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

Please read the datasheet carefully prior to use.

Version No.: Version 2.0

Website www.transgenbiotech.com
E-mail info@transgenbiotech.com
Customer Service +86-400-898-0321
Phone +86-10-57815027

Catalog No. KP201
Version No. Version 2.0
Storage: at -20℃ for one year

Description

*Trans*NGS® DNA Library Prep Kit for Illumina® is developed for the Illumina high-throughput sequencing platform, designed to efficiently and rapidly construct sequencing libraries from 1 ng to 1 μg of fragmented dsDNA. This reagent kit is suitable for various types of samples, including sonication fragmented genomic DNA, enzymatic digests, nucleic acid amplification products, chromatin immunoprecipitation DNA (ChIP DNA), and DNA from paraffin-embedded tissue sections (FFPE DNA), among others. It can be used in conjunction with exon capture or other target capture reagents (including Agilent SureSelect® and Roche NimbleGen SeqCap® EZ series) for exon sequencing or targeted sequencing.

Features

• Wide range of applicable sample types.
• High library conversion rate.

Applications

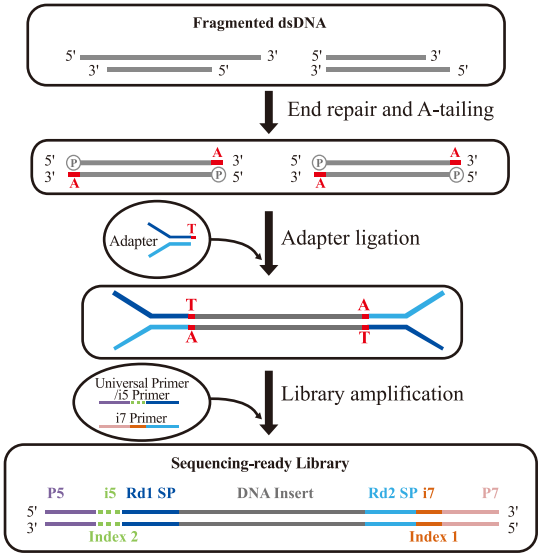
• Whole genome sequencing.
• Targeted gene sequencing.
• Exon sequencing / other target capture sequencing.
• Metagenomics sequencing.
• Immunoprecipitation sequencing.

Kit Contents

Component	KP201-11-V2 (12 rxns)	KP201-03-V2 (96 rxns)
End-repair and A-tailing Reaction Mix	120 μl	960 μl
End-repair and A-tailing Enzyme Mix	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 III	360 μl	4×720 μl
Adapter-ligation Enzyme II	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

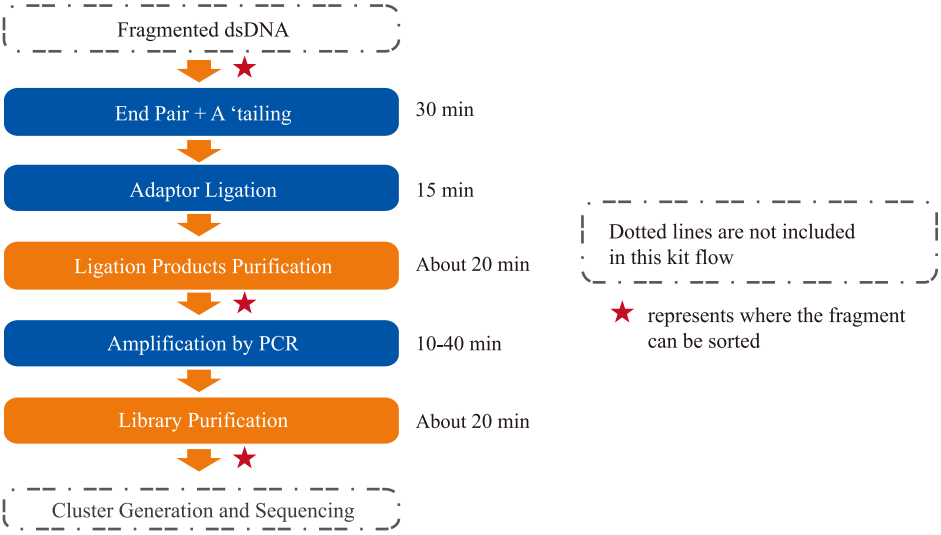
* The provided adapter and universal primer in this reagent kit are respectively compatible with short adapters and index-containing long adapters for library construction. Please choose one according to your needs and do not use them simultaneously.

