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

Please read the datasheet carefully prior to use.

Version No.: Version 3.0

Cat. No. KP231

Version No. Version 3.0

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, which simplifies the operation process and shortens the library preparation 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

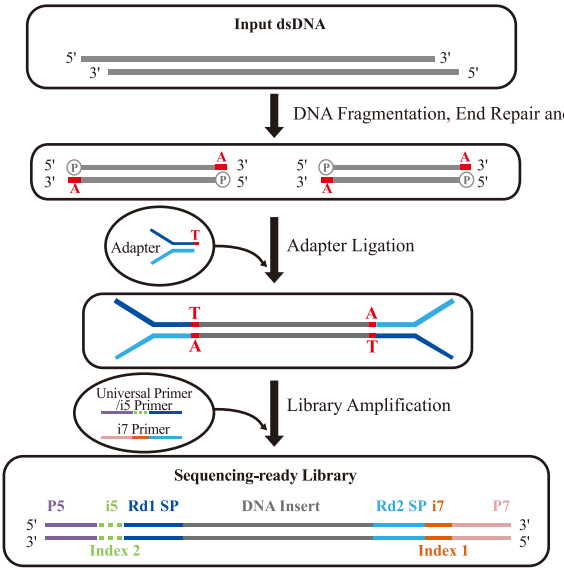
• Whole genome sequencing.
• Target gene sequencing.
• Exon sequencing / other targeted capture sequencing.
• Metagenomic sequencing.

Kit Contents

Component	KP231-01 (12 rxns)	KP231-02 (96 rxns)
10×Fragmentation Buffer II	60 μl	480 μl
Fragmentation Enzyme Mix II	120 μl	960 μl
*Trans*NGS® Adapter for Illumina®* (16 μM)	60 μl	480 μl
Adapter Dilution Buffer	600 μl	5 ml
Adapter-ligation Buffer for Illumina® II	480 μl	4×960 μl
Adapter-ligation Enzyme IV	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

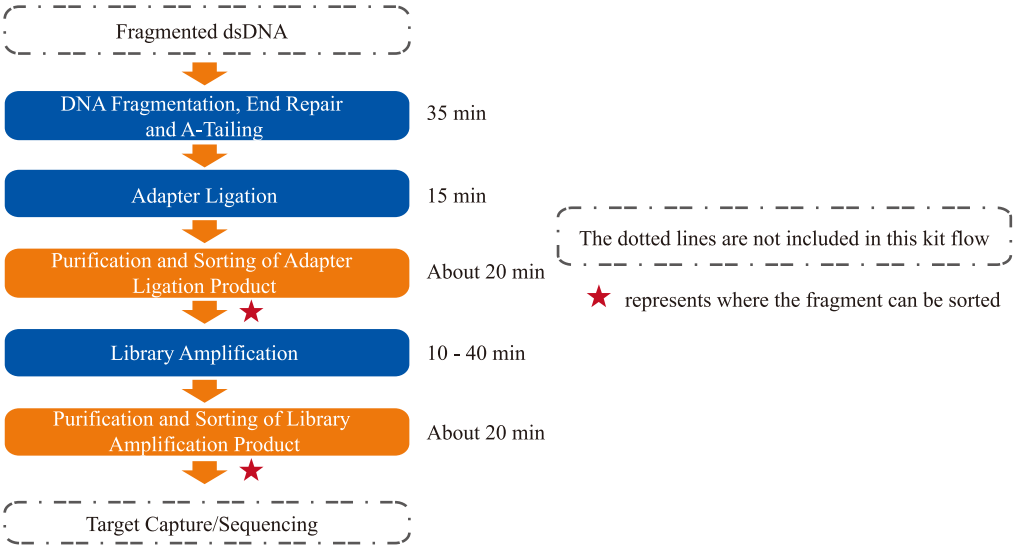
* *Trans*NGS® Adapter for Illumina® and *Trans*NGS® Universal Primer Mix for Illumina® do not contain an index and cannot be used together, and need to be used with an index-containing primer (e.g. KI241/KI251) or an adapter containing an index (e.g. KI341/KI351), respectively.

