NGS Fast DNA Library Prep Set for Ion Torrent

Project number: N665848

Storage conditions: -20℃ storage, dry ice transportation

Product content

Component	N665848-24T	N665848-96T
10×End Repair Buffer	200 μl	800 μl
End Repair Enzyme Mix	48 μl	192 μl
Ligation and Nick Repair Buffer	400μl	2×800μl
T4 DNA Ligase	48 μl	192 μl
Bst DNA Polymerase	48 μl	192 μl
2×HiFidelity PCR Mix	600 μl	2 x 1.2ml
10×Primer Mix (5μM each)	150 μl	600 μl

Product Introduction

Second Generation Sequencing Rapid DNA Library Construction Kit (Ion torrent) provides the enzyme premix system and reaction buffer required for constructing DNA libraries, including all components except junctions, and the prepared libraries can be used for sequencing on Ion torrent PGM and Ion Proton second generation sequencing platforms. Compared with conventional library construction methods, the kit combines multiple steps and omits multiple purification steps, thus significantly reducing the minimum amount of starting template DNA required and shortening the library construction time. In addition, the kit employs high-fidelity DNA polymerase for library enrichment and preference-free PCR amplification, which expands the region of sequence coverage and enables efficient preparation of DNA libraries for use in the Ion torrent second-generation sequencing platform.

Provide your own instruments, reagents and consumables

1. MAGNETIC FRAME
2. DNA Purification and 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: It is recommended to use PCR tubes with low adsorption and 1.5 ml centrifuge tubes; Tips: It is recommended to use high-quality filtration tips to prevent contamination of kits and library samples.

Pre-experiment Preparation and Important Notes

1. Avoid repeated freezing and thawing of the Buffer in the kit, and it is recommended to store the Buffer in portions for the first use. The enzyme should be put back to -20℃ for storage as soon as possible after use.
2. 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 PCR product purification area isolation, and the use of special pipettes, regular cleaning of the various experimental areas.

DNA end repair reactions:

1. Add the following components to a 200 μl PCR tube, gently mix the above solution with a lance tip, and centrifuge instantaneously to allow all components to collect at the bottom of the tube.

Reagent Name	volume
10×End Repair Buffer	6μl
End Repair Enzyme Mix	2μl
fragmented DNA	X (10ng-1μg)
RNase-Free Water	Up to 60μl

2. The tubes were placed in the PCR instrument with the thermal cap open and the reaction program was as follows:

20min@25℃
10min@70℃
Hold on 4℃

Adaptor connection:

The following is the procedure for connecting with adapter:

1. Add the following reagents directly to the above reaction solution, mix the above reagents with a lance tip and centrifuge briefly so that the solution collects at the bottom of the tube.

Reagent Name	volume
Ligation and Nick Repair Buffer	10μl
T4 DNA Ligase	2μl
Bst DNA Polymerase	2μl
Adaptor A	7μl
Adaptor P1	7μl
RNase-Free Water	12 μl
Total volume	40 μl

Note: It is recommended that the molar ratio of the amount of Adaptor added to the DNA fragments is 10:1-20:1, please refer to the following table for the specific concentration of Adaptor to be used. If the amount of DNA is 10-100ng, the recommended concentration of Adaptor is 1μM (less than 260bp) or 0.5μM (300-400bp).

Insertion DNA quantity/reaction	Different sizes of DNA suggest using Adaptor at different concentrations			
	130bp	260bp	320bp	410bp
1μg	10μM	10μM	5μM	5μM
500ng	5μM	5μM	2.5μM	2.5μM
100ng	1μM	1μM	0.5μM	0.5μM