Principle Schematic of Library Construction



Principle Schematic of Library Construction

(The dashed line at the i5 position indicates that some libraries do not have this index.)



Library construction workflow

Library Structure

The library structure obtained using *Trans*NGS® Index/UDI Primers Kit for Illumina® (Catalog No: KI241/KI251), or *Trans*NGS® UDI Indexed Adapter Kit for Illumina® (Catalog No: KI341), is as follows:

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

i5: Index 2, 8 bases;

i7: Index 1, 8 bases;

-XXXXXXXX-: Inserted sequence

Initial Sample Requirements

The starting sample should be 1 ng-1 μg of fragmented dsDNA dissolved in Nuclease-free Water or 10 mM Tris-HCl (pH 8.0). High purity samples with OD260/OD280 ratios between 1.8-2.0 are recommended. DNA concentration should be determined using fluorescence-based methods specific for dsDNA recognition, such as Qubit or fluorescent dyes like PicoGreen. The starting sample volume refers to the amount of fragmented dsDNA prepared for end repair and A-tailing. If there is any loss during fragmentation or if further processing steps such as purification or size selection are performed after fragmentation, it's advised to accurately quantify the DNA again before library construction begins.

Library Construction

For self-prepared reagents: freshly prepared 80% ethanol, *MagicPure*® Size Selection DNA Beads (Catalog No: EC401), primers containing indexes, *Trans*NGS® Index/UDI Primers Kit for Illumina® (Catalog No: KI241/KI251), or indexed adapters, *Trans*NGS® UDI Indexed Adapter Kit for Illumina® (Catalog No: KI341).

- The following steps do not include fragment selection. Fragment selection can be performed before end repair, after adapter ligation, or after library amplification, and it only needs to be performed once. It is recommended to perform fragment selection after adapter ligation or library amplification.
- Refer to Appendix Table 1 for recommended conditions for fragment selection using *MagicPure*® Size Selection DNA Beads (Catalog No: EC401).
- If the starting sample amount is ≤50 ng, fragment selection after adapter ligation is not recommended. It can be performed after library amplification.

1. End Repair and A-tailing

(1) Place sterile PCR tubes on ice and add the following components.

Component	Volume
Fragmented dsDNA	Variable
Nuclease-free Water	Variable
End-repair and A-tailing Reaction Mix	10 μl
End-repair and A-tailing Enzyme Mix	5 μl
Total volume	60 μl

Note: If there is more than one sample, you can first mix the reaction reagents in one tube and then distribute them into each reaction tube. The efficiency of the mixed reaction reagents may decrease with prolonged standing time, so it's best to prepare and use them immediately.

(2) Pipette up and down to mix thoroughly, then spin down any liquid from the tube walls.

(3) Place the reaction tubes in a PCR instrument and perform the following incubation steps (lid temperature ≥ 80°C).

28°C 15 min

68°C 15 min

≤10°C Hold

2. Adapter Ligation

(1) Thaw *Trans*NGS® Adapter for Illumina®/*Trans*NGS® UDI Indexed Adapter for Illumina® (KI341) on ice, and prepare adapters of appropriate concentrations according to the table below.

Initial Sample	Adapter Dilution Factor	Adapter Concentration after Dilution
100 ng ≤ x ≤1 μg	No dilution	16 μM
25 ng ≤ x ＜ 100 ng	2-fold dilution	8 μM
5 ng ≤ x ＜ 25 ng	10-fold dilution	1.6 μM
1 ng ≤ x ＜ 5 ng	25-fold dilution	0.6 μM

(2) Place the PCR tubes from the previous step on ice, and then add the following sequentially.

Component	Volume
Appropriate Concentration of Adapter	5 μl
Adapter-ligation Buffer III	30 μl
Adapter-ligation Enzyme II	5 μl
Total volume	100 μl

Note: Do not pre-mix the adapters with buffer and enzyme, as it may lead to the generation of numerous of adapter dimers.

(3) Pipette up and down to mix thoroughly. flick the tubes to collect any liquid from the walls, then centrifuge briefly.

(4) Place the reaction tubes in a PCR instrument and incubate at 20°C for 15 minutes (do not heat the lid). The ligation product can be immediately purified or stored at -20°C.

3. Size selection of library amplification product

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

*** When using the reagent kit with long adapters, it is recommended to use 0.4× *MagicPure*® Size Selection DNA beads for product purification.**

Note: If the sample input is low, fragment selection can be performed after library amplification, or proceed without purification. In this case, add Nuclease-free Water to bring the ligated system to a total volume of 150 μl. For fragment selection steps, refer to step 4 (Adapter Ligation Product Selection), and adjust the magnetic bead ratio according to Appendix Table 1.

