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NGS Fast DNA Library Prep kit for Ion Torrent

Product Number: PCK239

Shipping and Storage

Store at -20℃ and transport on dry ice.

Components

Component	PCK239 24rxns	PCK239 96rxns
10×End Repair Buffer	200μl	800μl
End Repair Enzyme Mix	48μl	192μl
Ligation and Nick Repair Buffer	400μl	2×800μl
T4 DNA Ligase	48μl	192μl
Bst DNA Polymerase	48μl	192μl
2×HiFidelity PCR Mix	600μl	2×1.2ml
10×Primer Mix (5μM each)	150μl	600μl

Description

The NGS Fast DNA Library Prep kit for Ion Torrent provides the enzyme premix system and reaction buffer required for constructing DNA libraries, including all components except connectors. The prepared library can be used for Ion Torrent PGM Sequencing of IonPoton second-generation sequencing platform. Compared with conventional library building methods, this kit combines multiple steps and omits multiple purification steps, significantly reducing the minimum requirement for starting template DNA and shortening the library construction time. In addition, the reagent kit uses high fidelity DNA polymerase for library enrichment and preference free PCR amplification, expanding the coverage area of the sequence and efficiently preparing DNA libraries for the Ion torrent second-generation sequencing platform.

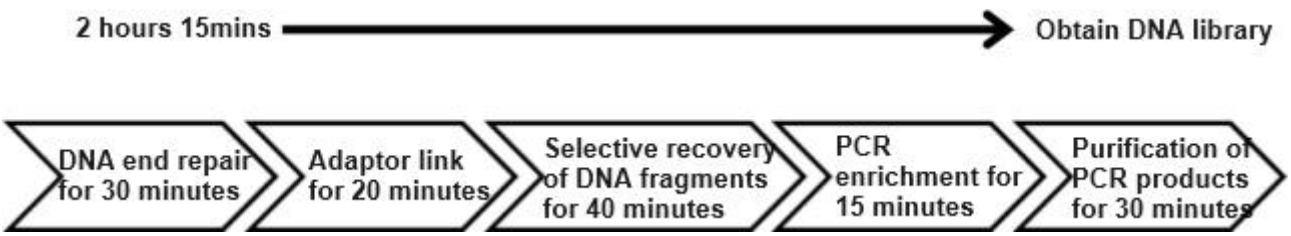
Self provided instruments, reagents, and consumables

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. Sample connector primer kit.
4. Anhydrous ethanol, EB (10 mMTris 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 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. To avoid repeated freezing and thawing of the buffer in the reagent kit, it is recommended to package and store the buffer for the first time of use. Enzymes should be stored at -20℃ as soon as possible after use,
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 library construction process



Starting material: 5ng-1μg of broken double stranded DNA, dissolved in EB (10 mM Tris HCl pH 8.0) or deionized water, DNA purity requirement: OD260/OD 280=1.8-2.0.

DNA end repair reaction

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

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

2. Place the tube into the PCR instrument, open the heat cap, and follow the reaction procedure as follows:

Time	Temperature
20 min	25℃
10 min	70℃
Hold on 4℃	

Adaptor connection

You can use Life, Kapa company's adapter, and the specific connection method can refer to the product user manual of each company. The following are the steps to connect using the adapter we use:

1. Directly add the following reagents to the above reaction solution, mix the above reagents with a gun head, centrifuge briefly, and collect the solution to the bottom of the tube.

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

Note: It is recommended to add an adapter with a molar ratio of 10:1-20:1 to DNA fragments. Please refer to the table below for the specific concentration of the adapter used. If the amount of DNA is 10-100ng, The adapter recommends using a concentration of 1uM (less than 260bp) or 0.5uM (300-400bp).

Insertion DNA	Different sizes of DNA suggest using Adaptor at different concentrations			
quantity/reaction	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 steps

Time	Temperature

15 min	25℃
5 min	65℃
Hold on 4℃	

Selective Recovery of Adaptor Connected DNA Fragments

When constructing DNA libraries of different sizes, selective recovery of DNA fragments is required. If the initial sample size is less than 50ng, selective DNA fragment recovery is not recommended. Another approach can be referred to for direct purification of DNA fragments. It is recommended to use the Magnetic Bear DNA Purification Kit (for NGS Size Selection) (DNK2508) for selective DNA fragment recovery. If you are not using our magnetic beads, you need to explore the optimal amount of magnetic beads on your own. The following steps use the Magnetic Bear DNA Purification Kit (for NGS Size Selection), which can selectively recover DNA fragments with a length range of 310-370bp (read length of 200bp) and a reaction starting volume of 100μl.

