

I. Introduction

SMARTer DNA HT dual index kits are designed for use with SMARTer ThruPLEX® and SMARTer 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
SMARTer DNA HT Dual Index Kit - 96N Set A	R400660	96N Set A	5 µM	5 µl
SMARTer DNA HT Dual Index Kit - 96N Set B	R400661	96N Set B	5 µM	5 µl
SMARTer DNA HT Dual Index Kit - 96N Set C	R400662	96N Set C	5 µM	5 µl
SMARTer DNA HT Dual Index Kit - 96N Set D	R400663	96N Set D	5 µM	5 µl
SMARTer 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.

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

III. General Considerations

A. Best Practices

- No more than four freeze/thaw cycles are recommended for the SMARTer 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 SMARTer DNA HT dual index kits are designed for use with the SMARTer 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 SMARTer DNA HT dual index kits.

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

C. Plate Handling Instructions for Low-Throughput Applications

The SMARTer 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. SMARTer DNA HT Dual Index Sequences and Sequence Combinations

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

Table I. SMARTer 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			

SMARTer® DNA HT Dual Index Kit Protocol-At-A-Glance

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

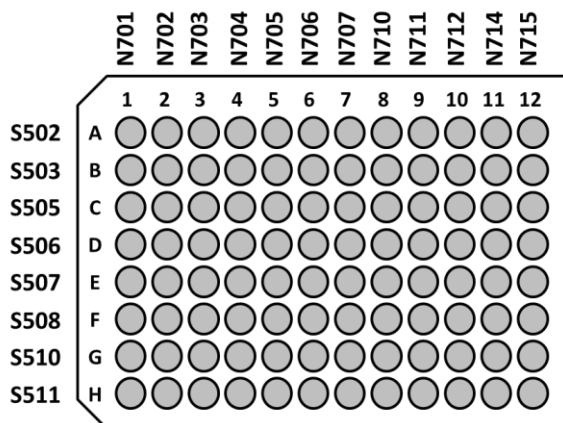
Table II. SMARTer DNA HT Dual Index Kit – 24N Index Primer Combinations

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

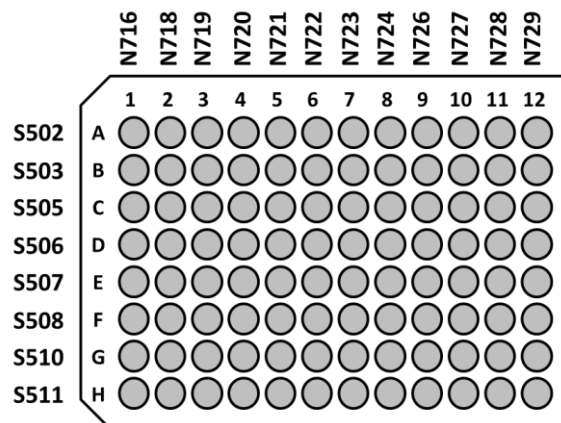
SMARTer® DNA HT Dual Index Kit Protocol-At-A-Glance

3. Each well of the **SMARTer 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 I. The dual index combination at each well position is indicated by the column (i7) and row (i5) labels on the plate maps (Figure 1).

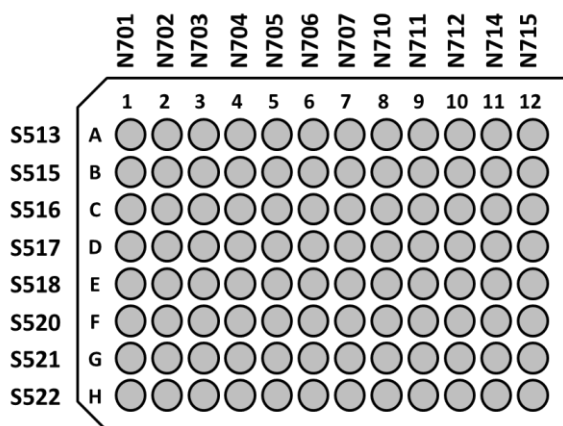
SMARTer DNA HT Dual Index Kit – 96N Set A



SMARTer DNA HT Dual Index Kit – 96N Set B



SMARTer DNA HT Dual Index Kit – 96N Set C



SMARTer DNA HT Dual Index Kit – 96N Set D

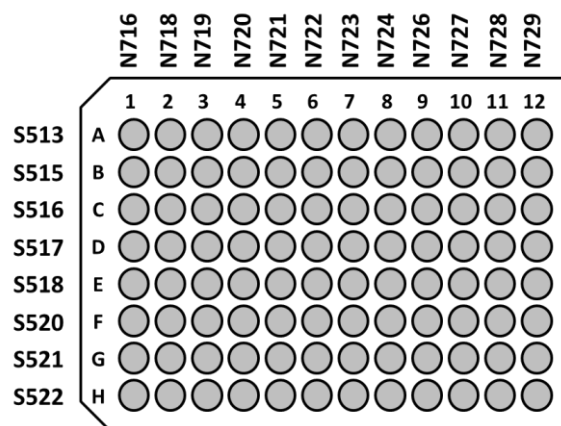


Figure 1. SMARTer DNA HT dual index kit plate maps.

IMPORTANT: When using the indexed PCR primers provided in the SMARTer 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.