*Trans*NGS® Fragmentase DNA Library Prep Kit for Illumina®

Please read the datasheet carefully prior to use.

Version No. Version 2.0

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Cat. No. KP231

Storage: at -20°C for one year

Description

The kit is designed to efficiently and quickly prepare DNA library from 1 ng to 1 μg double-stranded DNA for the Illumina high-throughput sequencing platform. The kit completes the DNA fragmentation, end repair and A-tailing in one step. The product can be directly used for adapter ligation without the purification steps. Due to the use of fragmentation enzymes to fragment the genome, libraries of inserts of different sizes can be obtained by adjusting the fragmentation time. And the kit is suitable for library construction of DNA from different sources and inputs.

Features

• Applicable to a wide range of sample types.

• High library conversion rate.

Application

• Whole genome sequencing.

• Target gene sequencing.

• Exon sequencing / other targeted capture sequencing.

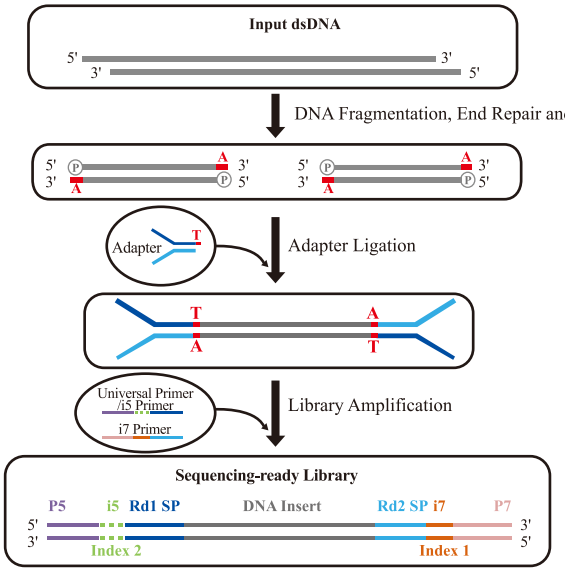
• Metagenomic sequencing.

Kit Contents

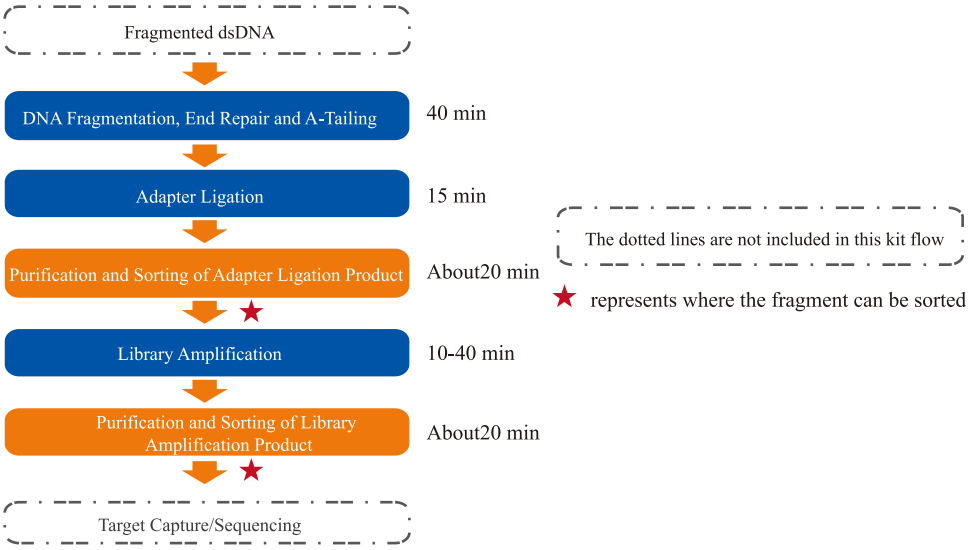
Component	KP231-01 (12 rxns)	KP231-02 (96 rxns)
Fragmentation Buffer (10×)	60 μl	480 μl
Fragmentation Enzyme Mix	120 μl	960 μl
Fragmentation Enhancer	60 μl	480 μl
*Trans*NGS® Adapter for Illumina® (16 μM)	60 μl	480 μl
Adapter Dilution Buffer	600 μl	5 ml
Adapter-ligation Buffer for Illumina	240 μl	4×480 μl
Adapter-ligation Enzyme	60 μl	480 μl
*Trans*NGS® Library Amplification SuperMix (2×)	300 μl	4×600 μl
*Trans*NGS® Universal Primer Mix for Illumina*	60 μl	480 μl
Library Elution Buffer	2 ml	4×4 ml
Nuclease-free Water	1 ml	5 ml

