TruePrep DNA Library Prep Kit V2 for Illumina®

TD501, TD502, TD503

Version 6.2

Vazyme biotech co., ltd.

Introduction

TruePrep DNA Library Prep Kit V2 for Illumina® is specifically designed for next-generation sequencing (NGS) onIllumina® platforms. This kit enables super fast and easy preparation of ready-to-use DNA library for sequencing by adopting a new-type transposase, which convert the complex steps of DNA fragmentation, end repair, dA-tailing, and adapter ligation into a one-step enzymatic reaction, significantly reducing the demanded amount of the initial DNA and shortening the time of library preparation. Three kinds of specifications of this kit are provided for different amount of input DNA: 50 ng(#TD501), 5 ng (#TD502), and 1 ng (#TD503).

Comtents of kits

Cat.No.	TD501-01/02	TD502-01/02	TD503-01/02
Size	24/96 rxn	24/96 rxn	24/96 rxn
InputDNA	50 ng	5 ng	1 ng
TTE Mix V50	120/480 μl	------	------
TTE Mix V5	------	120/480 μl	------
TTE Mix V1	------	------	120/480 μl
5×TTBL	240/960 μl	96/384 μl	96/384 μl
5×TS	------	120/480 μl	120/480 μl
PPM	120/480 μl	------	------
TAE	24/96 μl	24/96 μl	24/96 μl
5×TAB	240/960 μl	240/960 μl	240/960 μl
ControlDNA	10/10 μl	10/10 μl	10/10 μl

*TTE = TruePrep Tagment Enzyme; TTBL = TruePrep Tagment Buffer L; TS = Terminate Solution; PPM = PCR Primer Mix; TAE = TruePrep Amplify Enzyme; TAB = TruePrep Amplify Buffer.
*Control DNA, Mouse Genomic DNA, 50 ng/μl.

Storage

TD501: store at -20°C;
TD502: store 5× TS at room temperature and other components at -20°C;
TD503: store 5× TS at room temperature and other components at -20°C;

Additional Materials Required

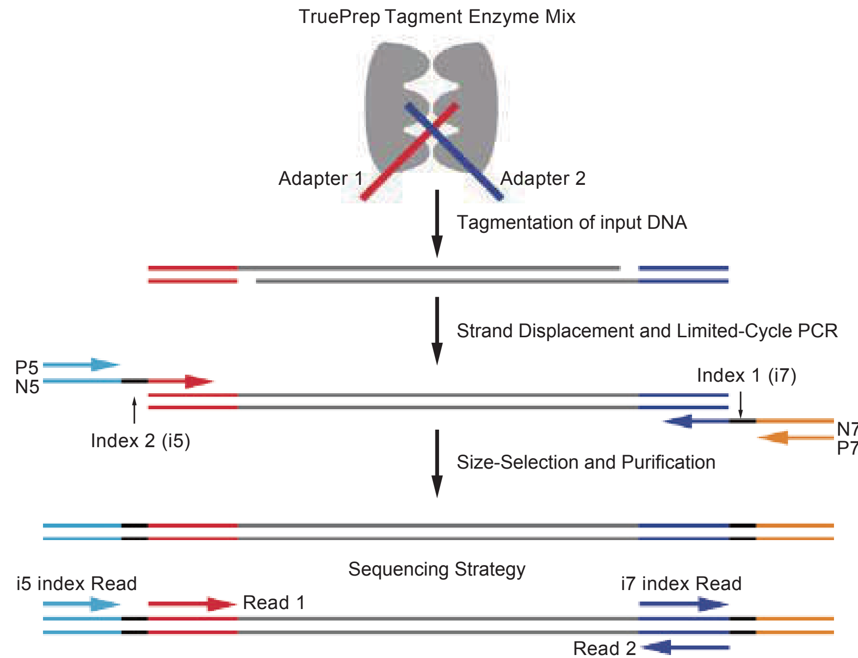
VAHTS DNA Clean Beads (Vazyme, #N411);
Magnetic stand;
Thermocycler (PCR Instrument);
Low absorption EP tubes and PCR tubes;
Ethanol (100%);
Sterilized ultra-pure water;
TruePrep Index Kit V2 for Illumina® (Vazyme, #TD202) or TruePrep Index Kit V3 for Illumina® (Vazyme, #TD203).

Application

Applicable to prepare ready-to-use DNA libraries for next-generation sequencing on the Illumina platform.

