

## Product Description

TruePrep Index Kit V2 for Illumina is specially designed for TruePrep DNA Library Prep Kit V2 for Illumina (Vazyme #TD501-TD503), UltraClean DNA Library Prep Kit V2 for Illumina (Vazyme #UTD501/UTD502/UTD503) and TruePrep Flexible DNA Library Prep Kit for Illumina (Vazyme #TD504). It contains 8 kinds of N5XX and 12 kinds of N7XX, providing 96 kinds of different dual-indexed adapter combinations. All the reagents provided in the kit have undergone rigorous quality control and functional testing to ensure the optimal stability and repeatability of library preparation.

## Components

| Components | Specifications<br>(192 rxns) | Index Sequence | Sample Sheet                           |                                                                      | Index Name |                |
|------------|------------------------------|----------------|----------------------------------------|----------------------------------------------------------------------|------------|----------------|
|            |                              |                | NovaSeq 1.0, MiSeq,<br>HiSeq 2000/2500 | NovaSeq 1.5, MiniSeq, NextSeq,<br>HiSeq 3000/4000, HiSeq X, iSeq 100 |            |                |
| N5XX       | N502                         | 120 µl         | CTCTCTAT                               | CTCTCTAT                                                             | ATAGAGAG   | ■              |
|            | N503                         | 120 µl         | TATCCTCT                               | TATCCTCT                                                             | AGAGGATA   | ■              |
|            | N505                         | 120 µl         | GTAAGGAG                               | GTAAGGAG                                                             | CTCCTTAC   | ■              |
|            | N506                         | 120 µl         | ACTGCATA                               | ACTGCATA                                                             | TATGCAGT   | Index 2 (i5) ■ |
|            | N507                         | 120 µl         | AAGGAGTA                               | AAGGAGTA                                                             | TACTCCTT   | ■              |
|            | N508                         | 120 µl         | CTAAGCCT                               | CTAAGCCT                                                             | AGGCTTAG   | ■              |
|            | N509                         | 120 µl         | TTCTAGCT                               | TTCTAGCT                                                             | AGCTAGAA   | ■              |
|            | N510                         | 120 µl         | CCTAGAGT                               | CCTAGAGT                                                             | ACTCTAGG   | ■              |
| N7XX       | N701                         | 80 µl          | TAAGGCGA                               | TAAGGCGA                                                             | TAAGGCGA   | ■              |
|            | N702                         | 80 µl          | CGTACTAG                               | CGTACTAG                                                             | CGTACTAG   | ■              |
|            | N703                         | 80 µl          | AGGCAGAA                               | AGGCAGAA                                                             | AGGCAGAA   | ■              |
|            | N705                         | 80 µl          | GGACTCCT                               | GGACTCCT                                                             | GGACTCCT   | ■              |
|            | N706                         | 80 µl          | TAGGCATG                               | TAGGCATG                                                             | TAGGCATG   | ■              |
|            | N707                         | 80 µl          | CTCTCTAC                               | CTCTCTAC                                                             | CTCTCTAC   | ■              |
|            | N711                         | 80 µl          | AAGAGGCA                               | AAGAGGCA                                                             | AAGAGGCA   | Index 1 (i7) ■ |
|            | N712                         | 80 µl          | GTAGAGGA                               | GTAGAGGA                                                             | GTAGAGGA   | ■              |
|            | N714                         | 80 µl          | ATCTCAGG                               | ATCTCAGG                                                             | ATCTCAGG   | ■              |
|            | N715                         | 80 µl          | ACTCGCTA                               | ACTCGCTA                                                             | ACTCGCTA   | ■              |
|            | N716                         | 80 µl          | GGAGCTAC                               | GGAGCTAC                                                             | GGAGCTAC   | ■              |
|            | N717                         | 80 µl          | CGATCAGT                               | CGATCAGT                                                             | CGATCAGT   | ■              |

▲ The usage of N5XX and N7XX were respectively 5 µl in individual DNA library.

▲ The color marked in the components table represents the color of each tube cover.

## Storage

Store at -30 ~ -15°C and transport at ≤0°C.