*** The Adapter and Universal Primer in the kit are respectively suitable for short adapter and long adapter for library construction. Choose one according to requirments and do not use them at the same time.**

Principle Chart and Flow Chart



Principle chart of library construction
(i5 position with dotted line indicates some libraries do not have this Index.)



Flow chart of library construction

Library Structure

If *Trans*NGS® Dual Index（or Index/UDI）Primers Kit for Illumina® (Cat. No. KI231/KI241/KI251), or TransNGS® UDI Indexed Adapter Kit for Illumina®（Cat. No. KI341）are used, the library has the following sequences:

5'-AATGATACGGCGACCACCGAGATCTACAC[i5]ACACTCTTTCCCTACACGACGCTCTTCCGATCT-XXXXXXX X-AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC[i7]ATCTCGTATGCCGTCTTCTGCTTG-3'

i5：Index 2，8 bases；

i7：Index 1，8 bases；

-XXXXXXXX-：insert sequence。

Input Sample Recommendations

If input sample is 1 ng -1 μg genomic DNA dissolved in Nuclease-free Water or 10 mM Tris-HCl (pH 8.0), the ratio of sample OD_{260}/OD_{280} should be 1.8-2.0. The DNA concentration should be measured using a fluorescent dye method based on specific recognition of dsDNA, such as Qubit or the fluorescent dye PicoGreen.

If sample is dissolved in TE buffer, please refer to the appendix for instructions on the presence of EDTA in DNA samples.

Protocol

Reagents not included in the kit: Freshly prepared 80% ethanol. *MagicPure*® Size Selection DNA Beads (Cat. No. EC401). Primers containing Index: *Trans*NGS® Dual Index/ UDI primers Kit for Illumina® (Cat. No. KI231/KI241/KI251). Or adapter containing Index: *Trans*NGS® UDI Indexed Adapter Kit for Illumina® (Cat. No. KI341).

This protocol uses 100 ng of human Hela cell genomic DNA as an example. The inserted fragment is about 300 bp. The fragments are sorted before library amplification, and the main peak position is about 450 bp. When the sample input and insert size are different, please refer to the fragmentation time table to adjust the reaction conditions.

1. DNA Fragmentation, End Repair and A-Tailing

(1) Place the sterile PCR tube on ice, add the following components to the tube:

Component	Volume	Volume
dsDNA	≤10 ng	11ng - 1000 ng
Fragmentation Buffer (10×)	5 μl	5 μl
Fragmentation Enhancer*	5 μl	-
Nuclease-free Water	to 40 μl	to 40 μl
Total Volume	40 μl	40 μl

** It is recommended to aliquot the Fragmentation Enhancer when using it for the first time to avoid repeated freezing and thawing during subsequent use.

(2) Mix by pipetting, briefly centrifuge to collect the liquid on the wall of the tube.

(3) Add 10 μl Fragmentation Enzyme Mix to each sample, mix by pipetting, and briefly centrifuge to collect the liquid on the wall of the tube.

(4) Place the tube in thermal cycler and perform the following incubation steps (with heated lid set at ≥80°C).

