

I. Introduction

DNA HT dual index kits are designed for use with ThruPLEX® and PicoPLEX® library preparation kits to construct libraries for multiplexed sequencing on Illumina® sequencers. These kits contain indexed PCR primers carrying the Illumina Nextera® XT v2 index sequences and offer a total of 384 dual indexes for multiplexing of up to 384 samples. The indexed PCR primers are supplied pre-dispensed in four different barcoded index plates, each of which contains one-fourth of the possible dual index combinations (Cat. Nos. R400660–R400663); or in a set of 24 individual tubes, each containing a different dual index combination (Cat. No. R400664). Each dual index tube (24N) contains sufficient volume for up to two uses. Each well of a dual index plate (96N Sets A–D) contains sufficient volume for a single use.

II. List of Components

Store all components at -20°C.

Product Name	Cat. No.	Dual index plate	Concentration	Volume per well or tube
DNA HT Dual Index Kit - 96N Set A	R400660	96N Set A	5 µM	5 µl
DNA HT Dual Index Kit - 96N Set B	R400661	96N Set B	5 µM	5 µl
DNA HT Dual Index Kit - 96N Set C	R400662	96N Set C	5 µM	5 µl
DNA HT Dual Index Kit - 96N Set D	R400663	96N Set D	5 µM	5 µl
DNA HT Dual Index Kit - 24N*	R400664	—	5 µM	10 µl

*Contains 24 different dual index primer combinations in individual tubes labeled as shown below.

DNA HT Dual Index Kit - 24N (Cat. No. R400664)	
N1 - DNA HT Dual Index	N13 - DNA HT Dual Index
N2 - DNA HT Dual Index	N14 - DNA HT Dual Index
N3 - DNA HT Dual Index	N15 - DNA HT Dual Index
N4 - DNA HT Dual Index	N16 - DNA HT Dual Index
N5 - DNA HT Dual Index	N17 - DNA HT Dual Index
N6 - DNA HT Dual Index	N18 - DNA HT Dual Index
N7 - DNA HT Dual Index	N19 - DNA HT Dual Index
N8 - DNA HT Dual Index	N20 - DNA HT Dual Index
N9 - DNA HT Dual Index	N21 - DNA HT Dual Index
N10 - DNA HT Dual Index	N22 - DNA HT Dual Index
N11 - DNA HT Dual Index	N23 - DNA HT Dual Index
N12 - DNA HT Dual Index	N24 - DNA HT Dual Index

III. General Considerations

A. Best Practices

- No more than four freeze/thaw cycles are recommended for the DNA HT dual index kits.
- Prior to use, remove the kit from the freezer and thaw for 10 min on the bench top. Spin in a tabletop centrifuge to collect contents at the bottom of the tubes or wells. Thoroughly wipe the tubes or index plate foil seal with 70% ethanol and allow it to dry.

B. Product Compatibility

The DNA HT dual index kits are designed for use with the NGS library preparation kits listed below. Please refer to the kit-specific user manual for instructions on using the indexed PCR primers provided in the DNA HT dual index kits.

- PicoPLEX DNA-seq Kit (Cat. No. R300381)
- ThruPLEX DNA-seq kits (Cat. Nos. R400406, R400407, R400427, R400428 & R400523)
- ThruPLEX Plasma-seq kits (Cat. Nos. R400490, R400491 & R400492)
- ThruPLEX Tag-seq kits (Cat. Nos. R400584, R400585 & R400586)

C. Plate Handling Instructions for Low-Throughput Applications

The DNA HT dual index plates are designed for high-throughput applications; therefore, the experiment should be designed to pool and sequence the full set of 96 dual indexes. If indexes from the entire plate are not used at the same time, it is critical to follow the instructions below to avoid cross-contamination:

- After removing indexing reagents of choice, cover any pierced or used index wells with scientific tape (e.g., VWR, Cat. No. 89097-920, General-Purpose Laboratory Labeling Tape, 0.5”).
- Thoroughly wipe the seal with 70% ethanol and allow it to dry completely.
- Replace the plastic lid, return the index plate to its sleeve and store at -20°C .
- The index plate should not be frozen and thawed more than four times.

