be ensured to be greater than 500bp. As transposase cannot act on the end of DNA, it is recommended to extend the two ends of the PCR product by 50-100 bp each when preparing the PCR product to avoid low end sequencing coverage.

Sample preparation

1. DNA purity requirement: A260/A280=1.8-2.0.
2. Sample DNA: Dissolved in ultrapure water.
3. DNA quantification: Excessive or insufficient DNA input can have an impact on the quality of the library. It is recommended to first use Nano to test the purity of genomic DNA, and then use Qubit to perform concentration testing on the genome (do not use any absorbance based measurement methods for template quantification).

Schematic diagram of DNA library construction process



Protocol

DNA Fragmentation and Ligation Reaction

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

Component	Volume
1ng DNA	Xμl
TPS V1	1.5μl
5×FA Reaction Buffer	4μl
ddH_2O	To 20μl

2. Gently blow and mix with a pipette, centrifuge briefly, and collect all components to the bottom of the tube.
3. Place the above PCR tube in the PCR instrument, open the Heat cap, and proceed with the following reaction procedure:

Temperature	Time
105℃	Heat cap
55℃	10 min
10℃	Hold

Inactivation reaction

After the completion of DNA fragmentation reaction, the enzyme is still in a high activity state. It should be immediately removed from the PCR instrument and added to the reaction termination buffer to terminate the reaction, in order to prevent the

library fragments from becoming smaller due to excessive DNA fragmentation.

1. Add 3μl TS Buffer to the PCR tube containing fragmented products.
2. Gently blow and mix with a pipette, centrifuge briefly, and collect all components to the bottom of the tube.
3. Incubate at room temperature for 5 minutes. If the indoor temperature is too low, it can be placed on the PCR instrument for reaction. At 25 ℃, close the Heat cap.

PCR amplification

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

Component	Volume
Fragmented products	23μl
Primer N5	1μl
Primer N7	1μl
2×PCR Mix	25μl

2. Gently blow and mix with a pipette, centrifuge briefly, and collect all components to the bottom of the tube.
3. Place the above PCR tube in the PCR instrument, open the Heat cap, and proceed with the following reaction procedure:

Step	Temperature	Time	
Extension	72℃	3 min	
Pre denaturation	98℃	30 s	
Denaturation	98℃	15 s	8-19cycles
Annealing	60℃	30 s	
Extension	72℃	30 s	
Final extension	72℃	5 min	
Preservation	4℃	Hold	

Selective recovery of library DNA fragments

It is recommended to use the Magnetic Bear DNA Purification Kit (for NGS Size Selection) (DNK2508) for selective DNA fragment recovery. When different sizes of DNA fragments are required, the amount of magnetic beads used varies. The specific amount of magnetic beads used can be found in the attached table (if using other brands of magnetic beads, the optimal amount of magnetic beads needs to be explored by oneself).

Note: Amplification products can also be sorted and purified using gel recovery kits for fragment length. If there are no special requirements for the length distribution range of the library, the amplified products may not undergo selective DNA fragment recovery. Please refer to page 4 of the instruction manual for direct purification of DNA fragments.

1. Magnetic beads should be shaken and mixed before use, and then placed at room temperature for 30 minutes to equilibrate.
2. Transfer the PCR product to a 1.5 ml centrifuge tube, add water to 100μl, add several volume balanced magnetic beads to room temperature, vortex for 5 seconds, and let it stand at room temperature for 5 minutes.
3. Centrifuge briefly and place the centrifuge tube on a magnetic rack to 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 a certain volume of magnetic beads to the supernatant, vortex for 5 seconds, and let it stand at room temperature for 5 minutes.
5. Short centrifugation, place the centrifuge tube on a magnetic rack to separate the magnetic beads from the supernatant until the solution is clear. Carefully aspirate the supernatant and discard it, avoiding contact with the magnetic beads that have already bound to the target DNA.

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

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

7. Repeat step 6 once.
8. Keep the centrifuge tube fixed on the magnetic frame, let it dry at room temperature until the surface of the magnetic beads cracks slightly, and add 20μl of ddH2O for dissolution.

Note: Do not excessively dry the magnetic beads to avoid affecting the elution efficiency.

9. Remove the centrifuge tube from the magnetic frame, vortex oscillate to completely resuspend the magnetic beads, and let it stand at room temperature for 5 minutes. Short centrifugation, place the centrifuge tube on a magnetic rack until the solution clears, and transfer the supernatant solution to a new centrifuge tube.

Appendix: Recommended dosage of magnetic beads for different fragment selection and recycling

DNA library size	Insert Fragment	230 bp	330 bp	430 bp
	(Insert fragment+adapter+prime)	350 bp	450 bp	550 bp
Magnetic bead usage	First choice	65μl	55μl	45μl
	Second choice	50μl	30μl	30μl

Purification of library DNA fragments

It is recommended to use the Magnetic Bear DNA Purification Kit (for NGS Size Selection) (DNK2508).

1. Magnetic beads should be shaken and mixed before use, and then placed at room temperature for 30 minutes to equilibrate.
2. Add 50μl of magnetic beads equilibrated to room temperature to the PCR product, vortex for 5 seconds, and let it stand at room temperature for 5 minutes.
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 3-5 minutes), carefully aspirate the supernatant and discard it, avoiding contact with the magnetic beads that have already bound to the target DNA.

Note: Do not discard the magnetic beads.

4. Continue to keep the centrifuge tube fixed on the magnetic frame, add 200μl of freshly prepared 80% ethanol to the centrifuge tube, let it sit at room temperature for 30 seconds, and carefully discard the supernatant.

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

5. Repeat step 4.
6. Keep the centrifuge tube fixed on the magnetic frame, let it dry at room temperature until the surface of the magnetic beads cracks slightly, and add 25μl of ddH2O for dissolution.

Note: Do not excessively dry the magnetic beads to avoid affecting the elution efficiency.

7. Remove the centrifuge tube from the magnetic frame, vortex oscillate to completely resuspend the magnetic beads, and let it stand at room temperature for 5 minutes. Short centrifugation, place the centrifuge tube on a magnetic rack until the solution clears, and transfer the supernatant solution to a new centrifuge tube.

Library quality inspection

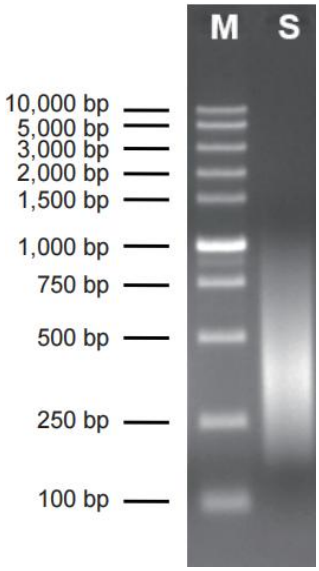
Library concentration measurement

In order to obtain high-quality sequencing results, precise quantification of the DNA library is necessary. Firstly, it is recommended to use Real time PCR method for absolute quantification of the DNA library. In addition, fluorescent dye methods such as Qubit method or fluorescent dye picogrreen method can also be used, and quantitative methods based on absorbance measurement should not be used here. The following approximate formula can ultimately be used to convert the molar concentration of DNA libraries.

Average total length of the library	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

Distribution of Library Fragments

The prepared DNA library can use agarose gel electrophoresis or Agilent 2100 Bioanalyzer to detect the distribution range of fragment length in the DNA library.



M:DNA Marker

S:Library DNA