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

(1) Pre-warm the DNA beads for 30mins from 2-8°C to room temperature.

(2) Vortex the magnetic beads to ensure thorough mixing. Add 80 μl DNA beads (0.8×) to the product of Adapter ligation.

(3) Mix the sample thoroughly by pipetting up and down, then let it stand at room temperature for 5 minutes.
Note: Insufficient mixing will lead to inconsistent size selection results.

(4) Place the reaction tube on a Magnetic Stand. Let it stand at room temperature for approximately 5 minutes until the solution becomes clear, allowing the magnetic beads to fully adhere to the tube wall close to the magnetic stand.
Note: If there is liquid on the tube wall, briefly centrifuge and then place it on the magnetic stand. Please make sure that all magnetic beads are fully attached to the tube wall.
(5) Keep the 1.5ml tube on the Magnetic Stand, add 200 µl of freshly prepared 80% ethanol into the tube and keep it at room temperature for 30 seconds without mixing. Then discard the supernatant.
Note: 80% ethanol must be freshly prepared, otherwise it may affect the experimental results.
(6) Repeat Step (5) once.
(7) Keep the 1.5ml tube on the Magnetic Stand with the lid open, dry the beads at room temperature.
Note: Do not heat-dry the tube, as it may affect the final yield.
(8) Remove the 1.5ml tube from the Magnetic Stand, add 23 µl Library Elution Buffer. Mix by pipetting up and down or by vortex. Incubate at room temperature for 2 minutes.
(9) Place the 1.5ml tube on the Magnetic Stand and wait for 2 minutes until the solution is clear, ensuring that all magnetic beads are fully attached to the tube walls.
Note: If there is liquid on the tube wall, briefly centrifuge and then place it on the magnetic stand. The room temperature stand time can be extended to 5 minutes, ensuring all magnetic beads are fully attached to the tube wall.
(10) Carefully transfer 20 µl of the supernatant into a fresh PCR tube. Proceed to library amplification step or store at -20°C.
Note: Low-concentration DNA is not stable. If the starting sample amount is ≤50 ng, it is recommended to proceed with library amplification immediately rather than store at -20°C. If you choose to continue with the selection step as per these instructions, it is suggested to use a 100 µl elution system. If the sample input is low and selection is needed after library amplification, then elution should be performed using a 20 µl elution system.

4. Adapter Ligation Product Selection (Optional)

Note: If the sample input is ≥50 ng, it is advisable to perform selection after ligation. If the sample input is <50 ng, it is recommended to perform selection after library amplification to minimize DNA loss.

Performing fragment selection according to the following steps and ratios, the final library peak position will be around 450 bp:

(1) Equilibrate the DNA beads for 30mins from 2-8°C to room temperature.
(2) Add Nuclease-free Water to the sample until the final volume is 100 µl
(3) Vortex the magnetic beads to ensure thorough mixing. Add 65 µl DNA beads (0.65×) to the sample for fragment selection.
(4) Adjust the pipette gun to ≥ 80 µl and mix the sample thoroughly by pipetting up and down, then let it stand at room temperature for 5 minutes.
(5) Place the reaction tube on a Magnetic Stand. Let it stand at room temperature for approximately 5 minutes until the solution becomes clear, allowing the magnetic beads to fully adhere to the tube wall close to the magnetic stand. Pipette up 160-162 µl supernatant to a new tube. Avoid pipetting the beads.

(6) Add 15 μl Beads (0.15×) to the supernatant of step 5, adjust the pipette gun to ≥ 80 μl to mix the sample thoroughly by pipetting up and down.

(7) Let it stand at room temperature for 5 minutes.

Note: Insufficient mixing will lead to inconsistent size selection results.

(8) Place the reaction tube on a Magnetic Stand. Let it stand at room temperature for approximately 5 minutes until the solution becomes clear, allowing the magnetic beads to fully adhere to the tube wall close to the magnetic stand. Discard the supernatant.

Note: If there is liquid on the tube wall, briefly centrifuge and then place it on the magnetic stand. Please make sure that all magnetic beads are fully attached to the tube wall. Do not pipette up the beads, as it may affect the final yield.

(9) Keep the reaction tube on the Magnetic Stand, add 200 μl of freshly prepared 80% ethanol into the tube and keep it at room temperature for 30 seconds without mixing. Then discard the supernatant.