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® Index/UDI Primers Kit for Illumina® (Cat. No. KI241/KI251), or *Trans*NGS® UDI Indexed Adapter Kit for Illumina® (Cat. No. KI341/KI351) are used, the library has the following sequences:

5'-AATGATACGGCGACCACCGAGATCTACAC[i5]ACACTCTTTCCCTACACGACGCTCTTCCGATCT
-XXXXXXXX-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®.

Library Construction

Reagents not included in the kit: Freshly prepared 80% ethanol. *MagicPure*® Size Selection DNA Beads (Cat. No. EC401). Primers containing Index: *Trans*NGS® Index/UDI Primers Kit for Illumina® (Cat. No. KI241/KI251). Or adapter containing Index: *Trans*NGS® UDI Indexed Adapter Kit for Illumina® (Cat. No. KI341/KI351).

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
dsDNA	Variable
10×Fragmentation Buffer II	5 μl
Fragmentation Enzyme Mix II	10 μl
Nuclease-free Water	to 50 μl
Total Volume	50 μl

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

(3) Place the tube in thermal cycler and perform the following incubation steps (with heated lid set at ≥90°C).

4°C	1 min
37°C	15 min (Insert size 300 bp; For other sizes, please refer to the fragmentation time table.)
72°C	20 min
4°C	Hold

Insert Size	Fragmented Time	Adjustable Range
450 - 550 bp	5 min	3 - 7 min
400 bp	10 min	7 - 12 min
300 bp	15 min	12 - 18 min
200 bp	20 min	18 - 25 min
150 bp	30 min	25 - 35 min

2. Adapter Ligation

(1) Thaw *Trans*NGS® Adapter for Illumina® or long adapter *Trans*NGS® UDI Indexed Adapter for Illumina® (Cat. No. KI341/KI351) on ice. Dilute the Adapter to the appropriate concentration with Adapter Dilution Buffer referring to the table below.

Sample Input	Adapter Dilution Factor	Adapter Concentration after Dilution
100 ng - 1 μg	No dilution	16 μM
25 ng - 100 ng	Diluted 2 times	8 μM
5 ng - 25 ng	Diluted 16 times	1 μM
100 pg - 5 ng	Diluted 160 times	0.1 μM

* Select the Adapter according to the needs. The quality and input of Adapter directly affect the efficiency and quality of library construction. It is recommended to use TransGen Adapter. Increasing input of Adapter can increase the library output at a certain extent, but too high will lead to residues of Adapter and form dimers; insufficient input of Adapter will affect the ligation efficiency and lead to poor library quality.

(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
Adapter: Appropriate concentration of Adapter	5 μl
Adapter-ligation Buffer for Illumina® II	40 μl
Adapter-ligation Enzyme IV	5 μl
Total Volume	100 μl

* Do not mix the Adapter with Adapter-ligation Buffer for Illumina® II and Adapter-ligation Enzyme IV in advance.

(3) Mix by pipetting, briefly spin 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.

3. Purification of Adapter Ligation Product

If this kit is used with *Trans*NGS® Adapter for Illumina®, it is recommended to use 0.8 × *MagicPure*® Size Selection DNA Beads (Cat. No. EC401) for purification. If this kit is used with TransNGS® UDI Indexed Adapter for Illumina® (Cat. No. KI341/KI351), it is recommended to use 0.6 × *MagicPure*® Size Selection DNA Beads for purification.

* If the input is low, it is recommended to sort the fragments after library amplification; or directly sorting without purification. For the sorting step, see step 4. **Sorting of Adapter Ligation Products**, and adjust the ratio of magnetic beads according to Appendix Table 1.

The specific procedures for purification 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 60 μl magnetic beads (0.6×) or 80 μl magnetic beads (0.8×) to the ligation product of the previous step.

(3) Mix well by pipetting, and stay still for 5 minutes at room temperature.

