Fast DNA Library Plus Prep Kit

Instruction for Use

【Product Name】

Fast DNA Library Plus Prep Kit

【Cat. No./Spec.】

K004-A/24 rxns; K004-B/96 rxns; sample sack/ 6 rxns

【Product Description】

Aiming at Illumina high-throughput sequencing platform, this kit provides a convenient and universal DNA library construction scheme in one tube. It combines fragmentation, end repair and A-Tailing into one step, greatly shortening the time of library construction and reducing the error caused by tedious steps. After fragmentation and end preparation, the product can be directly ligated with adapter without additional purification, and the subsequent procedure is the same as that of #K001 Fast DNA Library Prep Kit. Complete library quantification can be performed by dsDNA fluorescent dye method (e.g., Thermo Qubit Flex Fluorometer) or absolute quantification PCR after diluting the library to an appropriate concentration.

【Sample Type】

Table 1 Recommended Inputs for Common DNA

Application	Sample Type	Recommended Amount
Whole genome sequencing	High quality complex genomes	50ng-1μg
Target capture sequencing of whole exome	High quality complex genomes	10ng-1μg
Target capture sequencing of whole genome	FFPE DNA	≥50ng
Whole genome sequencing	Microbial genome	1ng-1μg
Whole genome sequencing （PCR-free）	High quality DNA	≥50ng (no size selection) ≥200ng (size selection)

【Storage Condition & Shelf Life】

All reagents should be stored at -20°C. Ligation Buffer is normal for crystals to precipitate at low temperatures, it should be balanced to room temperature before use. The product is valid for 12 months.

【Components】

Component	24 rxns	96 rxns
FEP Buffer	120 μl	480 μl
FEP Enzyme Mix	240 μl	2×480 μl
Fast DNA Ligase	120 μl	2×240 μl
Fast Ligation Buffer	600 μl	4×600 μl
2× HIFI Library PCR Master Mix	600 μl	4×600 μl
Primer Mix*	120 μl	480 μl
Neutralization Buffer	120 μl	480 μl

*FEP Buffer is the fragmentation and end preparation reaction buffer. FEP Enzyme Mix is a mixture of enzyme related to fragmentation and end preparation.

* If there are more than one sample, #K002 and #K003 adapter primer mix is recommended. This kit provides a set of primers with index, the primer sequence is as follows, in [] is 8bp index:

5'-AATGATACGGCGACCACCGAGATCTACAC[TGCTTCCA]ACACTCTTTCCCTACACGACGCTC-3'

5'-CAAGCAGAAGACGGCATACGAGAT[ATCGATCG]GTGACTGGAGTTCAGACGTGTGCTCT-3'

Note：recommended selection beads: #NC1011 GDSPure DNA Selection Magbeads or AMPure XP beads.

【Notes】

1. We offer two types of Universal Adapter primers set (GDS Adapter, #K002 and #K003, purchased separately), but customers can also choose from other manufacturers or synthesize their own Adapter for the Illumina sequencing platform. Too much Adapter will lead to the formation of Adapter dimer, and insufficient Adapter will lead to low library output. Therefore, appropriate Adapter concentration determines the concentration and quality of library. The recommended adapter concentrations for different amounts of DNA input are shown in the following table:

Table 2 Recommended Use Concentrations of Adapter

DNA Input	Recommended Conc. for Adapter	Adapter:Insert Mole Ratio*	GDS Adapter Dilution Degrees
1μg	10μM	10:1	No dilution
500ng	10μM	20:1	No dilution
250ng	10μM	40:1	No dilution
100ng	7.5μM	100:1	3:4
50ng	5μM	200:1	1:2
25ng	2.5μM	200:1	1:4
1ng	1μM	200:1	1:10

* Adapter:Insert mole ratio refers to the ratio of the Adapter molar number from other sources to the Input

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DNA molar number, which can be roughly calculated by referring to the following formula:
Input DNA number (pmol)≈Input DNA mass (ng)/[0.66×Input DNA average length (bp)]
*The quality and concentration of the Adapter greatly affect the output of the library, especially for low input libraries. The Adapter from a high-quality source should be selected and diluted to an appropriate concentration with 0.1×TE before ligation. For immediate use, ensure that each sample addition is a fixed 5 μl, avoid sample addition errors, and try to avoid repeated freeze-thaw.

