CREATIVE BIOMART®
Assay Kit

Tn5 DNA Library Prep Kit for Illumina

Product Information

Cat.No. Kit-3431

Product Overview

Tn5 DNA Library Prep Kit for Illumina (for 50 ng DNA) is an optimized genomic library construction kit for the Illumina high-throughput sequencing platform, which is suitable for the amount of 50 ng input DNA. Traditional library construction generally includes multi-step operations such as DNA fragmentation, end repair and linker ligation, while our kit adopts Tn5 transposase method which can be completed in one-step reaction. Our kits are easy to operate and greatly reduce library construction time, and significantly reducing the demanded amount of the initial DNA.

Storage

Store Transposase Mix at 4°C and other components at –20°C.

Kit Components

Components	24 rxns	96 rxns
Transposase Mix	120 µl	480 µl
2X Tagmentation Buffer	240 µl	960 µl
Amplify Enzyme	24 µl	96 µl
5X Amplify Buffer	240 µl	960 µl
dNTP Mixture	96 µl	384 µl
P5	60 µl	240 µl
P7	60 µl	240 µl
Control DNA	10 µl	10 µl

Note: Control DNA, Escherichia coli Genomic DNA, 50 ng/µl.

Materials Required but Not Supplied

1. Index Kit 1 for Illumina or Index Kit 2 for Illumina
2. Beads: Recommend DNA Clean and Selection Beads or Agencourt AMPure XP
3. Ethanol (100%)
4. Nuclease-free Water

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Preparation

The input DNA was purified and dissolved in Nuclease-free Water, requiring the absorbance of 260 nm/280 nm between 1.8 and 2.0. Accurate determination of DNA concentration is a critical step in the success of the experiment, and is recommended using fluorescent dye methods such as the Qubit® 2.0 rather than absorbent photometric methods (such as Nanodrop).

Assay Protocol

1. DNA Tagmentation

1) Thaw the 2X Tagmentation Buffer completely at room temperature and vortex to mix thoroughly (if it is precipitated, it can be heated at 37°C to help dissolve).

2) Place the sterilized PCR tube on ice and prepare the reaction system according to the following table:

Components Volume

2X Tagmentation Buffer 10 μl

Transposase Mix 5 μl

50 ng DNA X μl

Nuclease-free Water X μl

Total 20 μl

3) Use a pipette to gently blow until Mix thoroughly. The tube can be centrifuged instantaneously.

4) Put the reaction tube into a thermal cycler (choose the preheat lid option and set to 105°C), and run the following program: □

55°C for 10 minutes □

Hold at 10°C

5) Proceed to the next step immediately.

2. Purify the DNA fragments with Beads

1) Remove the beads from 4°C and equilibrate to room temperature before use.

2) Suspend the beads thoroughly with a vortex shaker, pipet 20 μl of beads into the PCR product, and mix thoroughly.

3) Incubate at room temperature for 5 min so that DNA binds to the beads.

4) The PCR tube is instantaneously centrifuged and placed on a magnetic stand. Wait until the

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solution becomes clear (about 5 min), the beads are completely adsorbed on the tube wall, carefully discard the supernatant.
5) Keep the tube on the magnetic stand, add 200 µl of the freshly prepared 80% ethanol to rinse the beads. Incubate 30 sec at room temperature, carefully remove the supernatant.
6) Repeat the step 5).
7) Keep the PCR tube on the magnetic stand and open the lid for 2 min at room temperature to completely evaporate the ethanol.
8) Remove the PCR tube from the magnetic stand, add 22 µl of Nuclease-free Water into the tube to elute DNA, vortex or blow until thoroughly mixed, Incubate at room temperature for 5 min.
9) Spin down the PCR tube briefly and put it on the magnetic stand. Wait until the solution becomes clear (about 5 min).
10) Carefully pipet 20 µl of the supernatant into a new sterile PCR tube, proceed to the next step immediately.
3. PCR Enrichment
1) Thaw the Index Kit for Illumina on ice, and mix it instantaneously.
2) Put the sterile PCR tube in the ice bath and prepare the PCR system according to the following table:

Components	Volume
Products of Step 1.5)	20 µl
5X Amplify Buffer	10 µl
N5XX	5 µl
N7XX	5 µl
P5	2.5 µl
P7	2.5 µl
dNTP Mixture	4 µl
Amplify Enzyme	1 µl
Total	50 µl

*Note: Index Kit 1 for Illumina provides 8 kinds of N5XX and 12 kinds of N7XX, Index Kit 2 for Illumina provides 16 kinds of N6XX and 24 kinds of N8XX. You can select according to the number of samples and the strategy of Index selection.