Note: 80% ethanol must be freshly prepared, otherwise it may affect the experimental results.

(10) Repeat Step (9) once.

(11) Keep the 1.5ml tube on the Magnetic Stand with the lid open, dry the beads at room temperature.

Note: Do not heat-dry the tube, as it may affect the final yield.

(12) Remove the 1.5ml tube from the Magnetic Stand, add 22 μl Library Elution Buffer. Mix by pipetting up and down or by vortex. Incubate at room temperature for 2 minutes.

(13) Place the 1.5ml tube on the Magnetic Stand and wait for 2 minutes until the solution is clear, ensuring that all magnetic beads are fully attached to the tube walls.

Note: If there is liquid on the tube wall, briefly centrifuge and then place it on the magnetic stand. The room temperature stand time can be extended to 5 minutes, ensuring all magnetic beads are fully attached to the tube wall.

(14) Carefully transfer 20 μl of the supernatant into a fresh PCR tube. Proceed to library amplification step or store at -20°C.

5. Library amplification

(1) Place the sterile PCR tube on ice and add the following components.

Component	For universal short adapter	For long adapter with index
Purification product from previous step	20 μl	20 μl
*Trans*NGS® Library Amplification SuperMix (2×)	25 μl	25 μl
*Trans*NGS® Universal Primer Mix for Illumina®	-	5 μl
i5 Primer	2.5 μl	-
i7 Primer	2.5 μl	-
Total volume	50 μl	50 μl

(2) Pipette up and down to mix thoroughly. Briefly spin the tubes to collect any liquid from the walls.

(3) Perform the following amplification program in the PCR instrument.

98°C	3 min	
98°C	30 sec	2-14 cycles*
60°C	30 sec	
72°C	30 sec	
72°C	3 min	
≤10°C	Hold	

* For different initial sample, refer to Appendix Table 2 for library yields. If fragment selection is performed before amplification, it is recommended to increase the number of amplification cycles by 1-2 cycles.

6. Library Amplification Product Purification

It is recommended to use 1.0× *MagicPure*® Size Selection DNA Beads (Catalog No: EC401) for product purification. *Trans*NGS® Library Amplification SuperMix (Catalog No: KA101) will not affect the fragment size during magnetic bead purification. This purification step can also be adjusted according to requirements, either to increase (to obtain libraries with smaller insert fragments) or decrease the magnetic bead ratio (to reduce primer residues). If fragment selection is required after purification, it is recommended to use 105 μl of Library Elution Buffer for elution. Transfer 100 μl of eluted product to a clean 1.5 ml centrifuge tube for further fragment selection. The specific steps for using 1.0× beads for purification are as follows:

(1) Pre-warm the DNA beads for 30mins from 2-8℃ to room temperature.

(2) Vortex the magnetic beads to ensure thorough mixing. Add 50 μl DNA beads (1.0×) to the product of previous step.

(3) Mix the sample thoroughly by pipetting up and down, then let it stand at room temperature for 5 minutes.
Note: Insufficient mixing will lead to inconsistent size selection results.

(4) Place the reaction tube on a Magnetic Stand. Let it stand at room temperature for approximately 5 minutes until the solution becomes clear, allowing the magnetic beads to fully adhere to the tube wall close to the magnetic stand.
Note: If there is liquid on the tube wall, briefly centrifuge and then place it on the magnetic stand. Please make sure that all magnetic beads are fully attached to the tube wall.

(5) Keep the 1.5ml tube on the Magnetic Stand, add 200 μl of freshly prepared 80% ethanol into the tube and keep it at room temperature for 30 seconds without mixing. Then discard the supernatant.
Note: 80% ethanol must be freshly prepared, otherwise it may affect the experimental results.

(6) Repeat Step (5) once.

(7) Keep the 1.5ml tube on the Magnetic Stand with the lid open, dry the beads at room temperature.
Note: Do not heat-dry the tube, as it may affect the final yield.

(8) Remove the 1.5ml tube from the Magnetic Stand, add 23 μl Library Elution Buffer. Mix by pipetting up and down or by vortex. Incubate at room temperature for 2 minutes.

(9) Place the 1.5ml tube on the Magnetic Stand and wait for 2 minutes until the solution is clear, ensuring that all magnetic beads are fully attached to the tube walls.
Note: If there is liquid on the tube wall, briefly centrifuge and then place it on the magnetic stand. The room temperature stand time can be extended to 5 minutes, ensuring all magnetic beads are fully attached to the tube wall.