2. The enzyme used in 2× HIFI Library PCR Master Mix is a B family DNA polymerase, which has 5 '-3' polymerase and 3 '-5' exonuclease activities, but lacks 5 '-3' exonuclease activities. It has high fidelity and homogeneity, and strong sustainable synthesis ability. Strict control of the number of amplification cycles is particularly important for library output. The following table shows the recommended number of amplification cycles corresponding to different amounts of DNA input:

Table 3 Recommended Number of Amplification Cycles Corresponding to Different Sample Inputs

Input DNA	Recommended Number of Amplification Cycles	
	100ng Library	1μg Library
1μg	0	2-5
500ng	0	2-5
250ng	1-3	5-7
100ng	2-4	6-8
50ng	4-6	8-10
25ng	5-7	9-12
10ng	7-9	11-13
5ng	9-11	13-14
2.5ng	10-12	14-16
1ng	11-13	15-17

Note: 1. The above table shows the test results using 150bp standard DNA, which is for reference only.
2. If incomplete connectors are used, a minimum number of cycles (1-3) should be amplified to obtain a complete library.
3. If the quality of the input DNA is poor, or the size selection is carried out during the library construction, the number of amplification cycles should be appropriately increased.

【Standard Library Construction Process】

Fragmentation and End Repair

1. Determine the solvent composition of template DNA, if no EDTA, proceed directly to Step 2; If EDTA is contained, 2.2× magnetic beads should be used for purification, or a corresponding volume of Neutralization Buffer should be added according to the content of EDTA in the following table for Neutralization:

EDTA Conc.	Volume of Neutralization Buffer
1mM	5 μl
0.8mM	4 μl
0.6mM	3 μl
0.5mM	2.5 μl
0.4mM	2 μl
0.2mM	1 μl
0.1mM	0.5 μl
<0.1mM	0 μl

2. Prepare the following reaction in a 200 μl PCR tube:

Reagents	Volume
Input DNA	X μl
FEP Buffer	5 μl
Neutralization Buffer	Y μl
ddH_2O	To 40 μl

3. Add 10 μl FEP Enzyme Mix to the above system, blow evenly, centrifuge briefly, and **immediately put into PCR instrument for the following reaction**:

Temperature	Time
37°C	Refer to Table 4
65°C	15min
4°C	∞

Table 4 Holding Time Required to Obtain Libraries of Different Sizes

Fragment Size	Time
150bp	20-30min
250bp	15-20min
350bp	10-15min
550bp	6-10min

Adapter Ligation

1. Proceed with the ligation reaction as soon as possible after fragmentation and end preparation.
2. Dilute the adapter according to Table 2.
3. Prepare the following reaction system:

Reagents	Volume

above products	50 μl
Fast Ligation Buffer	25 μl
Fast DNA Ligase	5 μl
Adapter X	5 μl
ddH_2O	15 μl
Total	100 μl

4. Vortex gently and spin down briefly to mix well, centrifuge briefly and collect all the liquid to the bottom of the tube.

5. Perform the following reaction in a thermal cycler:

Temperature	Time
20°C	15min
4°C	∞

Recommended Solution for PCR Cleanup/Size Selection (the specific magnetic bead volume should be adjusted according to the actual sample size)

1. Prepare 100 μl ligation products into an appropriate centrifuge tube.
2. Add 100 μl of resuspended DNA selection magnetic beads to the sample. Gently blow with a pipette for 10 times (or vortex for 30 s). Incubate samples for 5 min at room temperature.
3. Place the tube on an appropriate magnetic rack to separate the beads from the supernatant. When the solution is clear, carefully remove and discard the supernatant with a pipette (do not discard beads).
4. Add 200 μl of 80% freshly prepared ethanol to the tube while in the magnetic rack. Incubate at room temperature for 30 s, and then carefully remove and discard the supernatant (do not disturb beads).
5. Repeat Step 4 once for a total of two washes.

Note: Be sure to remove all visible liquid after the second wash.

6. Air dry the beads until the surface of the magnetic beads has no obvious gloss while the tube is on the magnetic rack with the lid open.

Note: Do not overdry the beads, this may result in lower recovery of DNA. When the beads start to crack, they are too dry.