4°C	1 min	
37°C*	10 min	（350 bp; For other lengths, please refer to the fragmentation time table.）
65°C	30 min	
4°C	Hold	

Insert Size	1-10 ng	100 ng	500 - 1000 ng
150 bp	25-35 min	20-30 min	15-25 min
250 bp	18-21 min	15-20 min	10-15 min
350 bp	12-18 min	10-15 min	5-10 min
550 bp	8-12 min	6-10 min	4-6 min

2. Adapter Ligation

(1) Thaw *Trans*NGS® Adapter for Illumina®/TransNGS® UDI Indexed Adapter for Illumina®(KI341) on ice, prepare the appropriate concentration of Adapter referring to the table below.

Sample Input	Adapter Dilution Factor	Adapter Concentration after Dilution
100 ng ≤ x ≤ 1 ug	No dilution	16 μM
25 ng ≤ x < 100 ng	Diluted 2 times	8 μM
5 ng ≤ x < 25 ng	Diluted 10 times	1.6 μM
1 ng ≤ x < 5 ng	Diluted 25 times	0.6 μM

(2) Place the PCR tube completed in the previous step on ice, and add the following reagents:

Component	Volume
Product of previous step	50 μl
Appropriate concentration of Adapter	5 μl
Adapter-ligation Buffer for Illumina	20 μl
Adapter-ligation Enzyme	5 μl
Nuclease-free Water	20 μl
Total Volume	100 μl

Note: If the number of samples is greater than 1, mix the reaction reagents in one tube first and then aliquot them into each reaction tube. The efficiency of the mixed reaction reagents will decrease as the storage time increases, so try to use freshly prepared reagents.

(3) Mix by pipetting, briefly centrifuge to collect the liquid on the wall of the tube.

(4) Place the tube in thermal cycler and incubate at 20°C for 15 minutes (the lid is not heated). Follow the following purification steps immediately after the ligation reaction.

*** If this kit is used with long adapter, it is recommended that the ligation reaction be performed at 20°C for 15 minutes.**

3. Purification of Adapter Ligation Product

It is recommended to use 0.8×*MagicPure*® Size Selection DNA Beads (Cat. No. EC401) for purification.

*** If this kit is used with long adapter, it is recommended to use 0.6×*MagicPure*® Size Selection DNA Beads (Cat. No. EC401) for purification.**

Note: If the input is low, it is recommended to sort the fragments after library amplification; or directly sorting without purification, that is, add Nuclease-free Water to make up the post-ligation system to 150 μl. For the sorting step, see step 4, and adjust the ratio of magnetic beads according to Appendix Table 1.

The specific procedures for purification using 0.8× magnetic beads are as follows:

(1) Take out the magnetic beads from 2-8°C and stay still for 30 minutes to equilibrate to room temperature.

(2) Vortex the magnetic beads to mix well, add 80 μl magnetic beads (0.8×) to the ligation product of the previous step.

(3) Adjust the volume to ≥80 μl with a pipette, mix by pipetting, and stay still for 5 minutes at room temperature.
Note: Be sure to mix thoroughly, otherwise it will affect the experimental results.

(4) Place the tube on the magnetic stand and stay still at room temperature, make sure the beads settle to the magnet completely. After the solution is clarified (about 5 minutes), discard the supernatant.
Note: Spin down briefly before put on magnetic stand if there is liquid on the wall. Make sure the beads settle to the magnet completely. Be careful not to disturb the beads, otherwise it will affect the final yield.

(5) Add 200 μl of freshly prepared 80% ethanol to the tube on the magnetic stand, do not pipet the beads. Stay still for 30 seconds at room temperature. Discard the supernatant.
Note: Be sure to use freshly prepared ethanol, otherwise it will affect the experimental results.

(6) Repeat step (5) once.

(7) Air dry the beads at room temperature for 2-5 minutes while the tube is on the magnetic stand.
Note: Do not heat to dry, otherwise the final yield will be affected.

