Product Description

AcuGenix™ One-Step Tn5 DNA Library Prep Kit is a DNA library construction kit specifically developed for Illumina high-throughput sequencing platforms. It is suitable for library preparation of various purified DNA samples, including genomic DNA, plasmid DNA and PCR fragments larger than 500 bp.By virtue of in vitro transposition technology, this kit simultaneously fragments DNA and integrates partial adapter sequences. Compared with traditional DNA library construction methods, it reduces the required template input and shortens the library construction time.Three specifications are available for this kit, corresponding to initial DNA inputs of 1 ng, 5 ng and 50 ng respectively.Libraries constructed with this kit can be directly applied to sequencing analysis on Illumina platforms after passing quality inspection.

Components

Product name	Cat	Specs	DNA Input
AcuGenix™ One-Step Tn5 DNA Library Prep Kit	BR3D301-01/03/06	8/24/96 T	1 ng
	BR3D302-01/03/06	8/24/96 T	5 ng
	BR3D303-01/03/06	8/24/96 T	50 ng

Composition		BR3D301-01/03/06	BR3D302-01/03/06	BR3D303-01/03/06
Tagment Enzyme V1		40/120/480 μL	------	------
Tagment Enzyme V5		------	40/120/480 μL	------
Tagment Enzyme V50		------	------	40/120/480 μL
4× Tagment Buffer		40/120/480 μL	40/120/480 μL	40/120/480 μL
5× Stop Buffer*		40/120/480 μL	40/120/480 μL	40/120/480 μL
4× Lib Amp Mix		100/300/1200 μL	100/300/1200 μL	100/300/1200 μL

Note: White precipitate may form in this component at low temperature, which is normal and will not affect product performance. Please dissolve it at room temperature before use. This component can be stored at room temperature.

Storage

Store at −20±5℃.

Application

This Kit is applicable to preparing purified DNA samples (including genomic DNA, plasmid DNA, PCR fragments larger than 500 bp, etc.) into libraries dedicated to Illumina high-throughput sequencing platforms.

Consumables Prepared by User

Reagents Prepared by User:Qubit reagent, DNase/RNase-free water, Biori®NGS DNA Clean Beads (BR3N401) for DNA purification and size selection, N5XX & N7XX index amplification primers (BR3N108), analytical pure absolute ethanol, etc.
Instruments Prepared by User:Magnetic stand, pipettes, PCR thermocycler, vortex mixer, microcentrifuge, etc.
Consumables Prepared by User:0.5 mL Qubit tubes, 1.5 mL centrifuge tubes, 0.2 mL PCR tubes, pipette tips, 50 mL centrifuge tubes, etc.

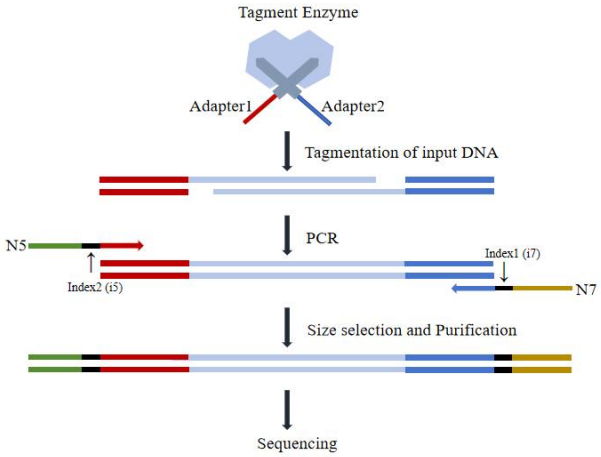
Notes

1.Purified DNA should be dissolved in sterilized ultrapure water, DEPC water or DNase/RNase-free water.
2.DNA purity requirement: A260/A280 = 1.8–2.0.
3.This kit is highly sensitive to DNA input. It is recommended to quantify DNA concentration using Qubit. Absorbance-based quantification methods are not allowed.
4.Mix reagents well before use and avoid repeated freeze-thaw cycles. White precipitate may form in 5× Stop Buffer at low temperature, which is normal and will not affect performance. It is recommended to store at room temperature after opening.
5.Equilibrate magnetic beads to room temperature before use. Fully resuspend magnetic beads thoroughly prior to each pipetting.

