TruePrep DNA Library Prep Kit V2 for Illumina

NB-54-0256
NB-54-0380
NB-54-0381

Contents

TruePrep DNA Library Prep Kit V2 for Illumina

#Cat: NB-54-0256-01 Size: 24rxns
#Cat: NB-54-0380-01/02 Size: 24/96rxns
#Cat: NB-54-0381-01/02 Size:24/96rxns

01/Product Description

TruePrep DNA Library Prep Kit V2 for Illumina is specifically designed for the Illumina high- throughput sequencing platform. The kit can be used to prepare DNA samples into sequencing libraries for Illumina high-throughput sequencing platform. Compared with traditional library construction methods, TruePrep kit uses a novel transposase method for DNA fragmentation, which transforms the tedious steps of DNA Fragmentation, End Repair and Adapter Ligation into a simple one-step enzymatic reaction, significantly reducing the demanded amount of the initial DNA and shortening the time of library preparation. The kit has three specifications, which are suitable for reactions with initial template DNA input of 1 ng. The user can freely choose according to the type of experiment. All the reagents provided in the kit have undergone rigorous quality control and functional testing, to ensure the optimal stability and repeatability of library construction.

02/Components

Cat.No. Size Input DNA	NB-54-0380-01/02 24/96 rxns 50 ng	NB-54-0381-01/02 24/96 rxns 5 ng	NB-54-0256-01 24 rxns 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
Control DNA	10/10 µl	10/10 µl	10/10 µl

▲The Color marked in the product components table represents the color of each tube lid.

▲ 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).

03/Storage

NB-54-0380: Store at -30 ~ -15°C and transport at ≤0°C.

NB-54-0381: Store 5 × TS at 15 ~ 25°C, store other components at -30 ~ -15°C and transport at ≤0°C.

NB-54-0256-01: Store 5 × TS at 15 ~ 25°C, store other components at -30 ~ -15°C and transport at ≤0°C.

04/Applications

This kit is suitable for preparing purified DNA samples into Illumina high-throughput sequencing platform dedicated libraries.

▲ If the DNA sample is a PCR product, the length should be >500 bp. Since transposase cannot act on the end of DNA, the sequencing coverage of the 50 bp at the end of the PCR product may be reduced. 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.

05/Self-prepared Materials

VAHTS DNA Clean Beads (Neo Biotech#NB-54-0060);
Magnetic stand;
PCR thermocycler;
Low absorption EP tubes and PCR tubes;
Absolute ethanol;
ddH_2O;
TruePrep Index Kit V2 for Illumina (Neo Biotech# NB-54-0194) or TruePrep Index Kit V3 for Illumina (Neo Biotech #NB-54-0195).

06/Notes

For research use only. Not for use in diagnostic procedures.

1. **Precautions for magnetic beads operation:**
   Equilibrate the beads to room temperature before use. All magnetic beads operations should be performed at room temperature. Do not store the magnetic beads at -20°C; The magnetic beads should be vortexed and mixed thoroughly each time before absorbing magnetic beads, and the DNA sample should be fully mixed with the magnetic beads after adding the magnetic beads;
   The supernatant should be removed carefully after the magnetic beads are completely adsorbed to avoid the influence of magnetic beads on subsequent experiments;
   Prepare fresh 80% ethanol. After rinsing the magnetic beads, try to absorb the remaining ethanol;

The magnetic beads should be fully dried before elution (the surface of the magnetic beads changed from bright brown to frostiness brown) to avoid ethanol residue affecting the subsequent experiments. However, avoid over-drying of beads, which resulting in the reduce of recovery efficiency.

2. Precautions for cross contamination of samples:

Change tips between samples;

Use tips with filter element.

3. Precautions for PCR products contamination:

It is recommended to isolate PCR reaction preparation area and PCR products detection area physically;

Use dedicated pipettors and other equipments;

Regularly clean all equipments and instruments (e.g. clean with 0.5% sodium hypochlorite or 10% bleach).

4. Precautions for reagent use

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

07/Sample Preparation

1. Starting material:

Dissolve purified DNA in ddH_2O.