(8) Remove the tube from the magnetic stand. Add 30 μl Library Elution Buffer. Mix the beads thoroughly by pipetting or vortexing. Stay still for 2 minutes at room temperature.

(9) Place the tube on the magnetic stand. Incubate at room temperature until the solution is clear (about 2 minutes). Make sure the beads settle to the magnet completely.
Note: Spin down briefly before put on magnetic stand if there is liquid on the wall. Incubation time can be extended to 5 minutes at room temperature. Make sure the beads settle to the magnet completely.

(10)Carefully transfer 28 μl of the eluate to a clean PCR tube for library amplification, or store at -20°C.
Note: DNA at low concentrations is unstable. For sample input is ≤ 50 ng, it is recommended to amplify the library immediately, and it is not recommended to store at -20°C. If sorting is performed according to this protocol, it is recommended to use 30 μl of the eluate; if the sample input is low, sorting should be performed after library amplification, and perform elution step with 20 μl eluate.

4. Sorting of Adapter Ligation Products (Optional)

Note: If the sample input is ≥ 100 ng, it is recommended to sort after ligation; if the sample input is < 100 ng, it is recommended to sort after library amplification to reduce DNA loss. Perform sort according to the following steps and ratios, and the main peak position of the final library is around 450 bp.

(1) Take out the magnetic beads from 2-8°C and stay still for 30 minutes to equilibrate to room temperature.

(2) Add Nuclease-free Water to the product to be sorted to a final volume of 100 μl.

(3) Vortex the magnetic beads to mix well, add 65 μl magnetic beads (0.65×) to the above library products to be sorted. Adjust the volume to ≥80 μl with a pipette, mix by pipetting.

(4) Stay still for 5 minutes at room temperature.

(5) Place the tube on the magnetic stand and stay still at room temperature, make sure the beads settle to the magnet completely. After the solution is clear (about 5 minutes), pipet 160-162 μl supernatant to a new tube. Be careful not to disturb the magnetic beads.

(6) Add 15 μl of magnetic beads (0.15×) to the supernatant of (5), adjust the volume to ≥80 μl with a pipette, mix by pipetting thoroughly.

(7) Stay still for 5 minutes at room temperature.
Note: Insufficient mixing will significantly affect the experimental results.

(8) Place the tube on the magnetic stand and stay still at room temperature, make sure the beads settle to the magnet completely. After the solution is clarified (about 5 minutes), discard the supernatant.
Note: Spin down briefly before put on magnetic stand if there is liquid on the wall. Make sure the beads settle to the magnet completely. Be careful not to disturb the beads, otherwise it will affect the final yield.

(9) Add 200 μl of freshly prepared 80% ethanol to the tube on the magnetic stand, do not pipet the beads. Stay still for 30 seconds at room temperature. Discard the supernatant.
Note: Be sure to use freshly prepared ethanol, otherwise it will affect the experimental results.

(10) Repeat step (9) once.

(11) Air dry the beads at room temperature for 2-5 minutes while the tube is on the magnetic stand.
Note: Do not heat to dry, otherwise the final yield will be affected.

(12) Remove the tube from the magnetic stand. Add 22 µl Library Elution Buffer. Mix the beads thoroughly by pipetting or vortexing. Stay still for 5 minutes at room temperature .

(13) Place the tube on the magnetic stand. Incubate at room temperature until the solution is clear (about 5 minutes). Make sure the beads settle to the magnet completely.

Note: Spin down briefly before put on magnetic stand if there is liquid on the wall. Incubation time can be extended to 5 minutes at room temperature. Make sure the beads settle to the magnet completely.

(14) Carefully transfer 20 µl of the eluate to a clean PCR tube. Product can be stored at -20°C.