7. Remove the tube from the magnetic rack. Add 22 μl elution buffer (10mM Tris-HCl, pH8.0-8.5) to the tube. Mix well by pipetting up and down at least 10 times or on a vortex mixer for 30 s. Incubate for 3-5 min at room temperature.
8. Place the tube on the magnetic rack. After 5 min (or when the solution is clear), transfer 20 μl supernatant to a new tube. The selection is completed, and the selected DNA can be used for subsequent experiments or stored at -20°C for a long time.

Library Amplification

1. Prepare the following reaction in a PCR tube:

Reagents	Volume
Ligation products after cleanup or size selection	20 μl
2× HIFI Library PCR Master Mix	25 μl
Primer mix	5 μl
Total	50 μl

2. Vortex gently and spin down briefly to mix well, centrifuge briefly and collect all the liquid to the bottom of the tube.

3. Perform the following reaction in a thermal cycler:

Temperature	Time	Cycle Number
95°C	3min	1
98°C	20sec	Select appropriate number of cycles according to Table 3
60°C	15sec	
72°C	30sec	
72°C	5min	1
4°C	∞	-

Recommended Solution for PCR Cleanup/Size Selection (the specific magnetic bead volume should be adjusted according to the actual sample size)

1. Prepare 50 μl ligation products into an appropriate centrifuge tube.
2. Add 45 μl of resuspended DNA selection magnetic beads to the sample. Gently blow with a pipette for 10 times (or vortex for 30 s). Incubate samples for 5 min at room temperature.
3. Place the tube on an appropriate magnetic rack to separate the beads from the supernatant. When the solution is clear, carefully remove and discard the supernatant with a pipette (do not discard beads).
4. Add 200 μl of 80% freshly prepared ethanol to the tube while in the magnetic rack. Incubate at room temperature for 30 s, and then carefully remove and discard the supernatant (do not disturb beads).
5. Repeat Step 4 once for a total of two washes.

Note: Be sure to remove all visible liquid after the second wash.

6. Air dry the beads until the surface of the magnetic beads has no obvious gloss while the tube is on the magnetic rack with the lid open.

Note: Do not overdry the beads, this may result in lower recovery of DNA. When the beads start to crack, they are too dry.

7. Remove the tube from the magnetic rack. Add 22 μl elution buffer (10mM Tris-HCl, pH8.0-8.5) to the tube. Mix well by pipetting up and down at least 10 times or on a vortex mixer for 30 s. Incubate for 3-5 min at room temperature.

8. Place the tube on the magnetic rack. After 5 min (or when the solution is clear), transfer 20 μl supernatant to a new tube. The selection is completed, and the selected DNA can be stored at 2-8°C for 1-2 weeks or stored at -20°C for a long time.

【Appendix】Recommended Scheme for Double-Sided Selection

If double-round selection is required, we provide the following scheme to select the appropriate magnetic bead volume according to the expected library size. The size selection can be performed before end repair or after amplification. Two or more double-round selection will greatly reduce the library yield.

Fill the library volume in the table below to 100 μl. Select the volume of magnetic beads in two rounds according to the expected library size. And carry out the selection operation according to the following instructions.

Table 5 Recommended Amount of Magnetic Beads for Double-Round Selection

Expected Library Size		150bp	200bp	250bp	300bp	400bp	500bp	600bp	700bp
Volume of Beads(μl)	Round 1	100	90	80	70	60	55	50	45
	Round 2	30	20	20	20	20	15	15	15

1. Fill the library volume to 100 μl in a 200μl PCR tube and labeled as A. Add a certain volume of Magnetic beads according to the Table 5 (Round 1) to the tube A. Gently blow with a pipette for 30 s. Incubate samples for 5 min at room temperature.

2. Place the tube A on an appropriate magnetic rack to separate the beads from the supernatant. When the solution is clear, carefully remove the supernatant to a new tube and label it as B. Discard beads.

3. Add a certain volume of Magnetic beads according to the Table 5 (Round 2) to the tube B. Gently blow with a pipette for 30 s. Incubate samples for 5 min at room temperature. Place the tube B on magnetic rack. When the solution is clear, carefully remove and discard the supernatant.

4. Add 200 μl of 80% freshly prepared ethanol to the tube B while in the magnetic rack. Incubate at room temperature for 30 s, and then carefully remove and discard the supernatant (do not disturb beads).

5. Repeat Step 6 once for a total of two washes.

Note: Be sure to remove all visible liquid after the second wash.

