NGS Fast DNA Library Prep Set for Illumina

Project number: N665633

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

Product content

Component	N665633-24T	N665633-96T
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	400μl	2×800μl
2×HiFidelity PCR Mix	600 μl	2 x 1.2ml

Product Introduction

This kit provides the enzyme premixing system and reaction buffer required for DNA library construction, containing all components except junctions and PCR primers, and is used for DNA library construction for illumina second-generation sequencing platform. Compared with general library construction methods, this kit is easy and convenient to operate, which greatly shortens the library construction time. In addition, the kit employs high-fidelity DNA polymerase for library enrichment and preference-free PCR amplification, which expands the coverage area of the sequences and allows efficient preparation of DNA libraries for illumina II sequencing platform. All reagents provided in the kit have been subjected to strict quality control and functional validation to maximize the stability of library construction.

Product Features

-Terminal leveling, phosphorylation, and addition of A in one step.
-No need to purify, just add the connector.
-Ultra-fidelity amplification minimizes amplification preference.
-Supports a wide range of samples and the resulting libraries can be used for sequencing on sequencing platforms such as Illumina GAIIx, HiSacnSQ, HiSeq 2500/2000/1000 and MiSeq sequencing.

Provide your own instruments, reagents and consumables

1. Magnetic frame.
2. DNA purification recovery kit.
3. Sample splice primer kit.
4. Anhydrous ethanol, EB (10 mM Tris-HCl, pH 8.0), deionized water (pH between 7.0 and 8.0).
5. Reaction tubes: low adsorption PCR tubes with 1.ml centrifuge tubes are recommended;
6. 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. To avoid repeated freezing and thawing of reagents, it is recommended that you store the remaining reagents in separate containers after the first use of the kit.

PCR products due to improper operation is very easy to produce pollution, resulting in inaccurate experimental results, it is recommended that the PCR

reaction system preparation area and PCR product purification area isolation, and the use of special pipettes, regular cleaning of the experimental areas.

Operation steps

Sample requirements: 5ng-1μg of interrupted double-stranded DNA, dissolved in EB (10mM Tris-HCl pH8.0) or deionized water.DNA purity requirement: OD_{260} /OD_{280} =1.8~2.0.

DNA end repair reaction

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

Reagent Name	volumetric
10×End Repair Reaction Buffer	6.5μl
End Prep Enzyme Mix	2μl
fragmented DNA	X (5ng-1μg)
RNase-free Water	Up to 65μl

2. Mix the above solutions by blowing gently with the tip of a gun 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 as follows:
15min @ 12℃
15min @ 37℃
20min @ 72℃
Hold on 4℃

Adaptor Connections

The following is the procedure for connecting the adapter:
1. Add the following reagents directly to the above reaction solution that has completed DNA end repair:

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

The total volume of solution in the tube at this point was 83.5 μl.
Note: If the starting sample volume is less than 100ng, please dilute the adaptor 10 times with deionized water to 1.5μM and use it.
2. Blow and mix the above reagents with a lance tip and centrifuge briefly so that the solution collects at the bottom of the tube.
3. 20° C warm bath for 15 minutes.
NOTE: If a pcr instrument is used for this operation, keep the thermal cover closed.

Selective recovery of DNA fragments

Selective recovery of DNA fragments is recommended using the Magnetic Bead Method DNA Purification and Recovery Kit.
Note: DNA fragment selective recovery is an optional step, if the starting sample size is lower than 50ng, it is not recommended to carry out DNA fragment selective recovery, you can refer to page 4 of the instruction manual and carry out the purification of DNA fragments directly. In addition, when constructing DNA libraries of different sizes, the amount of magnetic beads used for

selective recovery of DNA fragments is different, and the specific amount of magnetic beads can be referred to Exhibit 1.