5. Library Amplification

(1) Place the sterile PCR tube on ice, add reagents as follows:

Component	For *Trans*NGS® Adapter for Illumina	For *Trans*NGS® UDI Indexed Adapter for Illumina
Purified product of the previous step	20 µl	20 µl
*Trans*NGS® Library Amplification SuperMix (2×)	25 µl	25 µl
*Trans*NGS® Universal Primer for Illumina®	×	5 µl
i5 Primer	2.5 µl	×
i7 Primer	2.5 µl	×
Total volume	50 µl	50 µl

(2)Mix by pipetting, briefly centrifuge to collect the liquid on the wall of the tube.

(3) Perform the following amplification procedure in a thermal cycler.

98℃	3 min	
98℃	10 sec	2-14 cycles*
60℃	30 sec	
72℃	30 sec	
72℃	3 min	
4℃	Hold	

*Taking 100 ng of human Hela cell genomic DNA as an example, amplify for 4 cycles. For different inputs, the library yield is shown in Appendix Table 2.

6. Purification of Library Amplification Products

It is recommended to use 1.0×*MagicPure*® Size Selection DNA Beads (Cat. No. EC401). *Trans*NGS® Library Amplification SuperMix (Cat. No. KA101) will not affect the fragment size of magnetic bead purification. Increase (to obtain libraries with shorter inserts) or decrease the bead ratio (to reduce primer residue) as needed.

The specific steps of purification using 1.0× magnetic beads are as follows:

(1) Take out the magnetic beads from 2-8°C and stay still for 30 minutes to equilibrate to room temperature.

(2) Vortex the magnetic beads to mix well, add 50 µl magnetic beads (1.0×) to the product of the previous step.

(3) Adjust the volume to ≥50 µl with a pipette, pipet thoroughly to mix the magnetic beads-DNA mixture and stay still for 5 minutes at room temperature.

Note: Insufficient mixing will affect the experimental results.

(4) Place the tube on the magnetic stand and incubate at room temperature, make sure the beads settle to the magnet completely. After the solution is clarified (about 5 minutes), discard the supernatant.

Note: Spin down briefly before put on magnetic stand if there is liquid on the wall. Make sure the beads settle to the magnet completely. Be careful not to disturb the beads, otherwise will affect the final yield.

(5) Add 200 µl of freshly prepared 80% ethanol to the tube on the magnetic stand, do not pipet the beads. Incubate at room temperature for 30 seconds. Discard the supernatant.
Note: Be sure to use freshly prepared ethanol, otherwise it will affect the experimental results.
(6) Repeat step (5) once.
(7) Air dry the beads at room temperature for 2-5 minutes while the tube is on the magnetic stand.
Note: Do not heat to dry, otherwise the final yield will be affected.
(8) Remove the tube from the magnetic stand. Add 22 µl Library Elution Buffer. Mix the beads thoroughly by pipetting or vortexing. Incubate at room temperature for 2 minutes.
(9) Place the tube on the magnetic stand. Incubate at room temperature until the solution is clear (about 5 minutes). Make sure the beads settle to the magnet completely.
Note: Spin down briefly before put on magnetic stand if there is liquid on the wall. Incubation time can be extended to 5 minutes at room temperature. Make sure the beads settle to the magnet completely.
(10) Carefully transfer 20 µl of the eluate to a clean 1.5 ml centrifuge tube. The product can be stored at -20°C.

Appendix

A note about EDTA in DNA samples
Experiment preparation
(1) Confirm which solvent the DNA is dissolved in and whether the solvent contains EDTA. According to the EDTA content C_S, determine the added volume V_S, and calculate the EDTA concentration C_F in the fragmented system: $C_F = C_S \times V_S / 50$.
(2) If the CF concentration is less than 0.1 mM, fragmentation is performed directly;
If the C_F concentration is greater than 0.1 mM, it is first recommended to use 2.2× magnetic beads to purify the template DNA and elute with sterile ultrapure water. Or according to the calculated EDTA concentration, add 20 mM $MgCl_2$ for neutralization according to the table below.
If the sample is dissolved in AE buffer, neutralize it with 500 mM $MgCl_2$ in the following volume.