Notes: When using PCR products for library preparation, make sure the size of PCR products is > 500 bp. The transposase in TruePrep DNA Library Prep Kit V2 is not effective at the ends of DNA, leading to inevitable lower coverage of the 50 bp on each end. Therefore, to avoid the reduction of sequencing coverage on the ends, it is recommended to extend 50-100 bp at the both ends of the to-be-sequenced region when PCR products are prepared.

Overview of the Workflow

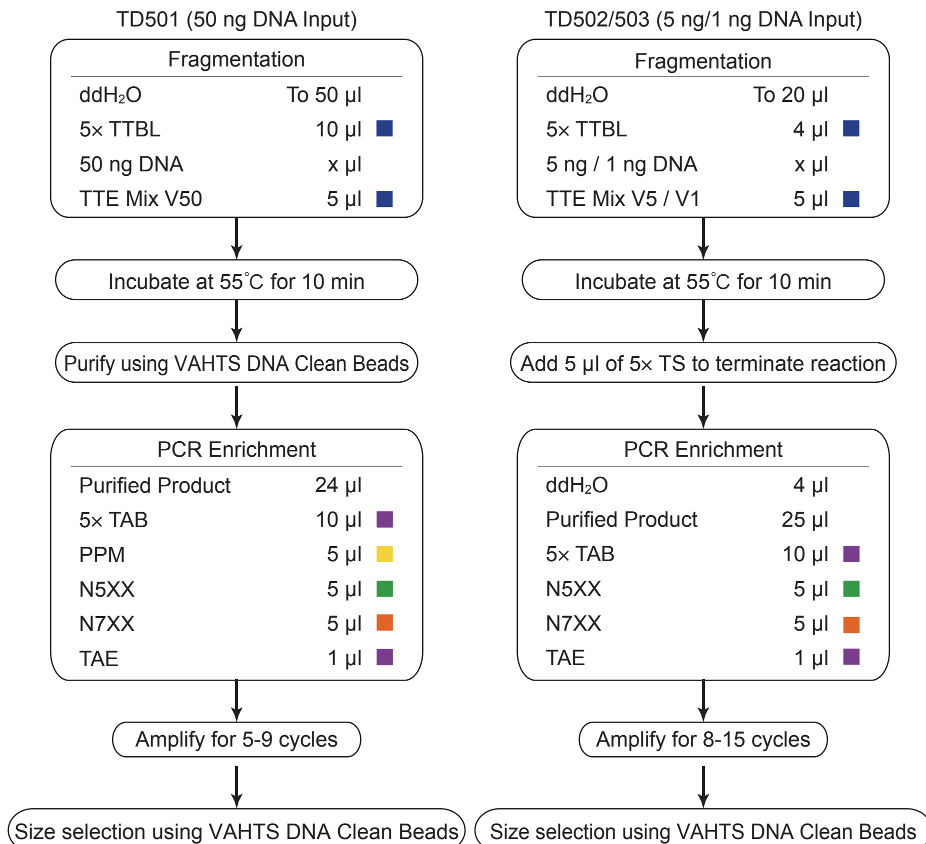


Adapter 1 and Adapter 2: two oligos embedded in TruePrep Tagment Enzyme.
P5 and P7: two universal PCR Primers.
N5 and N7: two index primers containing index2 (i5) and index 1 (i7) respectively.

Mechanism of Library Preparation with TruePrep

TruePrep Tagment Enzyme Mix (TTEMix) contains transposase and two kinds of adapters (Adapter 1 and Adapter 2) with equal molar. Input DNA are fragmented and linked with adapters on both ends just by mixing with TTE Mix, followed by a 10-minute incubation at 55°C. The tagged DNA fragments can be further amplified with two pairs of primers N5 (N5XX) / N7 (N7XX) and P5 / P7 (PCR Primer Mix, PPM). After size selection and purification, the library is ready for sequencing on the Illumina platform.

Overview of the Workflow



Library Structure

Structure of libraries prepared using TruePrep DNA Library Prep Kit V2 for Illumina®:

Index 2 (i5)

5'-AATGATACGGCGACCACCGAGATCTACACIIIIIIIITCGTCGGCAGCGTCAGATGTGTATAAGAG
ACAG-NNNNNN-CTGTCTCTTATACACATCTCCGAGCCCACGAGACIIIIIIIIATCTCGTATGCCGTC
TTCTGCTTG-3'

Index 1 (i7)

IIIIIIII: Index 2 (i5), 8 bases
IIIIIIII: Index 1 (i7), 8 bases
-NNNNNN-: Insert Sequence

Protocol

Starting Material: Input DNA dissolved in sterile distilled water.

