

TransNGS® DNA Library Prep Kit for Illumina®

Cat. No. KP201

Storage: at -20°C for one year

Description

TransNGS® DNA Library Prep Kit for Illumina® contains buffers and enzymes to generate next generation sequencing libraries for Illumina® SE or PE sequencing platform from 1 ng to 1 µg fragmented double-stranded DNA. This kit is suitable for fragmented genome DNA, amplification DNA products, ChIP DNA, or FFPE DNA.

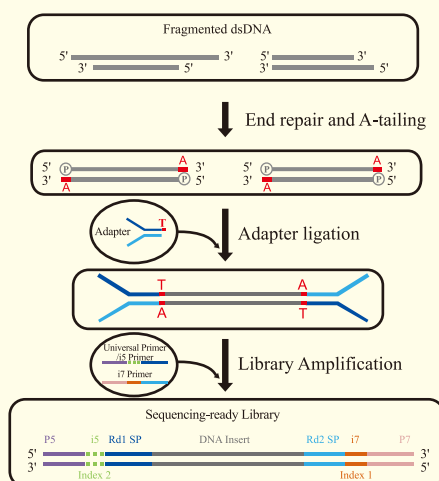
Highlights

- User-friendly and fast with minimal hands-on time;
- High efficiency with minimal amount of input DNA.

Kit Contents

Component	KP201-01 (6 rxns)	KP201-02 (24 rxns)	KP201-03 (96 rxns)
End-repair and A-tailing Reaction Mix	60 µl	240 µl	960 µl
End-repair and A-tailing Enzyme Mix	30 µl	120 µl	480 µl
End-repair and A-tailing Enhancer	18 µl	72 µl	288 µl
TransNGS® Adapter for Illumina® (16 µM)	30 µl	120 µl	480 µl
Adapter Dilution Buffer	300 µl	1.2 ml	5 ml
Adapter-ligation Buffer	180 µl	720 µl	4×720 µl
Adapter-ligation Enzyme	30 µl	120 µl	480 µl
TransNGS® Library Amplification SuperMix (2×)	150 µl	600 µl	4×600 µl
Library Elution Buffer	1 ml	4 ml	4×4 ml
Nuclease-free Water	1 ml	1 ml	5 ml

Flow Chart



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



Library construction flow chart

Library Structure

If *TransNGS*[®] Single Index Primers Kit for Illumina[®] Set 1-3 (Cat. No. KI201-KI221) is used, the resulting library has the following sequences:

5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-XXXXXXXX-AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC[i7]ATCTCGTATGCCGTCTTCTGCTTG-3'

i7: Index 1, 6 bases;

-XXXXXXXX-: insert DNA.

If *TransNGS*[®] Dual Index Primers Kit for Illumina[®] (Cat. No. KI231) is used, the resulting 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 DNA.

Input DNA Recommendations

The input DNA should be 1 ng to 1 µg, fragmented double-stranded DNA. We recommend to purified DNA dissolved in Nuclease-free Water or 10 mM Tris-HCl (pH8.0) with OD₂₆₀/OD₂₈₀ between 1.8~2.0.

Reagents not included in the kit:

1. 80% ethanol (freshly prepared);
2. DNA size selection beads like *MagicPure*[™] Size Selection DNA Beads (TransGen, EC401);
3. Index Primers Kit like *TransNGS*[®] Single Index Primers Kit for Illumina[®] (TransGen, KI201-KI221) or *TransNGS*[®] Dual Index Primers Kit for Illumina[®] (TransGen, KI231).

Library Construction Protocol

- Please refer to table 1 in the appendix for the conditions of size selection using *MagicPure*[™] Size Selection DNA Beads (TransGen, EC401).
- If input DNA is less than or equal to 50 ng, we recommend no size selection after post-ligation purification. If size selection is needed, we recommend to do it after the post-amplification purification.

1. End repair and A-tailing

(1) On ice, mix and add the following components to a clean PCR tube:

Component	Volume	Volume
Fragmented dsDNA	Variable (1 ng-10 ng)	Variable (10 ng-1 µg)
Nuclease-free Water	Variable	Variable
End-repair and A-tailing Reaction Mix	10 µl	10 µl
End-repair and A-tailing Enzyme Mix	5 µl	5 µl
End-repair and A-tailing Enhancer*	3 µl	-
Total volume	60 µl	60 µl

*If the input DNA is less than or equal to 10 ng, add End-repair and A-tail Enhancer to the mixture.

(2) Mix by pipetting followed by a quick spin.

(3) Place the tube in a thermocycler (with lid at 80°C), and run the following program:

28°C	15 min
68°C	15 min
≤10°C	Hold

2. Adapter ligation

(1) Thaw *TransNGS*® Adapter for Illumina® on ice, and dilute it in Adapter Dilution Buffer.

