Tinzyme Co., Limited

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

Fast NGS Library Kit for Illumina

Product Number: PCK85

Shipping and Storage

-20℃, dry ice transportation.

Components

Component	PCK85	PCK85
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

Description

This kit provides the enzyme premix system and reaction buffer required for DNA library construction, including all components except connectors and PCR primers, for the construction of Illumina second-generation sequencing platform DNA library. Compared with general library building methods, this kit is simple and convenient to operate, greatly shortening the library construction time. In addition, the kit uses high-fidelity DNA polymerase for library enrichment, with no preference for PCR amplification, expanding the coverage area of the sequence, and can efficiently prepare a DNA library for the Illumina second-generation sequencing platform. All reagents provided in the kit have undergone strict quality control and functional verification, ensuring the stability of library construction to the greatest extent possible.

Features

1. End leveling, phosphorylation, and addition of A are completed in one step.
2. No need for purification, add connectors directly.
3. Ultra fidelity amplification minimizes amplification preference.
4. Supports multiple samples, and the resulting library can be used for sequencing platforms such as Illumina GAIIx, HiSacnSQ, HiSeq 2500/2000/1000, and MiSeq sequencing.

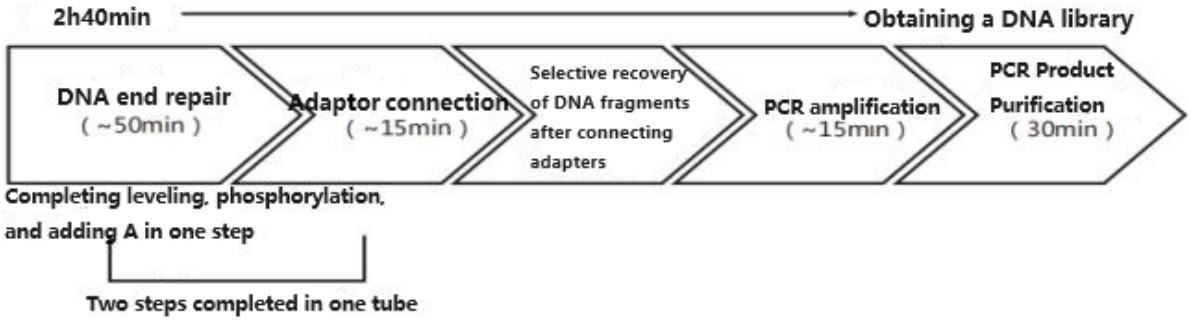
Self provided instruments, reagents, and consumables

1. Magnetic frame
2. DNA purification and recovery kit: It is recommended to use the Magnetic Bear DNA Purification Kit (for NGS Size Selection) (Cat. DNK2508).
3. Sample connector primer kit: It is recommended to use NGS Multiplex Oligos for Illumina (Index Primers Set I) (Cat. PCK86).
4. Anhydrous ethanol, EB (10mM Tris HCl, pH 8.0), deionized water (pH between 7.0 and 8.0).
5. Reaction tube: It is recommended to use a low adsorption PCR tube and a 1.5 ml centrifuge tube.
6. Gun head: It is recommended to use high-quality filter gun heads to prevent contamination of reagent kits and library samples.

Preparation and important precautions before the experiment

1. To avoid repeated freeze-thaw of reagents, it is recommended that you pack and store the remaining reagents after using the reagent kit for the first time.
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.

Schematic diagram of DNA database construction process



Protocol

Sample requirement: 5ng-1μg of interrupted double stranded DNA, soluble in EB (10 mM Tris HCl pH 8.0) or deionized water. DNA purity requirement: OD260/OD280=1.8~2.0.

1. DNA end repair reaction

1.1. Add the following reagents to the 200μl 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	Up to 65 μl

1.2. Use the gun head to gently blow and suck the above solution, mix well, and briefly centrifuge to collect all components to the bottom of the tube.

1.3. Place the above PCR tube in the PCR instrument, open the heat cover, and proceed with the following reaction procedure:

Temperature	Time
12℃	15 min
37℃	15 min
72℃	20 min
Hold on 4℃	

2. Adaptor connection

2.1. Directly add the following reagents to the reaction solution that has completed DNA end repair as mentioned above:

Reagent	Volume
T4 DNA ligase buffer for illumina	14 μl
T4 DNA ligase	2 μl
Adaptor	2.5 μl

At this point, the total volume of the solution in the tube is 83.5μl.