Determination of DNA Concentration: Since TTE Mix is sensitive to the concentration of DNA, the accuracy of DNA concentration determination is important to the success of the experiment. It is recommended to take advantage of Qubit® or PicoGreen® to determine the concentrations of DNA. Please avoid using methods based on the absorbance.

Requirement for DNA Purity: The absorbance of 260 nm / 280 nm = 1.8 - 2.0.

1. DNA Tagmentation (select according to the kit Cat. No.)

1-A: For 50 ng of input DNA (Cat. No. #TD501)

a. Thaw the 5 × TTBL at room temperature and mix thoroughly by inverting the sealed tube.
b. Add the following components to a sterile PCR tube in order:

ddH_2O	To 50 µl	
5 × TTBL	10 µl	■
50 ng DNA	x µl	
TTE Mix V50	5 µl	■

c. Mix thoroughly by pipetting gently for 20 times **(IMPORTANT!)**

d. Put the PCR tube into a PCR instrument and run the following program:

Heat Lid	On, 105°C
55°C	10 min
10°C	Hold

e. Purify the DNA fragments with VAHTS DNA Clean Beads:

①. Suspend the **VAHTS DNA Clean Beads** thoroughly by vortexing, and pipet 50 µl of beads into 50 µl fragmentation products. Mix thoroughly by gently pipetting up and down for 10 times. Incubate at room temperature for 5min.

②. Spin down the tube briefly to collect the liquid at bottom. Put the PCR tube on the magnetic stand to separate the beads and liquid. Wait until the solution becomes clear (about 5 min), and then carefully discard the supernatant.

③. Keep the tube on the magnetic stand. Add 200 µl of freshly prepared 80% ethanol to rinse the beads. Incubate at room temperature for 30 sec and **carefully discard** the supernatant.

④. Repeat the Step ③.

⑤. Keep the tube on the magnetic stand, open the EP tube lid and air-dry the beads for 10 min.

⑥. Take the EP tube off from magnetic stand. Add 26 µl of sterile ultra-pure water into the tube to elute DNA. Mix by vortexing or gently pipetting. Collect liquids at the bottom of the tube by a brief centrifugation, and then put the tube back on the magnetic stand. Wait until the solution becomes clear (about 5 min). Transfer 24 µl of supernatant to a new PCR tube carefully.

Note: The fragmented and tagged DNA can be purified with other methods, including magnetic beads or column.

⑦. Proceed to **Step 2. PCR Enrichment** immediately.

1-B: For 5 ng of input DNA (Cat.No. #TD502)

a. Thaw the 5× TTBL at room temperature and mix thoroughly by inverting the sealed tube. Bring the 5× TS to room temperature, and flick the tube wall to confirm if there is any possible precipitation. Precipitation can be dissolved by incubating at 37°C and vortexing.

b. Add the following components into a sterile PCR tube in order.

ddH_2O	To 20 μl	
5 × TTBL	4 μl	■
5 ng DNA	x μl	
TTE Mix V5	5 μl	■

c. Mix thoroughly by pipetting gently for 20 times **(IMPORTANT!)**

d. Put the PCR tube into a PCR instrument and run the following program:

Heat Lid	On, 105°C
55°C	10 min
10°C	Hold

e. Immediately pipet 5 μl of 5× TS into the PCR tube. Mix thoroughly by gently pipetting up and down. Incubate at room temperature for 5 min.

f. Proceed to **Step 2. PCR Enrichment** immediately.

1-C: For 1 ng input DNA (Cat.No. #TD503)

a. Thaw the 5× TTBL at room temperature and mix thoroughly by inverting the sealed tube. Bring the 5× TS to room temperature, and flick the tube wall to confirm if there is any possible precipitation. Precipitation can be dissolved by incubating at 37°C and vortexing.

b. Add the following components into a sterile PCR tube in order.

ddH_2O	To 20 μl	
5 × TTBL	4 μl	■
1 ng DNA	x μl	
TTE Mix V1	5 μl	■

c. Mix thoroughly by pipetting gently for 20 times **(IMPORTANT!)**

d. Put the PCR tube into a PCR instrument and run the following program:

Heat Lid	On, 105°C
55°C	10 min
10°C	Hold

e. Immediately pipet 5 μl of 5× TS into the PCR tube. Mix thoroughly by gently pipetting up and down. Incubate at room temperature for 5 min.

f. Proceed to **Step 2. PCR Enrichment** immediately.

