# *Trans*NGS® Tn5 DNA Library Prep Kit for Illumina® (for 1 ng DNA)

**Please read the manual carefully before use**

**Catalog No.** KP111

**Storage:** at -70℃ for one year (at -20℃ for 6 months)

## Description

*Trans*NGS® Tn5 DNA Library Prep Kit for Illumina® is a reagent kit designed for Illumina high-throughput sequencing platform, suitable for preparing DNA library from 1ng initial sample (such as acquiring small genome, plasmid and PCR amplification product larger than 300 bp). The resulting library can be used for paired-end sequencing. This kit employs an in vitro transposition technique, which can simultaneously complete DNA fragmentation and adapter ligation within 5 minutes. In comparison to conventional library preparation method that requires DNA fragmentation, end repair, and adapter ligation, the in vitro transposition technique significantly shortens library construction time and simplifies the operation.

## Feature

• Time saving

• Simple operation

## Application

Prepare short fragment DNA library for Illumina high-throughput sequencing platform from purified dsDNA (including genome from various species, plasmid and PCR amplification product larger than 300 bp, etc.).

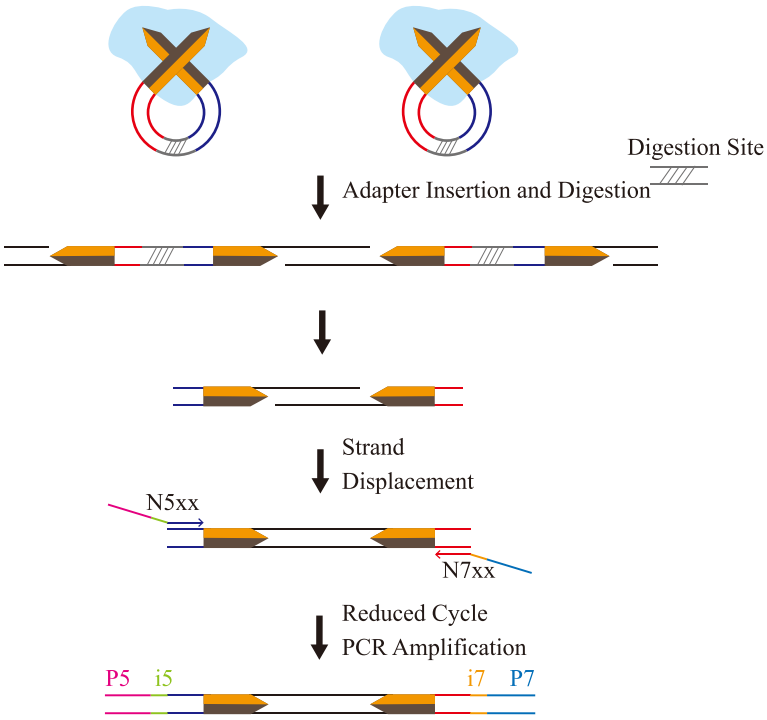
## Kit content

| Component | KP111-11 (12 rxns) | KP111-03 (96 rxns) |
|---|---|---|
| Tn5-1 Enzyme Mix | 24 µl | 192 µl |
| 5×Insertion and Digestion Buffer | 36 µl | 288 µl |
| 4×Stop Buffer | 60 µl | 480 µl |
| *Trans*NGS® Tn5 Library Amplification SuperMix (2×) | 300 µl | 4×600 µl |
| Library Elution Buffer (LEB) | 600 µl | 5 ml |
| Nuclease-free Water | 1 ml | 5 ml |

## Initial Sample Requirement

The initial sample should be purified DNA dissolved in Nuclease-free Water or 10 mM Tris-HCl (pH8.0), with an $OD_{260}/OD_{280}$ value between 1.8-2.0. Fluorescent dye method (Qubit or PicoGreen) is chosen for specific dsDNA detection and DNA concentration measurement. The ratio of the concentration measured by absorbance method (such as Nanodrop) to the concentration measured by fluorescent dye method for the same DNA sample is ≤2.