D. Multiplexing and Index Pooling

It is very important to select appropriate single indexes that are unique and meet the Illumina-recommended compatibility requirements. For low-plex (2- to 12-plex) pooling guidelines, please refer to Illumina's Nextera Low Plex Pooling Guide (Illumina, Publication Document #770-2011-044).

IV. DNA HT Dual Index Sequences and Sequence Combinations

- The DNA HT dual indexes are 8-nt long and employ the Illumina Nextera XT v2 i5 and i7 dual index sequences (Table 1).

Table 1. DNA HT Dual Index Sequences

i7 Index Name	i7 Bases for Sample Sheet	i5 index	i5 Bases for Sample Sheet (MiSeq®, NovaSeq, HiSeq® 2000/2500)	i5 Bases for Sample Sheet (MiniSeq™, NextSeq®, HiSeq 3000/4000)
N701	TAAGGCGA	S502	CTCTCTAT	ATAGAGAG
N702	CGTACTAG	S503	TATCCTCT	AGAGGATA
N703	AGGCAGAA	S505	GTAAGGAG	CTCCTTAC
N704	TCCTGAGC	S506	ACTGCATA	TATGCAGT
N705	GGACTCCT	S507	AAGGAGTA	TACTCCTT
N706	TAGGCATG	S508	CTAAGCCT	AGGCTTAG
N707	CTCTCTAC	S510	CGTCTAAT	ATTAGACG
N710	CGAGGCTG	S511	TCTCTCCG	CGGAGAGA
N711	AAGAGGCA	S513	TCGACTAG	CTAGTCGA
N712	GTAGAGGA	S515	TTCTAGCT	AGCTAGAA
N714	GCTCATGA	S516	CCTAGAGT	ACTCTAGG
N715	ATCTCAGG	S517	GCGTAAGA	TCTTACGC
N716	ACTCGCTA	S518	CTATTAAG	CTTAATAG
N718	GGAGCTAC	S520	AAGGCTAT	ATAGCCTT
N719	GCGTAGTA	S521	GAGCCTTA	TAAGGCTC
N720	CGGAGCCT	S522	TTATGCGA	TCGCATAA
N721	TACGCTGC			
N722	ATGCGCAG			
N723	TAGCGCTC			
N724	ACTGAGCG			
N726	CCTAAGAC			
N727	CGATCAGT			
N728	TGCAGCTA			
N729	TCGACGTC			

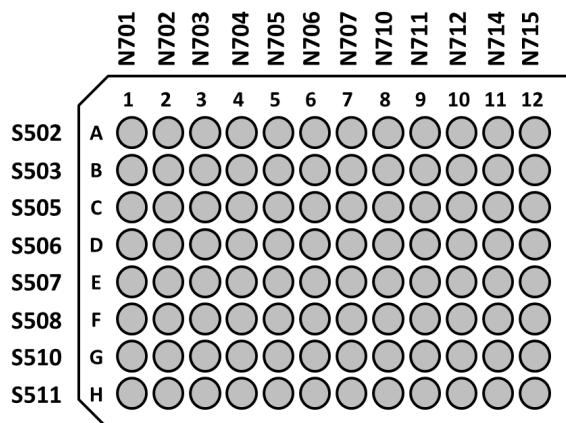
2. Each of the tubes in the **DNA HT Dual Index Kit - 24N** (Cat. No. R400664) contains a unique combination of i7 and i5 index primers (Table 2) carrying the 8-nt dual index sequences shown in Table 1.