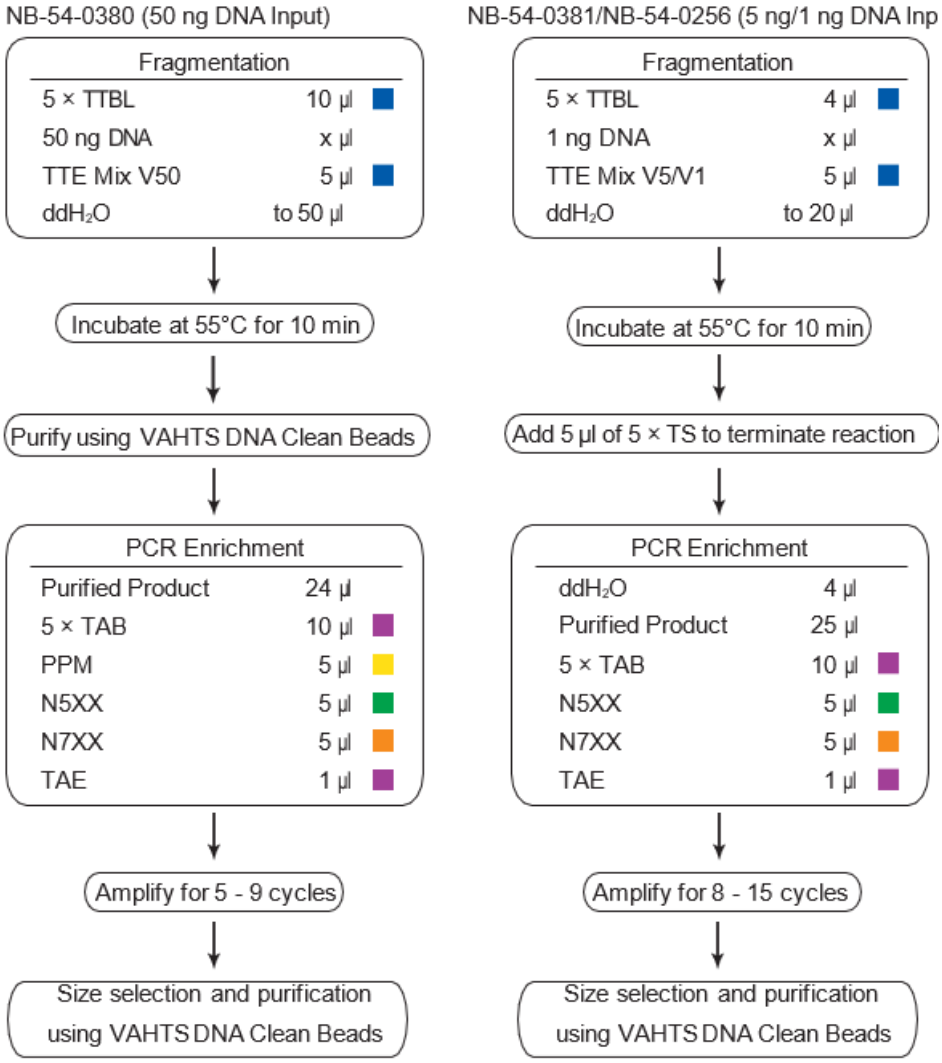
2. DNA concentration determination:

Since TTE Mix is very sensitive to DNA concentration, accurate DNA concentration determination is crucial to the success of the experiment. It is recommended to use Qubit or the fluorescent dye PicoGreen for the concentration determination of DNA samples. Do not use any method based on absorbance measurements.

3. DNA purity requirements:

A260/A280 = 1.8 - 2.0

08/Workflow & Library Structure



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

09/Experiment Process

Please read this instruction carefully before the experiment. This instruction is applicable to three kinds of kits, corresponding to the initial template DNA input of 50 ng, 5 ng and 1 ng respectively. Some components in the three specifications of the kit are different, and the corresponding reaction system is slightly different. In order to avoid unnecessary waste or delay of the experimental progress, it is not allowed to be mixed in the experimental operation process.

09-1 /DNA Fragmentation (Select according to the kit Cat. No.)

1-A: 50 ng initial DNA fragmentation (For kit NB-54-0380)

1. Thaw the 5x TTBL at room temperature and mix upside down for later use.

2.Prepare the following components in a sterile PCR tube:

Components	Volume
5 × TTBL	10 µl
50 ng DNA	x µl
TTE Mix V50	5 µl
ddH_2O	to 50 µl

3. Mix thoroughly by pipetting gently for 20 times. (**IMPORTANT!)**
4. Put the PCR tube into a PCR instrument and run the following program:

Temperature	Time
Hot lid of 105°C	On
55°C	10 min
10°C	Hold

▲ Purification of the product should be carried out immediately after the completion of the reaction, otherwise the DNA sample will be excessively fragmented, resulting in a smaller final library fragment.

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

① Vortex and shake to mix VAHTS DNA Clean Beads and pipet 50 μl of beads into 50 μl fragmented products. Mix thoroughly by gently pipetting up and down for 10 times. Incubate at room temperature for 5 min.

②Put 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.

③Keep 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.

④Repeat the Step ③ and rinse twice in total.

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

⑥ake the EP tube off from magnetic stand. Add 26 μl of ddH_2O into the tube to elute DNA. Mix by vortexing or gently pipetting up and down for 10 times. Incubate at room temperature for 5 min.

⑦Centrifuge the reaction tube briefly and place it on a magnetic stand to separate the magnetic beads from the liquid. Wait until the solution becomes clear (about 5 min). Transfer 24 μl of supernatant to a new PCR tube carefully.