## Application

Special for TruePrep DNA Library Prep Kit V2 for Illumina (Vazyme #TD501-TD503), UltraClean DNA Library Prep Kit V2 for Illumina (Vazyme #UTD501/UTD502/UTD503) and TruePrep Flexible DNA Library Prep Kit for Illumina (Vazyme #TD504).

## Notes

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

1. The N5XX index sequences of NovaSeq 1.5, MiniSeq, NextSeq, HiSeq 3000/4000, HiSeq X, iSeq 100 sequencing platforms need to be input in the sample sheet after reverse complementation.
2. Pay attention to prevent crosstalk between different indexes.



## Library Structure and Sequences

The structure of DNA library constructed by TruePrep Index Kit V2 for Illumina is as follows:

5' - **Index 2 (i5)** - Insert DNA Sequence - **Index 1 (i7)** - 3'

The kit contains 8 kinds of N5XX and 12 kinds of N7XX, which can be combined into 96 different double ended index combinations for distinguishing different samples during high-throughput sequencing.

## Strategy of Index Selection

Green fluorescent labeled dG/dT and red fluorescent labeled dC/dA were used in Illumina. To ensure successful sequencing, both green and red fluorescent signal must be detected in each sequencing cycle. Therefore, it is important to keep balance of the green and red fluorescence signals when choosing the indexes.

The following are some recommended combination of the indexes:

| Number of Sample(s) | N7XX Option [Index 1 (i7)]                                      | N5XX Option [Index 2 (i5)]    |
|---------------------|-----------------------------------------------------------------|-------------------------------|
| 1                   | Any N7XX                                                        | Any N5XX                      |
| 2                   | N701 and N702                                                   | Any N5XX                      |
| 3                   | Option 1: N701, N702 and N703                                   | Any N5XX                      |
|                     | Option 2: N705, N706 and N707                                   | Any N5XX                      |
| 4 - 5               | Option 1: N701, N702, N703 and any other N7XX                   | Any N5XX                      |
|                     | Option 2: N705, N706, N707 and any other N7XX                   | Any N5XX                      |
| 6                   | N701, N702, N703, N705, N706 and N707                           | Any N5XX                      |
| 7 - 12              | Option 1: N701, N702, N703, N705, N706, N707 and any other N7XX | Any N5XX                      |
|                     | Option 2: N701, N702, N703 and any other N7XX                   | N505 and N506                 |
|                     | Option 3: N705, N706, N707 and any other N7XX                   | N505 and N506                 |
| >12                 | N701, N702, N703, N705, N706, N707 and any other N7XX           | N505, N506 and any other N5XX |

The combinations of indexes listed above are only some examples of acceptable selections. In practice, you can check the sequence of each index (refer to the first table) to make sure that there are two kinds of fluorescence signals at each base position, exemplified in the following table.

| Examples of Right Selection |           |      |                |      | Examples of Wrong Selection |                   |           |      |                |      |                |
|-----------------------------|-----------|------|----------------|------|-----------------------------|-------------------|-----------|------|----------------|------|----------------|
| Number of Samples           | Sample ID | N7XX |                | N5XX |                             | Number of Samples | Sample ID | N7XX |                | N5XX |                |
|                             |           |      | [Index 1 (i7)] |      | [Index 2 (i5)]              |                   |           |      | [Index 1 (i7)] |      | [Index 2 (i5)] |
| 4                           | 1         | N705 | GGACTCCT       | N505 | GTAAAGGAG                   | 4                 | 1         | N705 | GGACTCCT       | N502 | CTCTCTAT       |
|                             | 2         | N706 | TAGGCATG       | N505 | GTAAAGGAG                   |                   | 2         | N706 | TAGGCATG       | N502 | CTCTCTAT       |
|                             | 3         | N701 | TAAGGCGA       | N506 | ACTGCATA                    |                   | 3         | N701 | TAAGGCGA       | N503 | TATCCTCT       |
|                             | 4         | N702 | CGTACTAG       | N506 | ACTGCATA                    |                   | 4         | N702 | CGTACTAG       | N503 | TATCCTCT       |
|                             |           |      | √√√√√√√        |      | √√√√√√√                     |                   |           |      | √√√√√√√        |      | √√√√××××       |

▲ √: Have both green and red fluorescence signals.

x: Missing green or red fluorescence signals.