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3) Mix gently by pipetting, centrifuge instantaneously, place the PCR tube into a thermal cycler, and run the following program:
Temperature Time Cycles
72°C 3 min 1
98°C 30 sec 1
98°C 15 sec 5-9
60°C 30 sec 5-9
72°C 3 min 5-9
72°C 5 min 1
4°C Hold 1
4) Proceed to the next step for size selection after the reaction is completed.
4. Purify the Amplified Library with Beads (Optional Step)
1) Remove the beads from 4°C and equilibrate to room temperature before use.
2) Suspend the beads thoroughly with a vortex shaker, pipet 50 μl of beads into the PCR product, and mix thoroughly (The product volume would be less than 50 μl due to the evaporation during PCR, make up the volume to 50 μl with Nuclease-free Water, to prevent obtained the unexpected selected size).
3) Incubate at room temperature for 5 min so that DNA binds to the beads.
4) The PCR tube is instantaneously centrifuged and placed on a magnetic stand. Wait until the solution becomes clear (about 5 min), the beads are completely adsorbed on the tube wall, carefully discard the supernatant.
5) Keep the tube on the magnetic stand, add 200 μl of the freshly prepared 80% ethanol to rinse the beads. Incubate 1 min at room temperature, carefully remove the supernatant.
6) Repeat the step 5).
7) Keep the PCR tube on the magnetic stand and open the lid for 2 min at room temperature to completely evaporate the ethanol.
8) Remove the PCR tube from the magnetic stand, add 25 μl of Nuclease-free Water into the tube to elute DNA, vortex or blow until thoroughly mixed, incubate at room temperature for 5 min.
9) Spin down the PCR tube briefly and put it on the magnetic stand. Wait about 5 min.
10) Carefully pipet 23 μl of the supernatant into a new sterile PCR tube, add Nuclease-free Water to

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the 50 μl system, and vortex to mix.
5. Select the Amplified Library with Beads
1) Accurately pipet the first amount of beads volume added to the system, fully blown and mix, and incubate at room temperature for 5 min. For the volume of beads needed in 1st round and 2nd round in selection process, please refer to the following table:
Average full length of the library (bp) ~350 ~450 ~550
Range of full length of the library (bp) 250 - 450 300 - 700 400 - 900
Volume of beads in the 1st round (μl) 35 (0.75X) 30 (0.6X) 25 (0.5X)
Volume of beads in the 2nd round (μl) 7.5 (0.15X) 7.5 (0.15X) 7.5 (0.15X)
*Note:
Volume of beads (μl) = Initial volume of PCR product (μl) × Volume ratio (for example, "0.75X" indicates 50 μl × 0.75 = 37.5 μl)
2) Spin down the tube briefly and put the PCR tube on the magnetic stand. Wait until the solution becomes clear (about 5 min), carefully transfer the supernatant to a new tube and discard the beads.
3) Suspend the beads thoroughly with a vortex oscillator, and pipet 2nd amount of beads volume into the supernatant collected in the precious step. Mix thoroughly and incubate at room temperature for 5 min.
4) Spin down the tube briefly and put the PCR tube on the magnetic stand. Wait until the solution becomes clear (about 5 min), discard the supernatant carefully.
5) Keep the tube on the magnetic stand, add 200 μl of the freshly prepared 80% ethanol to rinse the beads. Incubate 30 sec at room temperature, then carefully remove the supernatant.
6) Repeat the step 5).
7) Keep the tube on the magnetic stand, open the tube lid and air-dry the beads for 2 min at room temperature.
8) Remove the PCR tube from the magnetic stand, add 15 μl of Nuclease-free Water into the tube to elute DNA, vortex or blow until thoroughly mixed, Incubate at room temperature for 5 min.
9) Spin down the tube briefly and put the PCR tube on the magnetic stand. Wait until the solution becomes clear (about 5 min), transfer 13 μl of supernatant into a new tube carefully. The purified library can be stored at -20°C.

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*Note: If there is no special requirement for the length distribution of the library, the amplification products can be purified directly without size selection using beads purification or column purification. If a library with a more concentrated length distribution is required, the amplified product can be selected and purified by using the gel extraction kit.

Library Quality Control

1. Library Concentration In order to obtain a high quality of sequencing results, the library concentration must be accurately determined. In addition to accurate quantification of the library concentration by Real-time PCR, fluorescent dye method can also be used for measurement, and non-absorbance photometry (such as Nanodrop) is not recommended.
2. Library Distribution The constructed library can be subjected to length distribution detection using instruments such as Agilent 2100 Bioanalyzer, and the library concentration can also be detected.

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