Table 2. DNA HT Dual Index Kit – 24N Index Primer Combinations

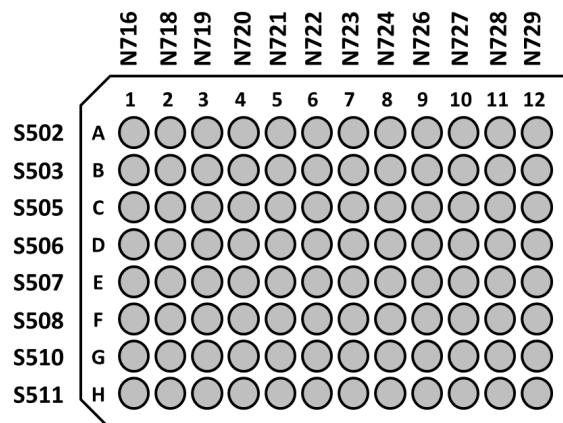
	i7 Index	i5 Index
N1 - DNA HT Dual Index	N701	S505
N2 - DNA HT Dual Index	N702	S505
N3 - DNA HT Dual Index	N703	S505
N4 - DNA HT Dual Index	N704	S505
N5 - DNA HT Dual Index	N705	S505
N6 - DNA HT Dual Index	N706	S505
N7 - DNA HT Dual Index	N701	S506
N8 - DNA HT Dual Index	N702	S506
N9 - DNA HT Dual Index	N703	S506
N10 - DNA HT Dual Index	N704	S506
N11 - DNA HT Dual Index	N705	S506
N12 - DNA HT Dual Index	N706	S506
N13 - DNA HT Dual Index	N701	S508
N14 - DNA HT Dual Index	N702	S508
N15 - DNA HT Dual Index	N703	S508
N16 - DNA HT Dual Index	N704	S508
N17 - DNA HT Dual Index	N705	S508
N18 - DNA HT Dual Index	N706	S508
N19 - DNA HT Dual Index	N701	S517
N20 - DNA HT Dual Index	N702	S517
N21 - DNA HT Dual Index	N703	S517
N22 - DNA HT Dual Index	N704	S517
N23 - DNA HT Dual Index	N705	S517
N24 - DNA HT Dual Index	N706	S517

3. Each well of the **DNA HT dual index kit plates** (Cat. Nos. R400660–R400663) contains a unique combination of i7 and i5 index primers carrying the 8-nt dual index sequences shown in Table 1. The dual index combination at each well position is indicated by the column (i7) and row (i5) labels on the plate maps (Figure 1).

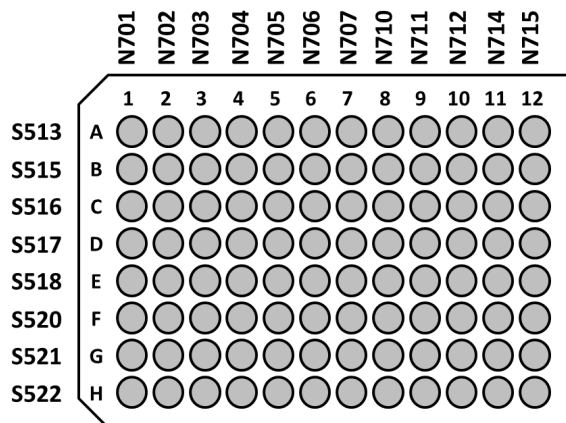
DNA HT Dual Index Kit – 96N Set A



DNA HT Dual Index Kit – 96N Set B



DNA HT Dual Index Kit – 96N Set C



DNA HT Dual Index Kit – 96N Set D

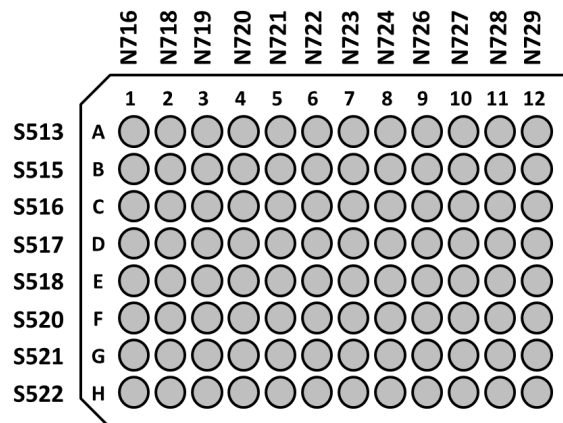


Figure 1. DNA HT dual index kit plate maps.

IMPORTANT: When using the indexed PCR primers provided in the DNA HT dual index kits, make sure to follow the instructions in the user manual that is specific for the kit you are using them with (Section II.B).

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This document has been reviewed and approved by the Quality Department.