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

2.2. Use the gun head to blow and suck the above reagents evenly, and briefly centrifuge to collect the solution to the bottom of the tube.

2.3. Take a warm bath at 20℃ for 15 minutes.

Note: If using a PCR instrument for this operation, please close the hot cover.

3. Selective Recovery of DNA Fragments

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

Note: Selective recovery of DNA fragments is an optional step. If the initial sample size is less than 50ng, it is not recommended to perform selective recovery of DNA fragments. Please refer to the instructions and directly purify the DNA

fragments. In addition, when constructing DNA libraries of different sizes, the amount of magnetic beads used for selective recovery of DNA fragments varies. The specific amount of magnetic beads can be referred to in Table 1.

In the following steps, the peak length of the recovered DNA fragment can be 320bp (insert fragment length 200bp), and the initial reaction volume is 100μl.

3.1. Vortex oscillate the magnetic beads for 20 seconds to thoroughly mix them into a uniform solution.

3.2. Add 16.5μl of deionized water to the connecting reaction solution to increase the volume of the adapter connecting reaction buffer to 100μl.

Note: If using NEB adapter, only 13.5μl of deionized water needs to be added.

3.3. Transfer the above adapter reaction buffer to a new 1.5ml centrifuge tube.

3.4. Add 70μl of evenly mixed magnetic beads, vortex oscillate for 5 seconds, and let stand at room temperature for 5 minutes.

3.5. Briefly centrifuge, place the centrifuge tube on a magnetic rack to separate the magnetic beads from the supernatant solution until the solution is clear (about 5 minutes), carefully transfer the supernatant solution to a new 1.5ml centrifuge tube, and discard the magnetic beads.

Note: Do not discard the upper clean.

3.6. Add 25μl of evenly mixed magnetic beads to the supernatant, vortex oscillate for 5 seconds, and then let it sit at room temperature for 5 minutes.

3.7. 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 5 minutes), carefully absorb 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.

3.8. Continue to keep the centrifuge tube fixed on the magnetic frame, add 250μl of newly prepared 80% ethanol to the centrifuge tube, and let it sit at room temperature for 30 seconds. After the suspended magnetic beads are fully adsorbed, carefully discard the supernatant.

3.9. Repeat step 8.

3.10. Keep the centrifuge tube fixed on the magnetic frame and let it stand at room temperature for 10 minutes to dry the magnetic beads in air.

3.11. Remove the centrifuge tube from the magnetic holder, add 28μl of 10mM Tris HCl (pH8.0) or deionized water (prepared), vortex oscillate to completely resuspend the magnetic beads in the eluent, and let stand at room temperature for 5 minutes.

3.12. Centrifuge briefly, place the centrifuge tube on a magnetic rack until the solution is clear (about 5 minutes), and transfer 23μl of eluent to a new PCR tube;

Note: Do not transfer magnetic beads, as trace contamination of magnetic beads can affect the normal progress of subsequent PCR reactions.

Another solution: purification of DNA fragments

1. Vortex oscillate the magnetic beads for 20 seconds to thoroughly mix them into a uniform solution.
2. Transfer the adapter connecting reaction solution to a new 1.5ml centrifuge tube.
3. Add magnetic beads that are twice the volume of the sample, vortex oscillate for 5 seconds, and let stand at room temperature for 5 minutes.
4. 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 5 minutes), carefully absorb 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.
5. Continue to keep the centrifuge tube fixed on the magnetic frame, add 250μl of freshly prepared 80% ethanol to the centrifuge tube, and let it sit at room temperature for 30 seconds. After the suspended magnetic beads are fully adsorbed, carefully discard

the supernatant.

6. Repeat step 5.
7. Keep the centrifuge tube fixed on the magnetic frame and let it stand at room temperature for 10 minutes to dry the magnetic beads in air.
8. Remove the centrifuge tube from the magnetic holder, add 28μl of EB (self prepared) or deionized water, vortex oscillate to completely resuspend the magnetic beads in the eluent, and let stand at room temperature for 5 minutes.
9. Centrifuge briefly, place the centrifuge tube on a magnetic rack until the solution clears (approximately 5 minutes), and transfer 23μl of eluent to a new PCR tube.