EDTA concentration in fragmentation system	$MgCl_2$ volume
1 mM	5 µl
0.8 mM	4 µl
0.6 mM	3 µl
0.5 mM	2.5 µl
0.4 mM	2 µl
0.2 mM	1 µl
0.1 mM	0.5 µl

(3) The fragmentation system is adjusted as follows:

Component	Volume
dsDNA	1-1000 ng
Fragmentation Buffer	5 µl
$MgCl_2$, 20 mM	Variable
Nuclease-free Water	Variable
Total Volume	40 µl

Table 1 Recommended conditions for size selection by *MagicPure*® Size Selection DNA Beads

Average Library Size (bp)		~320	~470	~670
Insert Size (bp)		~200	~350	~550
Purify adapter ligation products before sorting	1st beads ratio (DNA Beads: DNA)	0.9×	0.68×	0.56×
	2ed beads ratio (DNA Beads: DNA)	0.2×	0.15×	0.12×
Sort directly after ligation	1st beads ratio (DNA Beads: DNA)	0.35×	0.25×	0.15×
	2ed beads ratio (DNA Beads: DNA)	0.15×	0.15×	0.15×
Purify library amplification products before sorting	1st beads ratio (DNA Beads: DNA)	0.85×	0.65×	0.57×
	2ed beads ratio (DNA Beads: DNA)	0.15×	0.15 ×	0.1×

Note: Sorting can be performed at different stages according to the input. That is: Purify adapter ligation products before sorting, or sort directly after ligation; Purify library amplification products before sorting. Fragment sorting only needs to be performed at one of two optional positions. For the accuracy of fragment sorting, it is recommended that the volume of sample before fragment sorting should be exactly 100 μl. The difference in the ratio of magnetic beads at the two optional positions is caused by the different sequence sizes at both ends of the insert. Due to differences in the fragment sizes distribution of different samples, when the same conditions are used for sorting, the fragment sizes of the obtained products will also be different.

Table 2 Recommended cycles for 100 ng/1 μg library output with different inputs

Input	Recommended cycles**	
	100 ng	1 μg
100 pg	15-17	17-19
1 ng	11-13	14-16
10 ng	6-8	8-10
50 ng	4-5	6-8
100 ng	2-4	4-6
250 ng	2**	2-4**
500 ng	2**	2-3**
1 μg	2**	2**

* The recommended cycles in this table are empirical values for library construction using high-quality dsDNA derived from the human genome. If the DNA purity is poor or the DNA damage is severe, increase the cycles appropriately.
** *Trans*NGS® Adapter for Illumina® is a non-full-length adapter. In order to complete the Adapter sequence required for downstream sequencing, at least 2-3 cycles of amplification are needed.

Notes

- This product can be used with a short adapter or a long adapter. The short adapter is used with Index Primer, and the long adapter is used with Universal Primer. Choose one according to requirments.
- To obtain better sequencing data, it is recommended to sort the fragments after adaptor ligation or library amplification.
- Vibrant shaking should be avoided during mixing of the reaction solution to prevent the enzyme activity from decreasing which will result in a decrease in library construction efficiency.

- If magnetic beads are used for purification or fragment sorting, they should be well mixed during elution. The well-mixed magnetic beads should be uniformly suspended and free of visible particles. And no settling after stilling for 5 minutes.
- Samples with a concentration less than 1 ng/μl are recommended to be stored in a low-adsorption centrifuge tube or an ordinary centrifuge tube with 1× *Trans*NGS® Library Dilution Buffer (Cat. No. KB101) to prevent normal centrifuge tube from absorbing nucleic acid samples which will reduce the concentration of effective samples.
- The greater the number of library amplification cycles, the higher the repetition rate of the sequencing data, i.e., the less effective data. Therefore, it is recommended to use less amplification cycles on the basis of satisfying downstream applications.

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