Input	Adapter Dilution Times	Adapter Concentration after Dilution
100 ng < x ≤ 1 µg	No Dilution	16 µM
25 ng < x ≤ 100 ng	2 Fold	8 µM
5 ng < x ≤ 25 ng	10 Fold	1.6 µM
1 ng ≤ x ≤ 5 ng	25 Fold	0.6 µM

(2) Put the PCR tube on ice, add the following components to the tube:

Component	Volume
Adapter in appropriate concentration	5 µl
Adapter-ligation Buffer	30 µl
Adapter-ligation Enzyme	5 µl
Total volume	100 µl

(3) Mix by pipetting following by a quick spin.

(4) Place the tube in a thermocycler (with lid heat off) and incubate at 25°C for 15 minutes. Samples can proceed immediately to the next step, or store at -20°C.

3. Post-ligation Purification

We recommend to use 0.8×*MagicPure*™ Size Selection DNA Beads (TransGen, EC401) for purification. If size selection is needed after the purification, we recommend to elute the ligated DNA into 105 µl of Library Elution Buffer, and transfer 100 µl to a clean tube for size selection.

(1) Equilibrate the beads at room temperature for 30 minutes after take out from 2-8°C.

(2) Vortex beads to resuspend. Add 80 µl (0.8×) of resuspended DNA beads to the ligation reaction mixture.

- (3) Mix by pipetting up and down. Incubate at room temperature for 5 minutes.
Note: Insufficient mixing will affect the results significantly.
- (4) Place the tube on an appropriate magnetic stand to separate beads from the supernatant at room temperature. When the solution is clear, discard the supernatant.
Note: Spin down briefly before put the tube on the magnetic stand if there is liquid on the wall. Make sure that DNA beads settle to the magnet completely and not be aspirated when discard the supernatant. Beads loss will result in reduced yield.
- (5) Keep the tube still on the magnetic stand, add 200 μ l of freshly prepared 80% ethanol and incubate at room temperature for 30 seconds without pipetting up and down. Carefully discard the supernatant.
Note: Use freshly prepared 80% ethanol
- (6) Repeat step 5 once.
- (7) With the tube still on the magnetic stand, air dry beads at room temperature.
Note: Residual ethanol may influence the downstream reaction. Do not over dry or heat the beads, which may result in reduced yield.
- (8) Remove the tube from the magnetic stand and elute DNA with 22 μ l of Elution Buffer. Mix well by pipetting up and down or vortexing. Then incubate at room temperature for 2 minutes.
- (9) Put the tube back to the magnetic stand. Wait for 2 minutes until the solution is clear.
Note: Spin down briefly before put on magnetic stand if there is liquid on the wall. Make sure that DNA beads settle to the magnet completely.
- (10) Transfer 20 μ l of the supernatant to a new PCR tube. Proceed immediately to the next step or store the product at -20°C .
Note: We recommend to do the PCR enrichment immediately following the post purification if the initial DNA input is less than or equal to 50 ng because lower DNA with lower concentration is not stable at this stage.

4. PCR Enrichment of Adaptor Ligated DNA

- (1) On ice, add the following components:

Component	Volume
Purification DNA fragments	20 μ l
<i>TransNGS</i> [®] Library Amplification SuperMix (2 $\times$)	25 μ l
<i>TransNGS</i> [®] Universal Primer for Illumina [®] /i5 Primer*	2.5 μ l
i7 Primer*	2.5 μ l
Total volume	50 μ l

**TransNGS*[®] Single Index Primers Kit for Illumina[®] Set 1-3(*TransGen*, KI201-KI221) provide 48 different i7 Primers, *TransNGS*[®] Dual Index Primers Kit for Illumina[®] (*TransGen*, KI231) provides 8 different i5 Primers and 12 different i7 Primers. Please choose according to your applications.

- (2) Mix by pipetting following by a quick spin.
- (3) PCR cycling conditions:

98 $^{\circ}\text{C}$	3 min	
98 $^{\circ}\text{C}$	30 sec	} 2-14 cycles*
60 $^{\circ}\text{C}$	30 sec	
72 $^{\circ}\text{C}$	30 sec	
72 $^{\circ}\text{C}$	3 min	
$\leq 10^{\circ}\text{C}$	Hold	

*See Table 2 for recommended PCR cycles.

5. Cleanup of PCR Amplification

We recommend to use 1.0×*MagicPure*[™] Size Selection DNA Beads (TransGen, EC401) to do the Cleanup step.

If sizeselection is needed after the purification, we recommend to elute the ligated DNA into 10⁵ µl of Library Elution Buffer, and transfer 100 µl to a clean tube for size selection.

- (1) See post ligation purification section
- (2) Equilibrate the beads at room temperature for 30 minutes after take out from 2-8°C.
- (3) Vortex DNA beads to resuspend. Add 50 µl (1.0×) of resuspended DNA beads to the transposition reaction mixture.
- (4) Mix by pipetting up and down. Incubate at room temperature for 5 minutes.

Note: Insufficient mixing will affect the results significantly.

- (5) Place the tube on an appropriate magnetic stand to separate beads from the supernatant at room temperature. When the solution is clear, discard the supernatant.

