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

Rapid DNA Construction Kit for Second Generation Sequencing by Transposase Assay (Illumina, 1 ng)

Catalog Number:

N665954 (24 rxns)

N665954 (96 rxns)

Storage condition: -20℃ storage, dry ice transportation.

Products content

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

* This kit is suitable for human genomic DNA library construction, the starting template DNA input amount is 1 ng, our company also has 50 ng and 5 ng of human genomic DNA starting transposase method library construction kit, in order to get a higher quality library, different starting amount of DNA is recommended to use different kits.

Products Introduction

This kit is developed for Illumina's high-throughput sequencing platform and provides the enzyme premix system and reaction buffer for genomic DNA library construction, including all components except PCR primers. Compared with the traditional library construction kits, this kit adopts the new transposase method for library construction, which can complete DNA fragmentation, end repair and junction reaction in one simple enzymatic reaction, significantly reducing the amount of template, reducing the number of experimental steps, and shortening the time of library construction; it adopts the high-fidelity DNA polymerase for library enrichment, and the preference-free PCR amplification can expand the coverage area of the sequence, which can be used for efficient and effective sequencing. The use of high-fidelity DNA polymerase for library enrichment and preference-free PCR amplification broadens the coverage area of the sequence and enables efficient preparation of DNA libraries for Illumina's second-generation sequencing

platform. The kit is suitable for use with 1 ng of starting template DNA, and all reagents in the kit have been subjected to stringent quality control and functional validation to maximize the stability and reproducibility of library construction.

Product Features

- DNA fragmentation and junction ligation in one step.
- Ultra-fidelity amplification minimizes amplification preference.

Provide your own instruments, kits and consumables

1. Magnetic frame: DynaMagTM-2 is recommended.
2. DNA purification and recovery kit: It is recommended to use Kangwei DNA purification and recovery kit by magnetic bead method.
3. Library PCR primer kit: It is recommended to use Kangwei transposase method for second generation sequencing multi-sample primer kit.
4. Anhydrous ethanol, deionized water (pH between 7.0 and 8.0).
5. Reaction tubes: It is recommended to use low adsorption PCR tubes with 1.5 ml centrifuge tubes.

Tip: It is recommended to use a high quality filter tip to prevent contamination of kits and library samples.

Pre-experiment Preparation and Important Notes

1. Avoid repeated freezing and thawing of reagents.
2. PCR products are easily contaminated due to improper operation, resulting in inaccurate results. It is recommended to isolate the PCR reaction system preparation area from the PCR product purification area, and to use special pipettes to clean the experimental areas at regular intervals.
3. Bead purification: the beads should be equilibrated to room temperature before use, all operations on the beads should be carried out at room temperature, 80% ethanol should be dispensed freshly, the beads should be rinsed and dried until the surface is free of liquid reflections and has a frosted appearance, insufficient drying of the beads will cause ethanol residue that will affect the subsequent experiments, and over-drying of the beads will affect the efficiency of DNA recovery.
4. The kit is suitable for human genomic DNA library construction, if the DNA sample is a PCR product, it should be ensured that its length>.

500 bp, since transposases do not work on DNA ends, it is recommended to extend the PCR product by 50-100 bp at each end of the PCR product to avoid low coverage of the ends for sequencing.

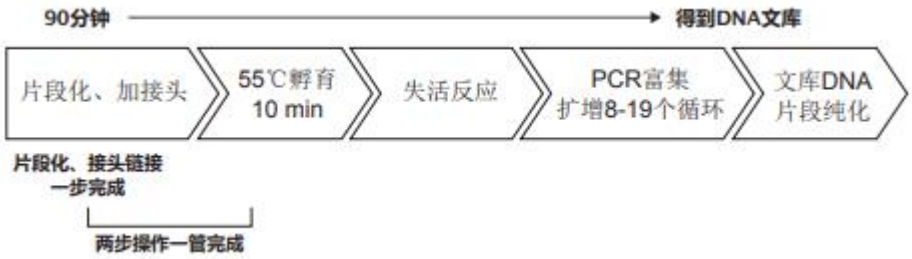
Sample Preparation

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

Sample DNA: dissolved in ultrapure water.

DNA quantification: Too much or too little DNA will affect the quality of the library. It is recommended to use Nano to test the purity of the genomic DNA and then use Qubit to test the concentration of the genome (do not use any absorbance-based assay for template quantification).

Schematic diagram of DNA banking process



procedure

DNA fragmentation, junction reaction

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

individual parts making up a compound	volumetric
1 ng DNA	X μl
TPS V1	1.5 μl
5×FA Reaction Buffer	4 μl
ddH2O	To 20 μl

2. Mix by gently blowing with a pipette and centrifuge briefly so that all components are collected at the bottom of the tube.

3. Place the above PCR tubes in the PCR instrument with the hot cap on and program the reaction as follows:

temp	timing
105°C	thermal cover
55°C	10 min
10°C	Hold

inactivation reaction

After the DNA is fragmented, the enzyme is still in a high active state, so it should be removed from the PCR instrument immediately and terminated by adding the Reaction Termination Buffer, in order to prevent the DNA from being fragmented too much and resulting in smaller library fragments.

1. Add 3 μl of TS Buffer to the PCR tube containing the fragmentation product.

2. Mix by gently blowing with a pipette and centrifuge briefly so that all components are collected at the bottom of the tube.