1. Vortex oscillate the Magnetic Bead for 20 seconds, thoroughly mixing it into a homogeneous solution;
2. Transfer 100μl adapter to reaction buffer and transfer it to a new 1.5ml centrifuge tube;
3. Add 60μl of evenly mixed Magnetic Bead, vortex for 5 seconds, and let it 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 transfer the supernatant solution to a new 1.5ml centrifuge tube, and discard the magnetic beads;

   Note: Do not discard the supernatant.
5. Add 20μl of evenly mixed Magnetic Bead to the supernatant, vortex for 5 seconds, and let it sit at room temperature for 5 minutes;
6. 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 remove the supernatant and discard it, avoiding contact with the magnetic beads that bind to the target DNA during this process;

   Note: Do not discard the magnetic beads.
7. 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;
8. Repeat step 7; To completely remove residual liquid, the centrifuge tube can be briefly centrifuged before removing the residual liquid again.
9. Keep the centrifuge tube fixed on the magnetic frame and let it stand at room temperature for 5 minutes to dry the magnetic beads in the air;
10. Remove the centrifuge tube from the magnetic rack, add 25μl of 10 mMTris HCl (pH 8.0) or deionized water (prepared), vortex oscillate to completely resuspend the magnetic beads in the eluent, and let it stand at room temperature for 5 minutes;
11. Short centrifugation, place the centrifuge tube on a magnetic rack until the solution clarifies (about 5 minutes), transfer 25μl of eluent to a new PCR tube;

Another solution: full recovery of adapter connected DNA fragments

1. Vortex oscillate the Magnetic Bead for 20 seconds to thoroughly mix it into a homogeneous solution.
2. Transfer the adapter to a new 1.5 ml centrifuge tube, connecting the reaction solution.
3. Add a Magnetic Bead that is twice the volume of the sample, vortex for 5 seconds, and let it 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 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.
5. Continue to keep the centrifuge tube fixed on the magnetic frame, add 250 μ l 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. To completely remove residual liquid, the centrifuge tube can be briefly centrifuged before removing the residual liquid again.
7. Keep the centrifuge tube fixed on the magnetic frame and let it stand at room temperature for 5 minutes to dry the magnetic beads in the air.
8. Remove the centrifuge tube from the magnetic rack, add 25μl EB (self prepared) or deionized water, vortex oscillate to completely resuspend the magnetic beads in the eluent, and let it stand at room temperature for 5 minutes.
9. Centrifuge briefly and place the centrifuge tube on a magnetic rack until the solution clears (about 5 minutes). Transfer 25μl of eluent to a new PCR tube.

PCR enrichment

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

Reagent	Volume
DNA fragments after connecting the adapter	20μl
2×HiFidelity PCR Mix	25μl
10×Primer Mix (5μM each)	5μl
Total	50μl

2. PCR reaction conditions

Step	Temperature	Time	Cycles
Pre denaturation	98°C	30s	
Denaturation	98°C	10s	4-12 cycles
Annealing	65°C	30 s	
Extend	72°C	30 s	
Final extension	72°C	5min	

Note: When the sample size is 1μg, there are 4-6 cycles; when the sample size is 100ng, there are 6-8 cycles; and when the sample size is 10ng, there are 10-12 cycles. The number of PCR cycles can be optimized based on experiments.

Purification of PCR products

1. Vortex oscillate the Magnetic Bead for 20 seconds, thoroughly mixing it into a homogeneous solution;
2. Transfer the PCR reaction solution to a new 1.5 ml centrifuge tube;
3. Add a Magnetic Bead that is twice the volume of the sample, vortex oscillate for 5 seconds, and let it 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 clears (approximately 5 minutes). Carefully remove the supernatant and discard it, avoiding contact with magnetic beads that bind to the target DNA during this process;

   Note: Do not discard the magnetic beads
5. Continue to keep the centrifuge tube fixed on the magnetic frame, add 250 μ l 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; To completely remove residual liquid, the centrifuge tube can be briefly centrifuged before removing the residual liquid again.
7. Keep the centrifuge tube fixed on the magnetic frame and let it stand at room temperature for 5 minutes to dry the magnetic beads in the air.
8. Remove the centrifuge tube from the magnetic rack, add 25μl of EB (self prepared) or deionized water, vortex oscillate to completely resuspend the magnetic beads in the eluent, let it stand at room temperature for 5 minutes, centrifuge briefly, place

the centrifuge tube on the magnetic rack until the solution clears (about 5 minutes), transfer 25μl of eluent to a new PCR tube, The DNA library is stored at -20℃.