6.80 % ethanol for bead washing should be freshly prepared before use.
7.Fully air-dry magnetic beads at room temperature before elution (the bead surface changes from shiny brown to matte brown). Avoid over-drying, which may cause bead cracking and reduce purification recovery.
8.To ensure experimental reproducibility, a PCR instrument with heated lid is recommended. Preheat the instrument to reaction temperature in advance before starting the reaction.
9.To prevent cross-contamination and aerosol contamination, filtered pipette tips are recommended during operation. After the experiment, disinfect the experimental area with chlorine-containing disinfectant or DNA decontaminant.

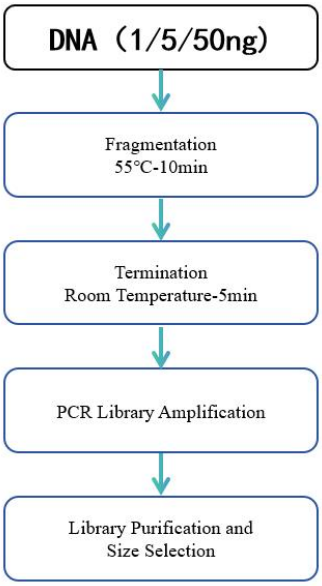
Experimental Principle

1. This product is developed based on the transposase method, with its core principle relying on transposase and transposition mechanism.The Tagment Enzyme contains a complete transposon composed of transposase and two equimolar adapters Adapter1 and Adapter2.During transposition, the transposon inserts Adapter1 and Adapter2 into the target DNA. DNA fragmentation is thus achieved simultaneously with adapter addition at fragment ends.
The fragmented products are amplified by PCR using N5XX and N7XX indexes, yielding libraries for high-throughput sequencing.



2.Library Structure
5'-AATGATACGGCGACCACCGAGATCTACAC(i5)TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-NNNNN(insert)-CTGTCTCTTATACACATCTCCGAGCCCACGAGAC(i7)ATCTCGTATGCCGTCTTCTGCTTG-3'

Experimental Procedure Overview



Protocol

Please read the manual carefully before conducting experiments. This manual applies to three specifications of the kit, corresponding to reaction starting amounts of DNA at 1 ng, 5 ng and 50 ng respectively.Certain components differ among the three kit specifications. Do not mix components from different kits, so as to avoid delaying experimental progress.

1. DNA Fragmentation

1-A: DNA Fragmentation with 1 ng Starting Material (Applicable to Kit BR3D301)

1.1Thaw 4× Tagment Buffer at room temperature or on ice. Mix gently by vortexing and perform brief centrifugation.Mix Tagment Enzyme by flicking the tube gently, centrifuge briefly, and keep on ice for later use.Verify that 5× Stop Buffer is at room temperature. If precipitation occurs, dissolve it by heating at 37 ℃, mix well and reserve for use.Prepare the following reaction system in an RNase-free centrifuge tube:

Component	Volume (μL)
4× Tagment Buffer	5
Tagment Enzyme V1	5
RNase-free ddH2O	10-X
1 ng DNA	X
Total	20

Note: The tagmentation reaction can also be performed at room temperature. Therefore, the reaction system must be prepared on ice, and incubation shall be initiated immediately after mixing.

1.2 Gently mix by pipetting up and down. Perform brief centrifugation to collect all reaction liquid at the bottom of the tube, and immediately conduct the following reaction on a PCR instrument:

Program	Temperature	Time	Cycles
Heated Lid	75℃	/	/
1	55℃	10min	/
2	4℃	∞	/

1.3 Immediately remove the PCR tube after reaction completion, add 5 μL 5× Stop Buffer.(This component should be dissolved at room temperature before use. Precipitation is normal; dissolve in a 37 ℃ water bath, mix thoroughly before use.)Vortex mix, centrifuge, and incubate at room temperature for 5 min.