(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.

* 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.

* 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.

* It is advisable to dry until the beads lose luster but are not cracked; 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. 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.

* 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 PCR tube for library amplification, or store at -20°C.

* 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 100 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)

If the sample input is ≥ 50 ng, it is recommended to sort after ligation; if the sample input is < 50 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.

The specific operations 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) 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.

(4) Mix well by pipetting, and 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 10 μl of magnetic beads (0.10×) to the supernatant of (5), mix by pipetting thoroughly, and stay still for 5 minutes at room temperature.

(7) 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.

* 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.

(8) 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.

* Be sure to use freshly prepared ethanol, otherwise it will affect the experimental results.

(9) Repeat step (8) once.

(10) Air dry the beads at room temperature for 2-5 minutes while the tube is on the magnetic stand.

* Do not heat to dry, otherwise the final yield will be affected.

(11) Remove the tube from the magnetic stand. Add 22 μl Library Elution Buffer. Mix the beads thoroughly by pipetting or vortexing. Stay still for 2 minutes at room temperature .

(12) 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.

* 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.

(13) Carefully transfer 20 µl of the eluate to a clean PCR tube for library amplification, or store 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°C	3 min	
98°C	30 sec	2 - 14 cycles*
60°C	30 sec	
72°C	30 sec	
72°C	3 min	
4°C	Hold	

* For different inputs, the recommended number of amplification cycles is shown in Appendix Table 2; if sorting is performed before amplification, it is recommended to increase the number of cycles by 1 - 2.

6. Purification of Library Amplification Products

It is recommended to use 0.9×*MagicPure*® Size Selection DNA Beads (Cat. No. EC401). Increase (to obtain libraries with shorter inserts) or decrease the bead ratio (to reduce primer residue) as needed.

The specific steps of purification 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 45 µl magnetic beads (0.9×) to the product of the previous step.

(3) Pipet thoroughly to mix and stay still for 5 minutes at room temperature.

(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.

* 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.

* 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.

* It is advisable to dry until the beads lose luster but are not cracked; 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. I ncubate at room temperature until the solution is clear (about 2 minutes). Make sure the beads settle to the magnet completely.

* 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

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

Average Library Size (bp)		~340	~440	~690
Insert Size (bp)		~200	~300	~550
Purify adapter ligation products before sorting	1st beads ratio (DNA Beads: DNA)	0.9×	0.65×	0.56×
	2ed beads ratio (DNA Beads: DNA)	0.2×	0.1×	0.1×
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×

* Fragment sorting only needs to be performed at one of two optional positions, either after adapter ligation or after library amplification. 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	13 - 15	15 - 17
1 ng	9 - 11	11 - 13
10 ng	5 - 7	7 - 9
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.

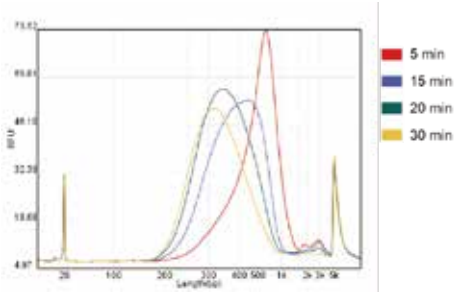


Figure 1 Library peak shapes for different digestion times

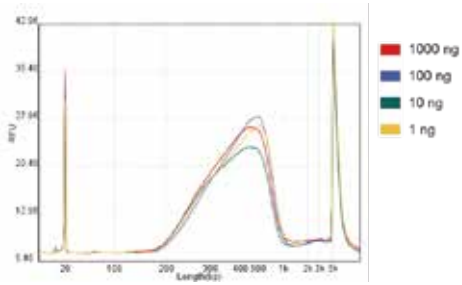


Figure 2 Library peak shapes for different inputs

Notes

- To obtain better sequencing data, it is recommended to sort the fragments after adapter 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.

For research use only, not for clinical diagnosis.

Version number: V3-202404

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