

## I. Introduction

The Unique Dual Index Kit - 48U (Cat. No. R400744) and Unique Dual Index Kit - 96U (Cat. No. R400745) contain unique dual-indexed PCR primers for amplification of indexed Illumina®-compatible NGS libraries. These kits are included in the SMART-Seq® HT PLUS Kit (Cat. No. R400748 & R400749), SMART-Seq v4 PLUS Kit (Cat. No. R400752 & R400753), and SMART-Seq Single Cell PLUS Kit (Cat. No. R400750 & R400751). The indexed PCR primers are supplied predispensed in 96-well plates and are available in two formats: one set of 96 unique dual indexes (Cat. No. R400745) and one set of 48 unique dual indexes (Cat. No. R400744). Each dual index is provided in a sufficient amount for a single use.

All indexes have been functionally validated to work with Illumina sequencing systems using two- or four-channel chemistry for base calling. They have not been validated with systems using one-channel chemistry.

## II. List of Components

Store all components at  $-20^{\circ}\text{C}$ .

**Table 1. UDI kits - 48U and - 96U information and specifications.**

Product name	Cat. No.	Conc.	Volume per well	Index ID
Unique Dual Index Kit - 48U	R400744	5 $\mu\text{M}$	12 $\mu\text{l}$	U001–U048
Unique Dual Index Kit - 96U	R400745	5 $\mu\text{M}$	12 $\mu\text{l}$	U001–U096

\*The indexes in the Unique Dual Index Kit - 48U are a subset of Unique Dual Index Kit - 96U.

## III. General Considerations

### A. Best Practices

- It is not recommended to subject the unique dual index kits to more than **four** freeze/thaw cycles.
- Prior to use, remove the 96-well plate containing the unique dual indexes from the freezer and bring to the benchtop. Thaw at room temperature for 10 minutes, then briefly spin in a tabletop centrifuge to bring the contents to the bottom of the wells.

### B. Product Compatibility

The Unique Dual Index Kit - 48U and Unique Dual Index Kit - 96U are designed for use with our SMART-Seq PLUS kits. Please refer to the appropriate PLUS kit user manual for instructions on using the indexed primers provided in the UDI kits.

### C. Multiplexing and Index Pooling

It is important to select appropriate indexes that are unique and meet the Illumina-recommended compatibility and color balance requirements. The UDIs should be chosen from a minimum number of columns to achieve greater color balance.

#### Low plexity (2–8 samples)

For low-plex pooling involving between two and eight samples per sequencing run, follow the guidelines in Illumina's Index Adapters Pooling Guide (Illumina, Document #1000000041074-v8) and as shown in Figure 1 on the next page. Pool libraries of plexity 2–8 down a column. Do not pool libraries across a row. For example, for a plexity of 2, use U001–U002 (green box); for a plexity of 3, use U001–U003 (blue box); and so on. Any combinations of UDIs from the same column is acceptable. For example, for a plexity of 2, U001–U002 or U007–U008 is valid. For a plexity of 4, U001–U004 or U005–U008 is valid.

**Higher plexity (>8 samples)**

If pooling more than eight samples, use UDIs from multiple columns. For example, for a plexity of 9, U001–U009 or U001–U004 + U009–U013 is valid.

	1	2	3	4	5	6	7	8	9	10	11	12
A	U001	U009	U017	U025	U033	U041	U049	U057	U065	U073	U081	U089
B	U002	U010	U018	U026	U034	U042	U050	U058	U066	U074	U082	U090
C	U003	U011	U019	U027	U035	U043	U051	U059	U067	U075	U083	U091
D	U004	U012	U020	U028	U036	U044	U052	U060	U068	U076	U084	U092
E	U005	U013	U021	U029	U037	U045	U053	U061	U069	U077	U085	U093
F	U006	U014	U022	U030	U038	U046	U054	U062	U070	U078	U086	U094
G	U007	U015	U023	U031	U039	U047	U055	U063	U071	U079	U087	U095
H	U008	U016	U024	U032	U040	U048	U056	U064	U072	U080	U088	U096

Figure 1. Index map and multiplexing strategy for the Unique Dual Index Kit - 96U. For multiplexing libraries of plexity 2–8, we recommend pooling indexes down a column (examples given in the colored boxes).

**IV. Unique Dual Index Sequences**

The Unique Dual Indexes are 8-nt long i5 and i7 dual index sequences, corresponding to the IDT for Illumina-TruSeq® DNA and RNA UD Indexes - UDI0001–UDI0096 according to the Illumina Adapter Sequences Document (1000000002694 v10).

The “Index ID” in Table 2 is shortened from the full index name: e.g., “U001 - Unique Dual Index (5 μM)” is “U001.”

A CSV file containing a full list of these indexes can also be downloaded from our website, [Unique Dual Index Kit Sequence Information](#).

For Illumina sequencing, enter indexes from the downloaded CSV file, or use 'IDT-ILMN TruSeq DNA UD Indexes (96 indexes)' from the dropdown menu of Illumina Experiment Sheet.