1.4 Immediately proceed to Step 9.2 PCR Library Amplification.

1-B:DNA Fragmentation with5 ng Starting Material (Applicable to Kit BR3D302)

1.1Thaw 4× Tagment Buffer at room temperature or on ice. Mix gently by vortexing and perform brief centrifugation.Mix Tagment Enzyme by flicking the tube gently, centrifuge briefly, and keep on ice for later use.Verify that 5× Stop Buffer is at room temperature. If precipitation occurs, dissolve it by heating at 37 ℃, mix well and reserve for use.Prepare the following reaction system in an RNase-free centrifuge tube:

Component	Volume (μL)
4× Tagment Buffer	5
Tagment Enzyme V5	5
RNase-free ddH2O	10-X
5 ng DNA	X
Total	20

Note: The tagmentation reaction can also be performed at room temperature. Therefore, the reaction system must be prepared on ice, and incubation shall be initiated immediately after mixing.

1.2 Gently mix by pipetting up and down. Perform brief centrifugation to collect all reaction liquid at the bottom of the tube, and immediately conduct the following reaction on a PCR instrument:

Program	Temperature	Time	Cycles
Heated Lid	75℃	/	/
1	55℃	10min	/
2	4℃	∞	/

1.3 Immediately remove the PCR tube after reaction completion, add 5 μL 5× Stop Buffer.(This component should be dissolved at room temperature before use. Precipitation is normal; dissolve in a 37 ℃ water bath, mix thoroughly before use.)Vortex mix, centrifuge, and incubate at room temperature for 5 min.

1.4 Immediately proceed to Step 9.2 PCR Library Amplification.

1-C: DNA Fragmentation with 50 ng Starting Material (Applicable to Kit BR3D303)

1.1Thaw 4× Tagment Buffer at room temperature or on ice. Mix gently by vortexing and perform brief centrifugation.Mix Tagment Enzyme by flicking the tube gently, centrifuge briefly, and keep on ice for later use.Verify that 5× Stop Buffer is at room temperature. If precipitation occurs, dissolve it by heating at 37 ℃, mix well and reserve for use.Prepare the following reaction system in an RNase-free centrifuge tube:

Component	Volume(μL)
4× Tagment Buffer	5
Tagment Enzyme V50	5
RNase-free ddH2O	10-X
50 ng DNA	X
Total	20

Note: The tagmentation reaction can also be performed at room temperature. Therefore, the reaction system must be prepared on ice, and incubation shall be initiated immediately after mixing.

1.2 Gently mix by pipetting up and down. Perform brief centrifugation to collect all reaction liquid at the bottom of the tube, and immediately conduct the following reaction on a PCR instrument:

Program	Temperature	Time	Cycles
Heated Lid	75℃	/	/
1	55℃	10min	/
2	4℃	∞	/

1.3 Immediately remove the PCR tube after reaction completion, add 5 μL 5× Stop Buffer.(This component should be dissolved at room temperature before use. Precipitation is normal; dissolve in a 37℃water bath, mix thoroughly before use.)Vortex mix, centrifuge, and incubate at room temperature for 5 min.

1.4 Immediately proceed to Step 9.2 PCR Library Amplification.

2. PCR Library Amplification

2.1 Thaw 4× Lib Amp Mix on ice, vortex thoroughly, perform brief centrifugation, and keep on ice for later use.

Prepare the following reaction system:

Component	Volume (μL)
Previous step	25
4× Lib Amp Mix	12.5
N5XX [a]	2.5
N7XX [a]	2.5
RNase-free ddH2O	7.5
Total	50

Note a: N5XX and N7XX amplification primers shall be provided by the customer or purchased separately from Biori NGS

Tagment Index Kit for Illumina (BR3N108).