In the following steps, the peak recovered DNA fragment length is optionally 320bp (insert fragment length 200bp) and the reaction starting volume is 100μl.
1. Vortex-shake the CMPure for 20 seconds to thoroughly mix it into a homogeneous solution.
2. Add 16.5μl of deionized water to the ligation reaction solution to bring the volume of adaptor ligation reaction buffer to 100μl.
Note: If using the NEB adaptor, only 13.5 μl of deionized water needs to be added.
3. Transfer the above adaptor reaction buffer to a new 1.5 ml centrifuge tube.
4. Add 70μl of well-mixed CMPure, 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 magnetic beads from the supernatant solution until the solution is clear (takes about 5 minutes), carefully transfer the supernatant solution to a new 1.5 ml centrifuge tube, and discard the magnetic beads.
Note: Do not discard the top clear.
6. Add 25μl of well-mixed CMPure to the supernatant, vortex and shake for 5 seconds and leave at room temperature for 5 minutes.
7. Centrifuge briefly, place the centrifuge tube on a magnetic rack to separate the beads from the supernatant solution until the solution is clear (takes about 5 minutes), carefully aspirate the supernatant and discard it, avoiding contact with the beads that have bound the target DNA during this time.
Note: Do not discard the beads.
8. Continuing to keep the centrifuge tube fixed on the magnetic rack, add 250μl of freshly configured 80% ethanol to the centrifuge tube and leave it at room temperature for 30 seconds, carefully discard the supernatant when the suspended magnetic beads are fully adsorbed.
9. Repeat step 8.
10. Keep the centrifuge tube fixed on the magnetic rack and let it stand at room temperature for 10 minutes to allow the magnetic beads to dry in the air.
11. Remove the centrifuge tube from the magnetic rack, add 28μl of 10mM Tris-HCl (pH8.0) or deionized water (self-provided), vortex and oscillate so that the magnetic beads are completely resuspended in the eluate, and let it stand for 5 minutes at room temperature.
12. Centrifuge briefly, place the tube on a magnetic rack until the solution is clear (takes about 5 minutes), and transfer 23 μl of the eluate to a new PCR tube;
Note: Be sure not to transfer the beads; trace bead contamination can affect the normal conduct of subsequent PCR reactions.

Another option: purification of DNA fragments

1. Vortex-shake the CMPure for 20 seconds to thoroughly mix it into a homogeneous solution.
2. Transfer the adaptor ligation reaction solution to a new 1.5 ml centrifuge tube.
3. Add 1x the sample volume of CMPure, vortex and shake for 5 seconds and let stand at room temperature for 5 minutes.

4. Centrifuge briefly, place the tube on a magnetic rack to separate the magnetic beads from the supernatant solution until the solution is clear (takes about 5 minutes), carefully aspirate the supernatant and discard, avoiding contact with magnetic beads that have bound the target DNA during this time. Note: Do not discard the beads.
5. Continuing to keep the centrifuge tube fixed on a magnetic rack, add 250μl of freshly configured 80% ethanol to the centrifuge tube and leave it at room temperature for 30 seconds and carefully discard the supernatant once the suspended magnetic beads are fully adsorbed.
6. Repeat step 5.
7. Keep the centrifuge tube fixed on a magnetic rack and let it stand at room temperature for 10 minutes to allow the magnetic beads to dry in the air.
8. Remove the centrifuge tube from the magnetic rack, add 28μl of EB (self-provided) or deionized water, vortex and oscillate to completely resuspend the magnetic beads in the eluent, and let stand for 5 minutes at room temperature.
9. Centrifuge briefly, place the tube on a magnetic rack until the solution is clear (takes about 5 minutes), and transfer 23 μl of the eluate to a new PCR tube.