▲ In addition, the fragmented product can also be purified using other magnetic beads or column purification kits.

6. Proceed to Step 09-2/PCR Enrichment immediately.

1-B: 5 ng initial DNA fragmentation (For kit NB-54-0381)

1. thaw the 5 × TTBL at room temperature and mix upside down for later use. Check that the 5 × TS is at room temperature and flick the tube wall to confirm if there is any possible precipitation. If there is precipitation, the precipitation can be dissolved by incubating at 37°C and vortexing.

2.Prepare the following components in a sterile PCR tube.

Components	Volume
5x TTBL	4 µl
5ng DNA	x µl
TTE Mix V5	5 µl
ddH_2O	to 20µl

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

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

Temperature	time
Hot lid of 105°C	On
55°C	10 min
10°C	Hold

1- C: 1 ng initial DNA fragmentation (For kit NB-54-0256-01)

1. Thaw the 5 × TTBL at room temperature and mix upside down for later use. Check that the 5 × TS is at room temperature and flick the tube wall to confirm if there is any possible precipitation. If there is precipitation, the precipitation can be dissolved by incubating at 37°C and vortexing.

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

Components	Volume
5x TTBL	4 µl ■
1ng DNA	x µl
TTE Mix V1	5 µl ■
ddH_2O	to 20µl

3. Mix thoroughly by pipetting gently for 20 times. (**IMPORTANT!**)
4. Put the PCR tube into a PCR instrument and run the following program:

Temperature	time
Hot lid of 105°C	On
55°C	10 min
10°C	Hold

5. Immediately add 5 µl of 5 × TS to the product after the reaction is completed. Mix thoroughly by gently pipetting up and down. Incubate at room temperature for 5 min.

▲ After completion of the reaction, 5 × TS should be added immediately to terminate the reaction, otherwise the DNA sample will be excessively fragmented, resulting in a small final library fragment.

6. Perform step 09-2/PCR Enrichment immediately.

09-2/ PCR Enrichment

1. Put the sterile PCR tube on ice to prepare the following reaction system:

Cat.No.	NB-54-0380	NB-54-0381/ NB-54-0256
ddH_2O	------	4 µl
Product from step 09-1	24 µl	25 µl
5 × TAB	10 µl	10 µl
PPM	5 µl	------
N5XX*	5 µl	5 µl
N7XX*	5 µl	5 µl
TAE	1 µl	1 µl

* TruePrep Index Kit V2 for Illumina (Neo Biotech # NB-54-0194) provides 8 kinds of N5XX and 12 kinds of N7XX; Select according to the number of samples and the strategy of Index selection.

2. Use a pipette to gently pipette and mix well. Put the PCR tube into a PCR instrument and run the following program:

Temperature	Time	Cycles
Hot lid of 105°C	On	
72°C*	3 min	
98°C	30 sec	
98°C 60°C 72°C	15 sec 30 sec 3 min	5-15
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 selected according to the actual situation, and the selection principle is as follows:

Input DNA	Applicable Kit	Cycles
50 ng	NB-54-0380	5-9
5 ng	NB-54-0381	8-12
1 ng	NB-54-0256-01	11 - 15

▲ The fewer amplification cycles, the lower the amplification Duplication, but also the corresponding lower library yield. Please refer to the 09-4/Library Quality Control for estimation of the amount of

3. Perform step 09-3/Size Selection of Amplified Products after PCR program.

09-3/Size Selection of Amplified Products

It is recommended to use VAHTS DNA Clean Beads for size selection of amplified products. Equilibrate the beads to room temperature before use.

▲The initial PCR product volume is 50 µl, and the product volume is less than 50 µl due to sample evaporation during PCR. Before size selection, ddH2O must be used to make up the volume to 50 µl, otherwise the length of the sorted fragments may be inconsistent with expectations.

Refer to the following table for the amount of magnetic beads (R1 and R2) used in both rounds of amplification products size selection:

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

1. Vortex and shake to mix VAHTS DNA Clean Beads and pipette the volume of R1 to 50 µl PCR product. Mix thoroughly by gently pipetting up and down for 10 times. Incubate at room temperature for 5 min.

▲Since magnetic beads are sticky, please pipette the appropriate volume accurately, otherwise the length of the selected fragments may be inconsistent with the expected.

2. The reaction tube is briefly centrifuged and placed on a magnetic rack to separate the beads from the liquid. Wait until the solution becomes clear (about 5 min), carefully transfer the supernatant to a new tube and discard the beads.

3.Vortex and shake to mix VAHTS DNA Clean Beads and pipette R2 volume into the supernatant. Mix thoroughly by gently pipetting up and down for 10 times. Incubate at room temperature for 5 min.

4.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 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 and rinse twice in total.