Table 1: Recommended dosage of magnetic beads for different fragments selection and recycling

DNA library size	Insert	150bp	200bp	250bp	300-400bp	400-500bp	500-700bp
	(insert+adaptor +primer)	270bp	320bp	400bp	400-500bp	500-600bp	600-800bp
Amount of magnetic beads used	First selection	85	70	55	50	45	35
	Second Choice	25	25	20	20	20	15

4. PCR amplification

4.1. Add the following reagents to the PCR tube and mix well

Reagent	Volume
DNA fragments after connecting the adapter	23 μl
2×HiFidelity PCR Mix	25 μl
Univesial primer	1 μl
Index primer	1 μl
Total	50 μl

4.2. PCR reaction conditions

Step	Temperature	Time	Cycles
Pre denaturation	98℃	30 s	
Denaturation	98℃	10 s	
Annealing	65℃	30 s	6-16
Extend	72℃	30 s	
Final extension	72℃	5 min	

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

5. Purification of PCR products

5.1. Vortex oscillate the magnetic beads for 20 seconds to thoroughly mix them into a uniform solution.

5.2. Transfer the PCR reaction solution to a new 1.5ml centrifuge tube.

5.3. Add one volume of magnetic beads to the sample, vortex oscillate for 5 seconds, and let it sit at room temperature for 5 minutes.

5.4. Short centrifugation, place the centrifuge tube on a magnetic rack to separate the magnetic beads from the supernatant solution until the solution clears (approximately 5 minutes). Carefully absorb the supernatant and discard it, avoiding contact with magnetic beads that have already bound to the target DNA.

Note: Do not discard the magnetic beads.

5.5. Continue to keep the centrifuge tube fixed on the magnetic frame, add 250μl of freshly prepared 80% ethanol to the centrifuge tube, and let it sit at room temperature for 30 seconds. After the suspended magnetic beads are fully adsorbed, carefully discard the supernatant.

5.6. Repeat step 5.

5.7. Keep the centrifuge tube fixed on the magnetic frame and let it stand at room temperature for 10 minutes to dry the magnetic beads in air.

5.8. Remove the centrifuge tube from the magnetic holder, add 30μl of EB (self prepared) or deionized water, vortex oscillate

to completely resuspend the magnetic beads in the eluent, and let stand at room temperature for 5 minutes.

5.9. Short centrifugation, place the centrifuge tube on a magnetic rack until the solution is clear (about 5 minutes), transfer the eluent to a new PCR tube of about 25μl, and store the DNA library at -20 ℃.

Library quality inspection

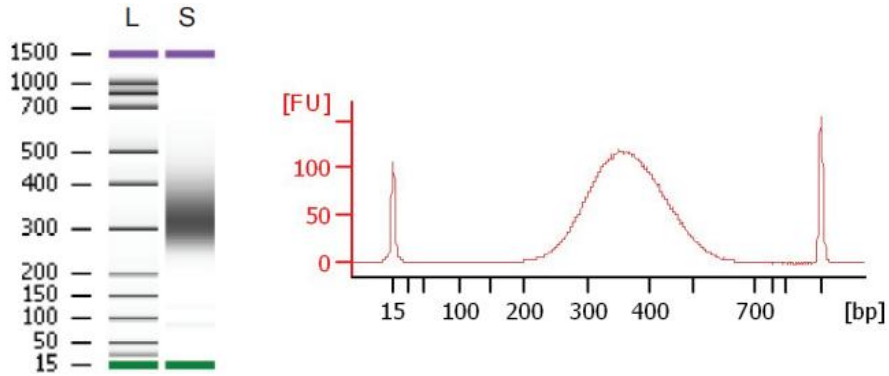
Library concentration

In order to obtain high-quality sequencing results, precise quantification of the DNA library is necessary. Firstly, it is recommended to use the 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	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

Library length distribution

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.



Picture 1: Analysis results of Agilent 2100 Bioanalyzer library

L: DNA Ladder

S: Using 200ng human genomic DNA to construct a library, magnetic beads were selected for recycling and the results were obtained.

Library structure

5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT [Target Sequence] AGATCGGAAGAGCACACGTCTGAACTCCAGTCACNNNNNNATCTCGTATGCCGTCTTCTGCTTG-3'

NNNNNN: index, 6bases