Version: 10/2023

NGS Fast DNA Library Prep Set for Ion Torrent

Cat. No. : CW2639S (24 rxns).
CW2639M (96 rxns)

Storage Conditions: Store at -20°C and transport on dry ice.

Components

Component	CW2639S 24rxns	CW2639M 96rxns
10×End Repair Buffer	200 μL	800 μL
End Repair Enzyme Mix	48 μL	192 μL
Ligation and Nick Repair Buffer	400 μL	2×800 μL
T4 DNA Ligase	48 μL	192 μL
Bst DNA Polymerase	48 μL	192 μL
2×HiFidelity PCR Mix	600 μL	2×1.2 mL
10×Primer Mix (5 μM each)	150 μL	600 μL

Introduction

Cowin NGS Fast DNA Library Prep Set (Ion Torrent) provides the enzyme premix system and reaction buffer for DNA library preparation, including all components except the adapters. The prepared library can be used for sequencing on the Ion torrent PGM and Ion Proton second-generation sequencing platforms. Compared with conventional library preparation methods, this kit combines multiple steps and omits multiple purification steps, thus significantly reduces the minimum requirement for starting template DNA and shortens library preparation time. In addition, this kit uses high-fidelity DNA polymerase for library enrichment and there is no perference for PCR amplification, expands the coverage area of the sequence, enables efficient preparation of DNA libraries for the Ion torrent next-generation sequencing platform.

Reagents to Be Supplied by User

1. Magnetic racks: Life Magnetic Racks are recommended.
2. DNA Purification Recovery Kit: Cowin MagBead DNA Purification Kit (CW2508) is recommended.
3. Sample adapter kit.
4. Absolute ethanol, EB (10 mMTris-HCl, pH 8.0), deionized water (pH between 7.0-8.0).
5. Reaction tubes: PCR tubes with low adsorption and 1.5 mL centrifuge tubes are recommended. It is recommended to use a high-quality filter tip to prevent contamination of kit and library samples.

Pre-experiment preparation and important precautions

1. To avoid repeated freeze-thaw of Buffer in the kit, it is recommended to store Buffer in small packs seperately when using it for the first time. The enzyme should be put back to -20°C as soon as possible after use.
2. PCR products can be easily contaminated due to improper handling, leading to inaccurate results. It is recommended to isolate the PCR reaction system preparation area from the PCR product purification area, prepare special pipettes, and clean each experimental area regularly.

2. PCR reaction program

steps	temperature	Time	
Pre-denaturation	98°C	30 s	
denaturation	98°C	10 s	
anneal	65°C	30 s	4-12 cycles
extend	72°C	30 s	
Final extension	72°C	5 min	

Note: Set 4-6 cycles when the sample size is 1ug, set 6-8 cycles when the sample size is 100ng, and set 10-12 cycles when the sample size is 10ng. The number of PCR cycles can be optimized according to the experiment.

Purification of PCR products

1. Vortex the CMPure for 20 sec to thoroughly mix into a homogeneous solution.
2. Transfer the PCR reaction to a new 1.5 mL centrifuge tube.
3. Add CMPure equal to the volume of the sample, vortex for 5 sec and let stand at room temperature for 5 min.
4. Centrifuge briefly. Place the centrifuge tube on a magnetic rack to separate the magnetic beads from the supernatant until the solution is clear (takes about 5 min). Carefully pipette the supernatant and discard, avoiding contact with magnetic beads binding to the target DNA.

   Note: Do not discard the magnetic beads.
5. Keep the centrifuge tube fixed on the magnetic rack, and add 250 μL of freshly configured 80% ethanol to the centrifuge tube. Leave it at room temperature for 30 seconds, and carefully discard the supernatant after the suspended magnetic beads are completely attracted.
6. Repeat step 5. To completely remove the residual liquid, the centrifuge tube can be centrifuged briefly and then the residual liquid can be removed again.
7. Keep the centrifuge tube fixed on the magnetic rack and let stand at room temperature for 5 min to allow the beads to dry in air.
8. Remove the centrifuge tube from the magnetic rack, add 25 μL of EB (self-provided) or deionized water, vortex and shake to completely resuspend the magnetic beads in the eluate and let stand for 5 min at room temperature. Centrifuge briefly. Place the centrifuge tube on the magnetic rack until the solution is clear (takes about 5 min), and transfer 25 μL of the eluate to a new PCR tube. DNA library should be stored at -20°C.

Another option: Complete recovery of adapter- ligated DNA fragments

1. Vortex the CMPure for 20 sec to thoroughly mix into a homogeneous solution.
2. Transfer the adapter ligation reaction mixture to a new 1.5 mL centrifuge tube.
3. Add CMPure equal to the volume of the sample, vortex for 5 sec and let stand at room temperature for 5 min.
4. Centrifuge briefly. Place the centrifuge tube on a magnetic rack to separate the magnetic beads from the supernatant until the solution is clear (takes about 5 min). Carefully pipette the supernatant and discard, avoiding contact with magnetic beads binding to the target DNA.