7. keep the tube on the magnetic stand, open the tube lid and air-dry the beads for 5 min.

8.Take the EP tube off from magnetic stand. Add 22 µl of ddH2O to elute DNA. Mix by vortexing or gently pipetting up and down for 10 times. Incubate at room temperature for 5 min.

9.Centrifuge the reaction tube briefly and place it on a magnetic stand to separate the magnetic beads from the liquid. Wait until the solution becomes clear (about 5 min), transfer 20 µl of supernatant into a new tube carefully and store at -20°C.

In addition, if you need to obtain a library with a more concentrated length distribution, the amplified products can be sorted and purified using a gel recovery kit. If there are no special requirements for the length distribution range of the library, the amplified products can be purified directly using magnetic beads or column purification kits without size selection.

09-4/Library Quality Control

Library Concentration Determination

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. Do not use any method based on absorbance measurements.

Average Total length of the Library	Approximate Conversion 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

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

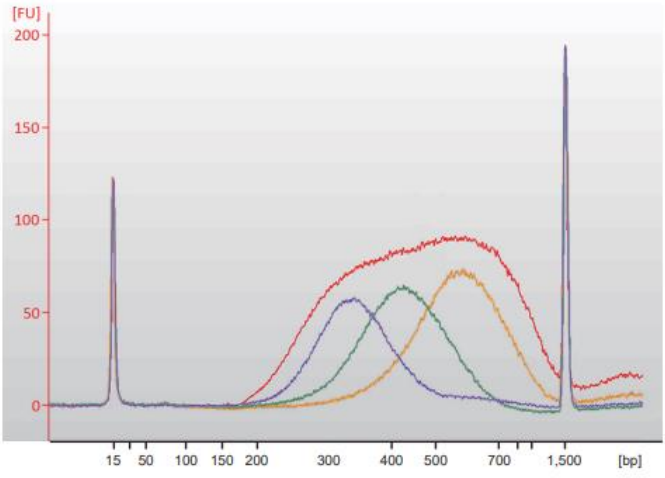
Amplification Cycles Using NB-54-0380 (50 ng Input DNA):	5	6	7	8	9
Amplification Cycles Using NB-54-0381 (5ng Input DNA):	8	9	10	11	12
Amplification Cycles Using NB-54-0256-01 (1 ng Input DNA):	11	12	13	14	15
Library Output (No Size Selection, ng):	250	400	600	1,000	1,500
Library Output (With Size Selection, ng):	100	150	250	500	800

▲The ng number in the table is the total mass of the library. The library mass concentration can be calculated by dividing this value by the library final volume. The mole concentration of the library can be

calculated from quality concentration of the library according the average size of library.

Library Length Distribution Detection

Detect the length distribution of the prepared libraries on an Agilent 2100 Bioanalyzer.



Library quality analysis with Agilent 2100 Bioanalyzer

Human genome library was prepared using TruePrep DNA Library Prep Kit V2 for Illumina (NB-54-0380, 9 cycles of PCR Amplification).

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.

10/FAQ & Troubleshooting

◇ Does the kit contain an interruption step?

Contains. The TruePrep kit use a new-type transposase, which convert the complex steps of DNA fragmentation, end repair, and adapter ligation into a one-step enzymatic reaction, significantly shortening the time of library preparation.

◇ Does the TruePrep kit have a bias for using transposase to disrupt DNA?

There is a certain bias when transposase was interrupted, but the test showed that it did not affect the sequencing results and data analysis.

◇ Can NB-54-0381 or NB-54-0256-01 be used to construct a 50 ng initial DNA library?

It is not recommended to do. For NB-54-0380, NB-54-0381 and NB-54-0256-01, the template input is and 50 ng, 5 ng and1 ng respectively. And the enzyme concentrations in the corresponding kits are different. If NB-54-0381 or NB-54-0256-01 is used to construct a DNA library starting from 50 ng, the template DNA fragment will be incomplete or the size will be too large, resulting in the final library far from the expected. Therefore, it is strongly recommended to select products of corresponding specifications according to the actual DNA starting quantity.

◇ When using NB-54-0256-01 to construct DNA library, can PCR library amplification

be performed directly without magnetic beads purification or TS solution terminating the reaction after 10 min reaction at 55°C?

Please follow the instructions for purification or termination of the reaction, otherwise the fragmentation reaction cannot be effectively terminated, which may result in a smaller total length of the final library than expected and poor library quality.

◇ How many index combinations can the TruePrep series provide?

A maximum of 384 index combinations are available. TruePrep Index Kit V2 for Illumina (Neo Biotech # NB-54-0194) contains 8 kinds of N5XX and 12 kinds of N7XX, providing 96 index combinations. TruePrep Index Kit V3 for Illumina (Neo Biotech # NB-54-0195) contains 16 N6XX and 24 N8XX and provides 384 index combinations.