(10) Carefully transfer 20 μl of the supernatant into a fresh PCR tube. Product can be stored at -20℃.

Appendix

Table 1 Reference of beads volume ratio (DNA beads : DNA) for fragment selection using *MagicPure*® Size Selection DNA Beads

Library average length (bp)		~320	~470	~670
Library insert average length (bp)		~200	~350	~550
Selection before end-repairing	1st Beads adding volume ratio	1.0×	0.7×	0.55×
	2nd Beads adding volume ratio	0.25×	0.2×	0.15×
Selection after purification of adapter ligation product	1st Beads adding volume ratio	0.9×	0.68×	0.56×
	2nd Beads adding volume ratio	0.2×	0.15×	0.12×
Direct selection after adapter ligation (no purification)	1st Beads adding volume ratio	0.35×	0.25×	0.15×
	2nd Beads adding volume ratio	0.15×	0.15×	0.15×
Selection after purification of library amplification product	1st Beads adding volume ratio	0.85×	0.65×	0.57×
	2nd Beads adding volume ratio	0.15×	0.15 ×	0.1×

Note: Fragment selection only needs to be performed at one of the three optional positions. For precise fragment selection, it is recommended that the sample before fragment selection is precisely 100 μl. The different magnetic bead ratios at the three optional positions are caused by the difference in sequence lengths at the ends of the insert fragments.

Due to differences in the length distribution of fragments in different samples, when using the same conditions for selection, the resulting product fragment lengths may also vary.

Table 2 Recommended amplification cycles for different starting sample amounts to achieve a library yield of 100 ng/1 μg are as follows

Initial sample	Recommended amplification cycles *	
	100 ng library	1 μg 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 recommended cycle numbers in this table are based on experimental data obtained from high-quality, human genomic DNA fragments with an average length of approximately 300 bp. The DNA is fragmented using sonication, and construct the library without fragment selection. In cases of poor DNA purity or severe DNA damage, it is advisable to increase the cycle numbers accordingly.

** When using incomplete length adapters such as *Trans*NGS®Adapter for Illumina®, it is recommended to perform at least 2-3 cycles to compensate for the downstream sequencing adapter sequences.

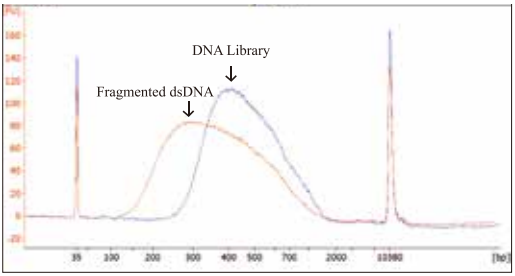


Figure 1 Schematic representation of changes in fragment length distribution before and after library construction
Red line: Human genomic DNA sample fragmented by sonication
Blue line: Library constructed using this reagent kit, without fragment selection during the library construction process.

Notes

- For obtaining higher quality sequencing data, it is recommended to perform fragment selection after adapter ligation or library amplification.
- Avoid vigorous shaking when mixing reaction solutions to prevent enzyme activity reduction, which may lead to decreased library construction efficiency.
- When using magnetic beads for purification or fragment selection, thoroughly mix the beads during elution. Well-mixed beads should be uniformly suspended, free of visible particles, and show no sedimentation after 2 minutes of standing.
- Samples with concentrations below 1 ng/μl are recommended to be stored in low-binding centrifuge tubes or tubes containing 1×*Trans*NGS® Library Dilution Buffer (Catalog No: KB101) to prevent reduced effective sample concentration due to nucleic acid adsorption to the walls of regular tubes.
- More amplification cycles during library amplification lead to higher repeat rates in sequencing data, resulting in fewer effective data. Therefore, it is advisable to use fewer amplification cycles while still meeting downstream application requirements.
- For higher library yields (e.g., for IVD), a 100 μl amplification system is recommended during library amplification (see table below).

Component	For universal short adapter	For indexed long adapter
Purification product of previous step	20 μl	20 μl
*Trans*NGS® Library Amplification SuperMix (2×)	50 μl	50 μl
*Trans*NGS® Universal Primer Mix for Illumina®	-	5 μl
i5 Primer	2.5 μl	-
i7 Primer	2.5 μl	-
Nuclease-free Water	25 μl	25 μl
Total volume	100 μl	100 μl

Note: When purifying after amplification, use 100 μl of *MagicPure*® Size Selection DNA Beads (1.0×) (Catalog No: EC401).

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Service telephone +86-10-57815020
Service email complaints@transgen.com