2. PCR Enrichment

2.1. Put the sterile PCR tube in the ice bath and add the following components in order:

Cat.No.	TD501		TD502 / TD503	
ddH_2O	------		4 μl	
Products of **Step 1**	24 μl		25 μl	
5×TAB	10 μl	■	10 μl	■
PPM	5 μl	■	------	
N5XX*	5 μl	■	5 μl	■
N7XX*	5 μl	■	5 μl	■
TEA	1 μl	■	1 μl	■

*TruePrep Index Kit V2 for Illumina® (Vazyme, #TD202) provides 8 kinds of N5XX and 12 kinds of N7XX;
TruePrep Index Kit V3 for Illumina® (Vazyme, #TD203)provides 16 kinds of N6XX and 24 kinds of N8XX;
Select according to the number of samples and the strategy of Index selection.

2.2. Gently pipet up and down to mix thoroughly. Put the PCR tube into a PCR instrument and run the following program:

	Heat Lid	On, 105˚C
	72˚C*	3 min
	98˚C	30 sec
5-15 cycles*	98˚C	15 sec
	60˚C	30 sec
	72˚C	3 min
	72˚C	5 min
	4˚C	Hold

*Incubation at 72˚C for 3 min is important for chain displacement reaction. Please DO NOT skip this step.
*The amplification cycle numbers should be adjusted accordingly, and the selection principle is as follows:
For 50 ng input DNA (#TD501), 5-9 cycles are recommended.
For 5 ng input DNA (#TD502), 8-12 cycles are recommended.
For 1 ng input DNA (#TD503), 11-15 cycles are recommended.

Note: The fewer amplification cycles, the less duplication can be achieved. Accordingly, the yield of the library will be lower. Please refer to the appendix for estimation of the amount of output DNA with different amount of input DNA and different amplification cycles.

2.3. Proceed to **Step 3. Purify the amplified library with VAHTS DNA Clean Beads** for size selection.

3. Purify the amplified library with VAHTS DNA Clean Beads

It is recommended to use **VAHTS DNA Clean Beads** to select and purify the amplified library. Equilibrate the beads to room temperature before use.

Before the next step, make up the volume to 50 μl with distilled water, for the product volume would be less than 50 μl due to the evaporation during PCR. It is important to make sure that the total volume is 50 μl, otherwise unexpected selected size may be obtained.

For the volume of beads needed in 1st round (R1)and 2nd round (R2) in selection process, please refer to the following table:

Average full length of the library	~ 350 bp	~ 450 bp	~ 550 bp
Average insert length of the library	~ 230 bp	~ 330 bp	~ 430 bp
Range of full length of the library	250 bp - 450 bp	300 bp - 700 bp	400 bp - 900 bp
Volume of beads in the 1st round	R1 = 35.0 μl (0.70 ×)*	R1 = 30.0 μl (0.60 ×)	R1 = 25.0 μl (0.50 ×)
Volume of beads in the 2nd round	R2 = 7.5 μl (0.15 ×)	R2 = 7.5 μl (0.15 ×)	R2 = 7.5 μl (0.15 ×)

* "×" were calculated from the volume of PCR products, for example, "0.60×" indicates 0.60 × 50 μl = 30.0 μl.

1. Suspend the VAHTS DNA Clean Beads thoroughly by vortexing, and pipet R1 volume of beads into 50 μl PCR products. Mix thoroughly by gently pipetting up and down for 10 times. Incubate at room temperature for 5 min.
2. Spin down the tube briefly to collect the liquid at the bottom for the tube. 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 VAHTS DNA Clean Beads thoroughly by vortexing, and transfer pipet R2 volume of beads into the supernatant collected in the precious step. Mix thoroughly by gently pipetting up and down for 10 times. Incubate at room temperature for 5 min.
4. Spin down the tube briefly to collect the liquid at the bottom of the tube. 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 separation rack. Add 200 μl of freshly prepared 80% ethanol to rinse the beads. Incubate at room temperature for 30 sec, and then discard the supernatant carefully.
6. Repeat the step 5.
7. Keep the tube on the magnetic stand, open the tube lid and air-dry the beads for 10 min.
8. Take the EP tube off from magnetic stand. Add 22 μl of sterile ultrapure water to elute DNA. Mix by vortexing or gently pipetting. Collect liquids at the bottom of the tube by a brief centrifugation, and then put the tube on the magnetic stand. Wait until the solution becomes clear (about 5 min), transfer 20 μl of supernatant into a new tube carefully. The purified library can be stored at -20˚C.

For a more concentrated library sized istribution, the amplification product can be size-selected and purified with a gel extraction kit. On the other hand, if there is no special need for the distribution range of library size, the amplification products can be directly purified without size selection using beads or column purification kits.