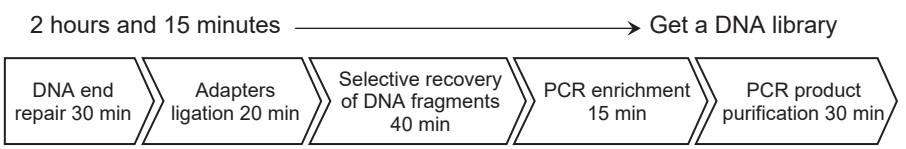
   Note: Do not discard the magnetic beads.
5. Keep the centrifuge tube fixed on the magnetic rack, and add 250 μL of freshly configured 80% ethanol to the centrifuge tube. Leave it at room temperature for 30 seconds, and carefully discard the supernatant after the suspended magnetic beads are completely attracted.
6. Repeat step 5. To completely remove the residual liquid, the centrifuge tube can be centrifuged briefly and then the residual liquid can be removed again.
7. Keep the centrifuge tube fixed on the magnetic rack and then let stand at room temperature for 5 min to dry the magnetic beads in air.
8. Remove the centrifuge tube from the magnetic rack, add 25 μL of EB (self-provided) or deionized water, vortex and shake to completely resuspend the magnetic beads in the eluate and let stand for 5 min at room temperature.
9. Centrifuge briefly. Place the centrifuge tube on the magnetic rack until the solution is clear (takes about 5 min), 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

Reagent name	volume
DNA fragments after ligation of adapter	20 μL
2×HiFidelity PCR Mix	25 μL
10×Primer Mix (5 μM each)	5 μL
Total volume	50 μL

Schematic diagram of the DNA library preparation process



Starting material: 5 ng-1 μg of fragmented double-stranded DNA, dissolved in EB (10 mM Tris-HCl pH 8.0) or deionized water
DNA purity requirements: OD 260/OD 280=1.8-2.0.

DNA end repair reaction:

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

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

2. Place the tube in the thermal cycler, open the hot lid, and set the reaction procedure as follows:
   20 min @ 25°C
   10 min @ 70°C
   Hold on 4°C

Adapter Ligation:

It is recommended to use Cowin's adapter for ligation, or you can choose to use Life or Kapa's adapter, and the specific ligate method can refer to the product instruction manual of each company. The following is how to ligate using Cowin's adapter:

1. Add the following reagents directly to the above reaction solution, mix with the tip and centrifuge briefly to collect all components to the bottom of the tube.

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

Note: It is recommended that the molar ratio of the amount of adapter to the DNA fragment is 10:1-20:1. Please refer to the table below for the specific concentration of the adapter. If the amount of DNA is 10-100 ng, adapter concentration of 1 μM (less than 260 bp) or 0.5 μM (300-400 bp) is recommended.

Insert DNA amount /reaction	Adapter concentrations recommended for DNA of different sizes			
	130 bp	260 bp	320 bp	410 bp
1 μg	10 μM	10 μM	5 μM	5 μM
500 ng	5 μM	5 μM	2.5 μM	2.5 μM
100 ng	1 μM	1 μM	0.5 μM	0.5 μM

2. Reaction steps
   15 min @ 25°C
   5 min @ 65°C
   Hold on 4°C

Selective recovery of adapter- ligated DNA fragments

When preparing DNA libraries of different sizes, selective recovery of DNA fragments is required. If the starting sample size is lower than 50 ng, selective recovery of DNA fragments is not recommended. Another protocol can be used to directly purify DNA fragments. Using Cowin's MagBead DNA Purification Kit (for NGS Size Selection) (CW2508) for selective DNA fragment recovery is recommended. If you use magnetic beads from manufacturers other than Cowin, you need to find the optimal bead dosage by yourself. The following procedure uses Cowin's MagBead DNA Purification Kit. The length of the selectively recovered DNA fragments ranges from 310-370 bp (read length 200 bp) and the initial reaction volume is 100 μL.

1. Vortex the CMPure for 20 sec to thoroughly mix into a homogeneous solution.
2. Transfer 100 μL of the adapter ligation reaction buffer to a new 1.5 mL centrifuge tube.
3. Add 60 μL of well-mixed CMPure, vortex for 5 sec and let stand at room temperature for 5 min.
4. Centrifuge briefly. Place the centrifuge tube on a magnetic rack to separate the magnetic beads from the supernatant until the solution is clear (takes about 5 min). Carefully transfer the supernatant to a new 1.5 mL centrifuge tube, and discard the magnetic beads.

   Note: Do not discard the supernatant.
5. Add 20 μL of well-mixed CMPure to the supernatant, vortex for 5 seconds and then let stand at room temperature for 5 minutes.
6. Centrifuge briefly. Place the centrifuge tube on a magnetic rack to separate the magnetic beads from the supernatant until the solution is clear (takes about 5 min). Carefully pipette the supernatant and discard, avoiding contact with magnetic beads binding to the target DNA.

   Note: Do not discard the magnetic beads.
7. Keep the centrifuge tube fixed on the magnetic rack, and add 250 μL of freshly configured 80% ethanol to the centrifuge tube. Leave it at room temperature for 30 seconds, and carefully discard the supernatant after the suspended magnetic beads are completely attracted.
8. Repeat step 7. To completely remove the residual liquid, the centrifuge tube can be centrifuged briefly and then the residual liquid can be removed again.
9. Keep the centrifuge tube fixed on the magnetic rack and then let stand at room temperature for 5 min to dry the magnetic beads in air.
10. Remove the centrifuge tube from the magnetic rack, add 25 μL of 10 mMTris-HCl (pH 8.0) or deionized water (self-provided), vortex and shake to completely resuspend the magnetic beads in the eluate and let stand for 5 min at room temperature.
11. Centrifuge briefly. Place the centrifuge tube on the magnetic rack until the solution is clear (takes about 5 min), and transfer 25 μL of the eluate to a new PCR tube.