3. Incubate at room temperature for 5 min, or if the room temperature is too low, place the reaction on a PCR instrument at 25°C with the thermal cover closed.

PCR amplification

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

Agent: Component	volumetric
segmentation product	23 µl
Primer N5	1 µl
Primer N7	1 µl
2×PCR Mix	25 µl

2. Mix by gently blowing with a pipette and centrifuge briefly so that all components are collected at the bottom of the tube.

3. Place the above PCR tubes in the PCR instrument with the thermal cap open, and the reaction program is as follows:

move	temp	timing	
reach	72°C	3 min	
premutability	98°C	30 s	
denaturation	98°C	15 s	8-19 cycles
annealing (metallurgy)	60°C	30 s	8-19 cycles
reach	72°C	30s	8-19 cycles
ultimate extension	72°C	5 min	
save (a file etc) (computing)	4°C	Hold	

Selective recovery of library DNA fragments

It is recommended to use CombiVision Magnetic Beads DNA Purification and Recovery Kit for selective recovery of DNA fragments. When different sizes of DNA fragments are required, the amount of magnetic beads used is different, please refer to the attached table for the specific amount of magnetic beads used.

(If using other brands of magnetic beads, you need to figure out the optimal amount of magnetic beads by yourself).

Note: Amplification products can also be fragment length sorted and purified using the Gum Recovery Kit. If there is no special requirement for library length distribution, amplification products can also be purified directly from DNA fragments without selective recovery of DNA fragments as described on page 4 of the manual.

1. CMPure should be equilibrated at room temperature for 30 min after shaking and mixing before use.

2. Transfer the PCR products to a 1.5 ml centrifuge tube, rehydrate to 100 µl, add several volumes 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 tube on a magnetic rack to separate the beads from the supernatant until the solution is clear, and carefully aspirate the supernatant and transfer it to a new 1.5 ml

centrifuge tube.

Note: Do not discard the top clear.

4. Add several volumes of magnetic beads to the supernatant, vortex and shake for 5 seconds, then let stand at room temperature for 5 minutes.

5. Centrifuge briefly, place the tube on a magnetic rack to separate the beads from the supernatant until the solution is clear, carefully aspirate the supernatant and discard it, avoiding contact with the beads that have bound the target DNA.

Note: Do not discard the beads.

6. Continue to keep the centrifuge tube fixed on a magnetic rack and add 200 μl of freshly prepared 80% ethanol to the tube and allow to stand at room temperature for 30 seconds, carefully discarding the supernatant.

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

7. Repeat step 6 once.

8. Keep the centrifuge tube fixed on a magnetic rack and leave to dry at room temperature until the surface of the beads is slightly cracked, add 20 μl of ddH2O to solubilize.

Note: Do not over-dry the beads as this may affect the elution efficiency.

9. Remove the tube from the magnetic rack, vortex to completely resuspend the beads, and allow to stand at room temperature for 5 minutes. Centrifuge briefly, place the tube on the magnetic rack until the solution is clear, and transfer the supernatant solution to a new tube.

Table: Suggested amount of magnetic beads for different segment selection recovery

DNA library	Insert clip	230 bp	330 bp	430 bp
DNA library size	(insert fragment + adaptor + primer)	350 bp	450 bp	550 bp
Magnetic	First choice	65 μl	55 μl	45 μl
Magnetic	Second choice	50 μl	30 μl	30 μl

Library DNA fragment purification

We recommend the use of the Kangwei Century Magnetic Bead Method DNA Purification and Recovery Kit.

1. CMPure should be equilibrated at room temperature for 30 min after shaking and mixing before use.

2. 50 μl of magnetic beads equilibrated to room temperature were added to the PCR product, vortexed and shaken for 5 seconds, and then left to stand at room temperature for 5 minutes.

3. Centrifuge briefly, place the tube on a magnetic rack to separate the beads from the supernatant solution until the solution is clear (approximately 3-5 minutes), carefully aspirate the supernatant and discard it, avoiding contact with the beads that have bound the target DNA. Note: Do not discard the beads.

4. Continue to keep the centrifuge tube fixed on a magnetic rack and add 200 μl of freshly prepared 80% ethanol to the centrifuge tube and allow to stand at room temperature for 30 seconds, carefully discarding the supernatant.

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

5. Repeat step 4.

6. Keep the centrifuge tube fixed on a magnetic rack and leave to dry at room temperature until the surface of the beads is slightly cracked, add 25 μl of ddH2O to solubilize.

Note: Do not over-dry the beads as this may affect the elution efficiency.

7. Remove the tube from the magnetic rack, vortex to completely resuspend the beads, and allow to stand at room temperature for 5 minutes. Centrifuge briefly, place the tube on the magnetic rack until the solution is clear, and transfer the supernatant solution to a new tube.

Library quality control

Determination of library concentration

In order to obtain high-quality sequencing results, accurate quantification of DNA libraries is required, and the first recommendation is to use Real-time

PCR methods are used for absolute quantification of DNA libraries. Additionally, fluorescent dye methods such as the Qubit method or the fluorescent dye picogreen method can be used; do not use quantification methods based on absorbance measurements here. The following approximate formula can be used to convert the molar concentration of the DNA library.

Average total length	Approximate
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 prepared DNA libraries can be detected by agarose gel electrophoresis or Agilent 2100 Bioanalyzer.

Range of segment length distributions.