4. Library Quality Control

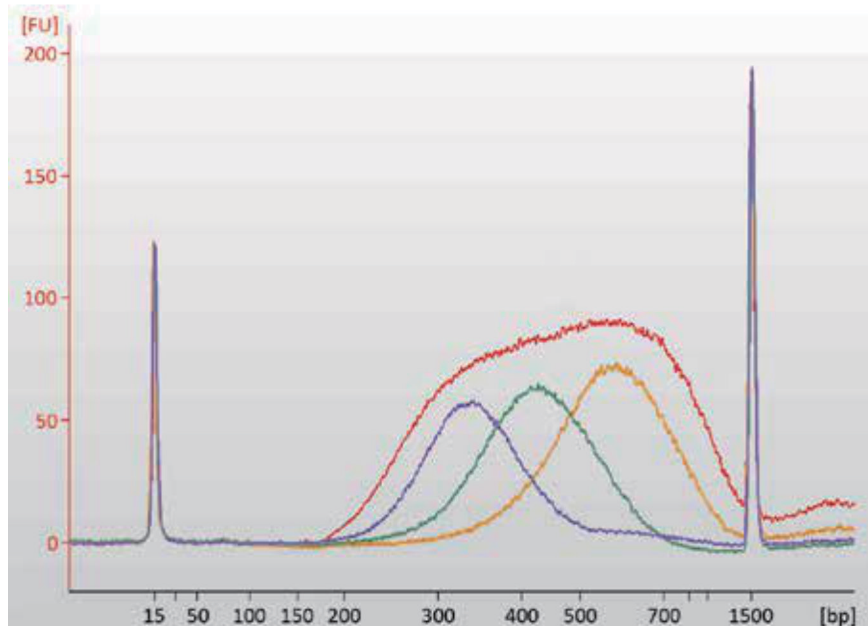
Library Concentration

In order to obtain a high quality of sequencing data, it is necessary to determine the accuracy concentration of the library. Real-time PCR was recommended to definitely quantify the concentration of the library. Besides, fluorescent dye methods (such as Qubit® and PicoGreen®) based on special recognition on double stranded DNA can also be utilized. It is recommended to use the approximate formulas in the following table to calculate the molar concentration of the library:

Average full length of the library	Approximate Formula
350 bp	1 ng / μl = 4.3 nM
450 bp	1 ng / μl = 3.3 nM
550 bp	1 ng / μl = 2.7 nM

Library Distribution

The library distribution is determined using an Agilent Technologies 2100 Bioanalyzer.



Human genomic library prepared with TruePrep TM DNA Library Prep Kit V2 for Illumina®(TD501, 9 cycles of PCR Amplification) was analyzed by an Agilent Technologies 2100 Bioanalyzer.

Red line: Library prepared without size selection and purified directly with1 × beads.
Purple line: 350 bp size of library obtained from size selection with beads.
Green line: 450 bp size of library obtained from size selection with beads.
Yellow line: 550 bp size of library obtained from size selection with beads.

Tips

1. VAHTS DNA Clean Beads Tips

Equilibrate the beads to room temperature before use.
Mix the beads thoroughly every time before pipetting.
Thoroughly mix the beads with DNA samples.
All the DNA size selection and procedures using beads should be performed at room temperature.
Do not pipet any VAHTS DNA Clean Beads when transferring th esupernatant.
Prepare fresh 80% ethanol and discard after use.
Try to remove all the 80% ethanol after washing.
Thoroughly air-dry the beads before DNA elution.

2. Avoid cross contamination between samples.

Change tips between samples.
Use filtered pipette tips.

3. Aliquot reagents after the first use to avoid repeated freeze-thaw cycles.

4. Prevent contamination of PCR products.

Isolate the experimental area and carefully clean all equipments and instruments (e.g. clean with 0.5% sodium hypochlorite or 10% bleach)to avoid possible contamination in PCR system.

Appendix

Referenced table of library output of TruePrep DNA Library Prep Kit V2 for Illumina®:

Amplification cycles using TD501 (50 ng Input DNA):	5	6	7	8	9
Amplification cycles using TD502 (5 ng Input DNA):	8	9	10	11	12
Amplification cycles using TD503 (1 ng Input DNA):	11	12	13	14	15
Library Output (No Size Selection, ng)*:	250	400	600	1000	1500
Library Output (With Size Selection, ng)*:	100	150	250	500	800

* The “ng” stated in the table is the total mass of the library. The quality concentration of the library can be calculated by dividing the volume of the library. The mole concentration of the library can be calculated from quality concentration of the library according the average size of library.

ISO 9001: 2015