2.2 Gently mix by pipetting up and down. Perform brief centrifugation to collect the reaction mixture at the bottom of the tube, and run the following reaction on a PCR instrument:

Program	Temperature	Time	Cycles	Volume (μL)
Heated Lid	105℃	/	/	50 μL
1	72℃ [a]	5 min	1 cycle	
2	98℃	1 min	1 cycle	
3	98℃	10 s	6-13 cycles [b] (Program 3-5)	
4	60℃	30 s		
5	72℃	30 s		
6	72℃	1 min	1 cycle	
7	4℃	∞	/	

Note a: Incubation at 72 ℃ is used to generate mature PCR templates. This step cannot be omitted.

Note b: The number of amplification cycles shall be selected according to the actual input amount of nucleic acid, following the principles below:

DNA input	Applicable Kit	Reference Cycles
1 ng	BR3D301	11-13
5 ng	BR3D302	9-11
50 ng	BR3D303	6-9

3. Library Purification or Size Selection

If there is no special requirement for library length distribution, the amplified product can be directly purified with 1.2× magnetic beads.It is recommended to purify and select the amplified product using Biori® NGS DNA Clean Beads (BR3N401).

3.1 Invert and mix Biori® NGS DNA Clean Beads equilibrated to room temperature thoroughly.

3.2 Perform two-round size selection according to the required DNA fragment length. Make up the PCR product to 100 μL with water. Add magnetic beads for the first round selection referring to the table below, vortex well, and incubate at room temperature for 5 min.

Average Library Insert Size	250bp	350bp	450bp
First-round Bead Volume	70 μL (0.7×)	60 μL (0.6×)	50 μL (0.5×)
Second-round Bead Volume	20 μL (0.2×)	15 μL (0.15×)	15 μL (0.15×)

3.3 Perform brief centrifugation, place the sample on a magnetic rack for 2 min until the solution is clear, and carefully transfer the supernatant to a clean centrifuge tube.

3.4 Add magnetic beads for second-round selection to the supernatant according to the table above, vortex thoroughly, and incubate at room temperature for 5 min.

3.5 Perform brief centrifugation, place the sample on a magnetic rack for 2 min until the solution is clear, and carefully discard the supernatant.

3.6 Add 200 μL freshly prepared 80% ethanol, stand for 30 s, and carefully aspirate and discard the supernatant.

3.7 Repeat step 3.6 once.

3.8 After brief centrifugation, carefully remove residual liquid, and air-dry at room temperature until the beads appear matte on the surface.

3.9 Add 22 μL nuclease-free water, vortex for 10 s to ensure thorough mixing of beads, and stand at room temperature for 2 min.

3.10 After brief centrifugation, place on the magnetic rack for 2 min until clear. Carefully transfer 20 μL supernatant to a new centrifuge tube.

4.Library Quality Control

4.1 Library Concentration Quantification:

Determine library concentration using Qubit 4.0 Fluorometer with Qubit dsDNA HS Assay Kit. Absolute quantification of library concentration via qPCR is recommended.

4.2 Library Fragment Distribution Analysis:

Detect fragment distribution of the prepared library using Qsep1 Fully Automated Nucleic Acid & Protein Analyzer or agarose gel electrophoresis.

5. Troubleshooting

5.1 Why are the fragmented fragments excessively large?

Answer: The transposase is highly sensitive to DNA input amount. Accurate determination of DNA concentration is critical to experimental success.

Large library fragments may result from excessive DNA input or inhibitors existing in DNA samples.

It is recommended to accurately measure DNA concentration by fluorescence method, and adopt reliable DNA purification to remove inhibitors that interfere with transposase activity.

5.2 Why are the fragmented fragments excessively small?

Answer: Small library fragments are mainly caused by insufficient DNA input or degraded DNA samples.

It is suggested to increase DNA input amount and use high-quality intact DNA.

5.3 What types of DNA can be fragmented with this product?

Answer: In addition to genomic DNA fragmentation, this product is also applicable to plasmid and PCR product fragmentation.For PCR product fragmentation, it is recommended that the PCR product length be greater than 500 bp.