Note: Spin down briefly before put on magnetic stand if there is liquid on the wall. Make sure that DNA beads settle to the magnet completely and not be aspirated when discard the supernatant. Beads loss will result in reduced yield.

- (6) Keep the tube still on the magnetic stand, add 200 µl of freshly prepared 80% ethanol and incubate at room temperature for 30 seconds without pipetting up and down. Carefully discard the supernatant.

Note: Not freshly prepared 80% ethanol may result in reduced yield.

- (7) Repeat previous step for a total of 2 washes.

- (8) With the tube still on the magnetic stand, air dry beads at room temperature.

Note: Residual ethanol may influence the downstream reaction. Do not over dry or heat the beads, which may result in reduced yield.

- (9) Remove the tube from the magnetic stand and elute DNA with 22 µl EB. Mix well by pipetting up and down or vortex. Then incubate at room temperature for 2 minutes.

- (10) Put the tube back to the magnetic stand. Wait for 2 minutes until the solution is clear.

Note: Spin down briefly before put on magnetic stand if there is liquid on the wall. Make sure that DNA beads settle to the magnet completely.

- (11) Transfer 20 µl of the supernatant to a new tube. The DNA product can be stored at -20°C.

Appendix

TransNGS[®] Adapter for Illumina[®]:

5'ACACTCTTTCCCTACACGACGCTCTTCCGATC-s-T

3' CTGACCTCAAGTCTGCACACGAGAAGGCTAG-(P)

-s- indicates phosphorothioate bond, -(P) indicates phosphorylation.

Table 1 Recommended conditions for size selection by *MagicPure*[™] Size Selection DNA Beads

Average Size of Selected Library (bp)		~320	~470	~670
Insert Size of Selected Library (bp)		~200	~350	~550
Size selection prior to end repair and A-tailing	1st beads ratio (DNA Beads: DNA)	1.0×	0.7×	0.55×
	2ed beads ratio (DNA Beads: DNA)	0.25×	0.2×	0.15×
Size selection after post-ligation purification	1st beads ratio (DNA Beads: DNA)	0.9×	0.68×	0.56×
	2ed beads ratio (DNA Beads: DNA)	0.2×	0.15×	0.12×
Size selection after post-amplification purification	1st beads ratio (DNA Beads: DNA)	0.85×	0.65×	0.57×
	2ed beads ratio (DNA Beads: DNA)	0.15×	0.1×	0.1×

Note: Size selection only needed to be carried out once. For the accuracy of size selection, we recommend to use 100 µl of the DNA sample. Dual to the difference size flanked to the insert DNA, the ratios used in the three points are different. The average size of selected libraries may vary slightly dual to the different size distribution of the libraries.

Table 2. Recommended cycle numbers to generate 100 ng or 1 µg of library from

Input	Number of cycles required to generate*	
	100 ng of library	1 µg of library
1 µg	--**	2-3**
500 ng	--**	3-4
100 ng	2-3**	5-6
50 ng	2-3	6-7
10 ng	6-7	9-10
5 ng	7-8	10-11
1 ng	10-11	13-14

*The number of cycles is generated by input fragmented dsDNA with an average size of 300 bp without size selection.

**A minimum of 2-3 amplification cycles is needed to add the complete adapter sequences for sequencing on Illumina sequencing platform.

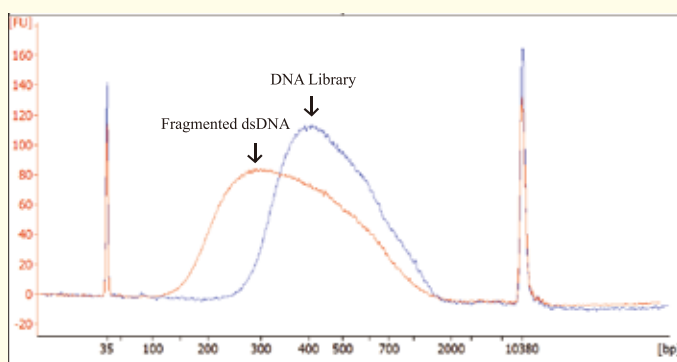


Figure 1 DNA size distribution before and after library construction

Red line-Human gDNA fragmented by sonication;

Blue line-Library constructed without size selection.

Notes

- 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 standing for 2 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× *TransNGS*[®] 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.
- In order to obtain higher production of the library (e.g. for molecular diagnosis), 100 µl amplification system is recommended for DNA amplification (see table below).

Component	Volume
The purified product of the previous step	20 μ l
<i>TransNGS</i> [®] Library Amplification SuperMix (2 $\times$)	50 μ l
<i>TransNGS</i> [®] Universal Primer for Illumina [®] /i5 Primer*	2.5 μ l
i7 Primer*	2.5 μ l
Nuclease-free Water	25 μ l
Total volume	100 μ l

Note: When purified after amplification, use 100 μ l *MagicPure*[™] Size Selection DNA Beads (1.0 $\times$) (Cat No.: EC401).

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