6. Air dry the beads until the surface of the magnetic beads has no obvious gloss while the tube B is on the magnetic rack with the lid open.

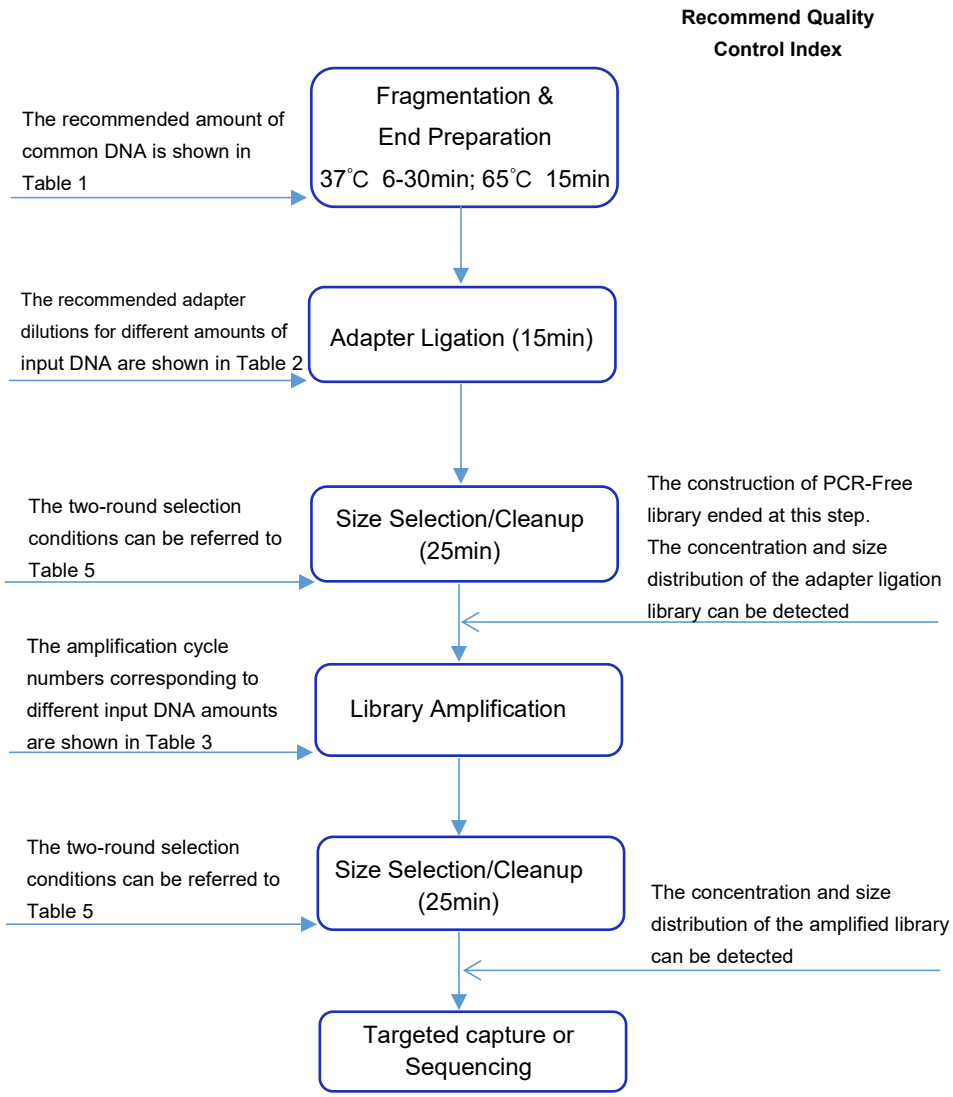
Note: Do not overdry the beads, this may result in lower recovery of DNA. When the beads start to crack, they are too dry.

7. Remove the tube B from the magnetic rack. Add 22 μl elution buffer to the tube. Mix well by pipetting up and down at least 10 times or on a vortex mixer for 30 s. Incubate for 3-5 min at room temperature.

Note: If targeted capture will not be performed, add elution buffer (10mM Tris-HCl, ph 8.0-8.5) for elution. Otherwise, sterilized ultrapure water should be used for elution.

8. Place tube B on the magnetic rack. Transfer 20 μl supernatant to a new tube.

Fig.1 Standard Library Construction Process



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