## Principle Diagram of Library Construction




## Library Construction

5’ -AATGATACGGCGACCACCGAGATCTACAC[i5]TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG
-XXXXXXXX-CTGTCTCTTATACACATCTCCGAGCCCACGAGAC[i7]ATCTCGTATGCCGTCTTCTGCTTG-3’

i5：Index 2，8 bases

i7：Index 1，8 bases

-XXXXXXXX-：inserted DNA.

## Protocol

Prepare the following reagents: freshly prepared 80% ethanol; *MagicPure*® Size Selection DNA Beads (Cat. No. EC401); ***Trans***NGS® Tn5 Index Kit for Illumina® (Cat. No. KI101).

### 1. Adapter Insertion and DNA Fragmentation

The transposase Tn5 can react slowly at room temperature; therefore, the reaction mixture should be prepared on ice, mixed by pipetting, and immediately move to thermocycler for reaction. Thaw the 4×Stop Buffer completely at room temperature.

(1) Assemble the following reaction set in a sterile PCR tube on ice:

| Component | Volume |
|---|---|
| Tn5-1 Enzyme Mix | 2 μl |
| 1 ng DNA | Variable |
| 5×Insertion and Digestion Buffer | 3 μl |
| Nuclease-free Water | Variable |
| Total volume | 15 μl |

(2) Mix by pipetting up and down, if there is liquid on the walls of the tube, spin briefly and immediately proceed to the next step of the reaction.

(3) Place the reaction tube in a thermocycler and incubate at 55°C for 5 minutes (lid temperature at 70°C).

(4) Immediately place the reaction tube on ice, and immediately add 5 μl of 4× Stop Buffer to the tube. Mix by pipetting up and down, and place the tube on ice.

**2. Library Amplification**

(1) Add the following reaction components to the PCR tube on ice:

| Component | Volume |
|---|---|
| Output of previous reaction | 20 μl |
| *Trans*NGS® Tn5 Library Amplification SuperMix (2×) | 25 μl |
| N5xx* | 2.5 μl |
| N7xx* | 2.5 μl |
| Total volume | 50 μl |

* *Trans*NGS® Tn5 Index Kit for Illumina® (Cat. No. KI101) provides 8 different N5xx barcode oligos and 12 different N7xx barcode oligos. Please choose as needed.

(2) Mix by pipetting up and down. Spin briefly.

(3) Recommended PCR amplification program:

| | | |
|---|---|---|
| 72°C | 3 min* | |
| 98°C | 3 min | |
| 98°C | 30 sec | 12 cycles |
| 62°C | 30 sec | |
| 72°C | 30 sec | |
| 72°C | 3 min | |
| ≤10°C | Hold | |

* This step is indispensable. The transposition reaction product is not complete double-stranded DNA. Incubate at 72°C for 3 minutes to generate complete PCR templates.

**3. Size selection of library amplification product**

If there is no special requirement for fragment length, the enriched library can directly be purified with 1.0× DNA beads, rather than going through size selection steps.

*MagicPure*® Size Selection DNA Beads (Cat. No. EC401) is recommended for size selection of library amplification product. To avoid residual adapter or residual long fragments, it is recommended to perform fragment size selection twice, or to conduct DNA purification with 1.0× magnetic beads before size selection. The specific steps for fragment size selection are as follows:

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

(2) Transfer 50ul output from library amplification step into a 1.5ml sterile centrifuge tube. If the output is less than 50 μl, it should be supplemented with Nuclease-free Water.

(3) Vortex the magnetic beads to ensure thorough mixing. Add the appropriate volume of DNA beads to the PCR products (refer to table1).

Volume of DNA beads to add = volume of DNA solution × 1st volume ratio.

For example: 30 μl beads = 50 μl DNA solution × 0.6

(4) 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.