2. reaction step

15min@25℃
5min@65℃
Hold on 4℃

Selective recovery of Adaptor ligated DNA fragments

Selective recovery of DNA fragments is required for the construction of DNA libraries of different sizes. If the starting sample size is lower than 50ng, selective recovery of DNA fragments is not recommended. Another solution can be referred to for direct purification of DNA fragments. The following procedure uses the Magnetic Bead Method DNA Purification and Recovery Kit, which can

selectively recover DNA fragments in the length range of 310-370bp (read length of 200bp), and the starting volume of the reaction is 100μl.
1. Vortex-shake the CMPure for 20 seconds to thoroughly mix it into a homogeneous solution;
2. Transfer 100 μl of adaptor ligation reaction buffer to a new 1.5 ml centrifuge tube;
3. Add 60μl of well-mixed 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 transfer the supernatant solution to a new 1.5 ml centrifuge tube, and discard the magnetic beads;
Note: Do not discard the top clear.
5. Add 20μl of well-mixed CMPure to the supernatant, vortex and shake for 5 seconds and leave at room temperature for 5 minutes;
6. 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 remove the supernatant and discard it, avoiding contact with the magnetic beads that bind the target DNA during this time;
Note: Do not discard the beads.
7. 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;
8. Repeat step 7; for complete removal of residual liquid, the tube may be centrifuged briefly and the residual liquid removed again.
9. Keep the centrifuge tube fixed on a magnetic rack and let it stand at room temperature for 5 minutes to allow the magnetic beads to dry in the air;
10. Remove the centrifuge tube from the magnetic rack, add 25μl of 10mM Tris-HCl (pH 8.0) or deionized water (self-provided), vortex and oscillate to completely resuspend the magnetic beads in the eluate, and allow to stand at room temperature for 5 minutes;
11. Centrifuge briefly, place the tube on a magnetic rack until the solution is clear (takes about 5 minutes), and transfer 25 μl of the eluate to a new PCR tube;

Another option: full recovery of Adaptor ligated 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 250 μl of freshly configured 80% ethanol to the centrifuge tube and leave it at room

temperature for 30 s. After the suspended magnetic beads are fully adsorbed, carefully discard the supernatant.
6. Repeat step 5. To completely remove the residual liquid, centrifuge the tube briefly and then remove the residual liquid again.
7. Keep the centrifuge tube fixed on a magnetic rack and let it stand at room temperature for 5 minutes to allow the magnetic beads to dry in the air.
8. Remove the centrifuge tube from the magnetic rack, add 25 μl of EB (self-provided) or deionized water, vortex and oscillate to completely resuspend the magnetic beads in the eluent, and allow to 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 25 μl of the eluate to a new PCR tube.

PCR enrichment

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

reagents	volume
DNA fragments after ligating adaptor	20μl
2×HiFidelity PCR Mix	25 μl
10×Primer Mix (5μM each)	5 μl
total volume	50 μl

2. PCR reaction conditions

Step	Temperature	time	
Pre denaturation	98° C	30s	
denaturation	98° C	10s	4-12 cycles
annealing	65° C	30s	
Extend	72° C	30s	
Final extension	72° C	5min	

Note: 4-6 cycles for a sample size of lug, 6-8 cycles for a sample size of 100ng, and 10-12 cycles for a sample size of 10ng.The number of PCR cycles can be optimized according to the experiment.

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 the 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 remove and discard the supernatant, avoiding contact with the magnetic beads that bind the target DNA during this time; note: do not discard the magnetic 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; for complete removal of residual liquid, the tube may be centrifuged briefly and the residual liquid removed again.
7. Keep the centrifuge tube fixed on a magnetic rack and let it stand at room temperature for 5 minutes to allow the magnetic beads to dry in the air.
8. Remove the centrifuge tube from the magnetic rack, add 25μl of EB (self-provided) or deionized water, vortex and oscillate to completely resuspend the magnetic beads in the eluent solution, let it stand at room temperature for 5 minutes, centrifuge briefly, place the tube on the magnetic rack until the solution is clear (takes about 5 minutes), transfer 25μl of elution solution into a new PCR tube, and store the DNA libraries at -20℃.