Exhibit 1: Suggested Magnetic Bead Amount for Different Segment Selection Recovery

DNA library size	Insert clip	150bp	200bp	250bp	300-400bp	400-500bp	500-700bp
	(insert fragment + adaptor + primer)	270bp	320bp	400bp	400-500bp	500-600bp	600-800bp
Magnetic bead dosage	First choice	85	70	55	50	45	35
	Second choice	25	25	20	20	20	15

PCR amplification

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

Reagent Name	volumetric
DNA fragments after ligating adaptor	23 μl
2×HiFidelity PCR Mix	25 μl
Univesial primer	1μl
Univesial primer	1μl
total volume	50 μl

2. PCR reaction conditions.

procedure	Temperature	Time	
Prevarication	98℃	30s	
Denaturation	98℃	10s	6-16 cycles
Annealing	65℃	30s	
Extension	72℃	30s	
Final extension	72℃	5min	

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

Purification of PCR products

1. Vortex-shake the CMPure for 20 seconds to thoroughly mix it into a homogeneous solution.
2. The PCR reaction solution was transferred to a new 1.5 ml centrifuge tube.
3. Add 1x sample volume of CMPure, vortex and shake for 5 seconds and let stand at room temperature for 5 minutes.
4. Centrifuge briefly and place the tube on a magnetic rack to separate the magnetic beads from the supernatant solution until the solution is clear (takes about 5 minutes). Carefully aspirate the supernatant and discard, avoiding contact with magnetic beads that have bound target DNA during this time.
Note: Do not discard the beads.
5. Continuing to keep the centrifuge tube fixed on a magnetic rack, add 250μl of freshly configured 80% ethanol to the centrifuge tube and leave it at room temperature for 30 seconds and carefully discard the supernatant once the suspended magnetic beads are fully adsorbed.
6. Repeat step 5.
7. Keep the centrifuge tube fixed on a magnetic rack and let it stand at room temperature for 10 minutes to allow the magnetic beads to dry in the air.
8. Remove the centrifuge tube from the magnetic rack, add 30μl of EB (self-provided) or deionized water, vortex and oscillate to completely resuspend the magnetic beads in the eluent, and let stand for 5 minutes at room temperature.
9. Centrifuge briefly, place the tube on a magnetic rack until the solution is clear (takes about 5 min), transfer the eluate to a new PCR tube of about 25 μl, and store the DNA library at -20° C.

Library quality control

library concentration

In order to obtain high-quality sequencing results, precise quantification of the DNA library is required, and the Real-time PCR method is first recommended for absolute quantification of the DNA library. In addition, 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. Ultimately, the molar concentration of the DNA library can be converted using the following approximate formula.

Average total length of libraries	Approximate conversion formula	Concentration of cluster-responsive DNA libraries
200bp	1ng/μl = 7.5nM	6-12pM
300bp	1ng/μl = 5.0nM	6-12pM
400bp	1ng/μl = 3.8nM	6-12pM
500bp	1ng/μl = 3.0nM	6-12pM

Library length distribution

The prepared DNA libraries can be examined by agarose gel electrophoresis or Agilent 2100 Bioanalyzer to detect the range of fragment length distribution in the DNA libraries.

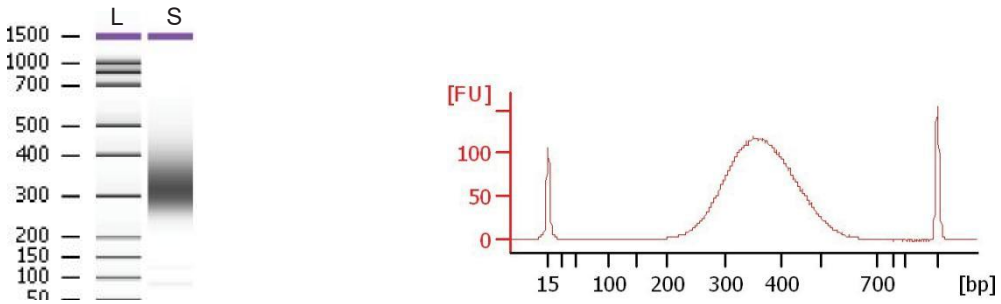

Figure 1: Agilent 2100 Bioanalyzer library analysis results

L: DNA Ladder;

S: library construction using 200 ng of human genomic DNA, results after selective recovery by magnetic beads.

library structure

5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACACGACGCTCTTCC GATCT [Target Sequence] AGATCGGAAGAGAGCACACGTCTGAACTCCAGTCACNNNN NNNATCTCGTATGCCGTCTTCTGCTTG-3''

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