(5) Place the 1.5ml 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 rack.
Note: If there is liquid on the tube wall, briefly centrifuge and then place it on the magnetic rack. Please make sure that all magnetic beads are fully attached to the tube wall.

(6) Keep the 1.5ml tube on the Magnetic Stand, transfer the supernatant into another clean 1.5 ml tube. Discard the beads.

(7) Add the appropriate volume of beads into the supernatant (refer to Table 1).
Volume of the beads to add = initial volume of DNA solution × 2nd volume ratio
For example: 7.5 μl beads = 50 μl DNA solution × 0.15

(8) Mix by pipetting up and down. Incubate at room temperature for 5 minutes.
Note: Insufficient mixing will lead to inconsistent size selection results.

(9) Place the 1.5 ml centrifuge tube on the magnetic rack and 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 rack. Discard the supernatant.
Note: If there is liquid on the tube wall, briefly centrifuge and then place it on the magnetic rack, ensuring all magnetic beads are fully attached to the tube wall. Do not pipette the magnetic beads, as this may affect the final yield.

(10) 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, as it may affect the experimental results.

(11) Repeat Step (10) once.

(12) 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.

(13) 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.

(14) 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 rack. The room temperature stand time can be extended to 5 minutes, ensuring all magnetic beads are fully attached to the tube wall.

(15) Carefully transfer 20 μl of the supernatant into a fresh EP tube, and store at -20℃.

## Appendix

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

| **Average Size of Selected Library (bp)** | ~330 | ~480 | ~680 |
|---|---|---|---|
| **Insert Size of Selected Library (bp)** | ~300 | ~350 | ~550 |
| **1st volume ratio (DNA Beads : DNA)** | 0.65× | 0.6× | 0.55× |
| **2nd volume ratio (DNA Beads : DNA)** | 0.15× | 0.15× | 0.12× |

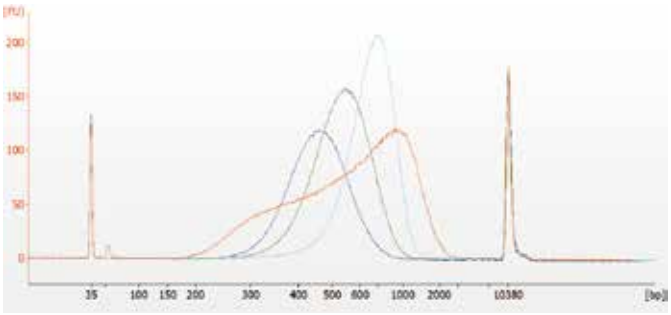



Figure 1 Reference of size selected library by *MagicPure*® Size Selection DNA Beads.
Library amplification output - Bacteria library prepared by this kit, after 12 PCR cycles and 1.0× beads purification (red curve); or after 12 PCR cycles and size selection by 0.6×+0.1× (blue curve); 0.6×+0.15× (green curve); or 0.55×+0.12× beads (light blue curve).

**Note**

The transposase recognition sites exhibit a certain preference, resulting in each inserted fragment having 9 nucleotides at both ends that are not entirely randomly distributed, which means the first 9 nucleotides of each sequencing read show a certain preference (Table 2).

Table 2 Transposase recognition site preference statistics table

| Probability Statistics (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Base position** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** |
| **A** | 20.0375 | 21.08 | 24.8825 | 16.1325 | 36.29 | 38.2125 | 30.3475 | 34.4075 | 15.02 |
| **T** | 10.3075 | 36.355 | 29.0425 | 39.1625 | 35.6925 | 17.7975 | 23.9275 | 21.42 | 21.6425 |
| **C** | 25.125 | 24.66 | 25.1125 | 31.4525 | 13.8025 | 13.2325 | 19.8475 | 19.0425 | 39.17 |
| **G** | 44.345 | 17.3975 | 20.96 | 13.21 | 14.2175 | 30.7575 | 25.8775 | 25.13 | 24.165 |

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Version number: V2.0-202008

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