**NOTE:** Do NOT select 'IDT-ILMN TruSeq DNA UD Indexes v2 - 96 Indexes' from the dropdown.

Table 2. UDI i5 and i7 bases correlating to the Index IDs, for use in sample sheets.

Index ID	i7 bases for sample sheet	i5 bases for sample sheet (HiSeq® 2000/2500, MiSeq®, NextSeq® 2000 (Sample Sheet v2), NovaSeq™ 6000 with v1.0 reagent kits.)	i5 bases for sample sheet (HiSeq 3000/4000/X, MiniSeq™, NextSeq 500/550, NextSeq 2000 (Sample Sheet v1), NovaSeq 6000 with v1.5 reagent kits)
U001	CCGCGGTT	AGCGCTAG	CTAGCGCT
U002	TTATAACC	GATATCGA	TCGATATC
U003	GGACTTGG	CGCAGACG	CGTCTGCG
U004	AAGTCCAA	TATGAGTA	TACTCATA
U005	ATCCACTG	AGGTGCGT	ACGCACCT
U006	GCTTGTC A	GAACATAC	GTATGTTC
U007	CAAGCTAG	ACATAGCG	CGCTATGT
U008	TGGATCGA	GTGCGATA	TATCGCAC
U009	AGTTCAGG	CCAACAGA	TCTGTTGG
U010	GACCTGAA	TTGGTGAG	CTCACCAA

## Unique Dual Index Kit Protocol-At-A-Glance

Index ID	i7 bases for sample sheet	i5 bases for sample sheet (HiSeq® 2000/2500, MiSeq®, NextSeq® 2000 (Sample Sheet v2), NovaSeq™ 6000 with v1.0 reagent kits.)	i5 bases for sample sheet (HiSeq 3000/4000/X, MiniSeq™, NextSeq 500/550, NextSeq 2000 (Sample Sheet v1), NovaSeq 6000 with v1.5 reagent kits)
U011	TCTCTACT	CGCGG TTC	GAACCGCG
U012	CTCTCGTC	TATAACCT	AGGTTATA
U013	CCAAGTCT	AAGGATGA	TCATCCTT
U014	TTGGACTC	GGAAGCAG	CTGCTTCC
U015	GGCTTAAG	TCGTGACC	GGTCACGA
U016	AATCCGGA	CTACAGTT	AACTGTAG
U017	TAATACAG	ATATTCAC	GTGAATAT
U018	CGGCGTGA	GCGCCTGT	ACAGGCGC
U019	ATGTAAGT	ACTCTATG	CATAGAGT
U020	GCACGGAC	GTCTCGCA	TGCGAGAC
U021	GGTACCTT	AAGACGTC	GACGTCTT
U022	AACGTTCC	GGAGTACT	AGTACTCC
U023	GCAGAATT	ACCGGCCA	TGGCCGGT
U024	ATGAGGCC	GTTAATTG	CAATTAAC
U025	ACTAAGAT	AACCGCGG	CCGCGGTT
U026	GTCGGAGC	GGTTATAA	TTATAACC
U027	CTTGGTAT	CCAAGTCC	GGACTTGG
U028	TCCAACGC	TTGGACTT	AAGTCCAA
U029	CCGTGAAG	CAGTGGAT	ATCCACTG
U030	TTACAGGA	TGACAAGC	GCTTG TCA
U031	GGCATTCT	CTAGCTTG	CAAGCTAG
U032	AATGCCTC	TCGATCCA	TGGATCGA
U033	TACCGAGG	CCTGAACT	AGTTCAGG
U034	CGTTAGAA	TTCAGGTC	GACCTGAA
U035	AGCCTCAT	AGTAGAGA	TCTCTACT
U036	GATTCTGC	GACGAGAG	CTCTCGTC
U037	TCGTAGTG	AGACTTGG	CCAAGTCT
U038	CTACGACA	GAGTCCAA	TTGGACTC
U039	TAAGTGGT	CTTAAGCC	GGCTTAAG
U040	CGGACAAC	TCCGGATT	AATCCGGA
U041	ATATGGAT	CTGTATTA	TAATACAG
U042	GCGCAAGC	TCACGCCG	CGGCGTGA
U043	AAGATACT	ACTTACAT	ATGTAAGT
U044	GGAGCGTC	GTCCGTGC	GCACGGAC
U045	ATGGCATG	AAGGTACC	GGTACCTT
U046	GCAATGCA	GGAACGTT	AACGTTCC
U047	GTTCCAAT	AATTCTGC	GCAGAATT
U048	ACCTTGGC	GGCCTCAT	ATGAGGCC
U049	ATATCTCG	ATCTTAGT	ACTAAGAT
U050	GCGCTCTA	GCTCCGAC	GTCGGAGC

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U051	AACAGGTT	ATACCAAG	CTTGGTAT
U052	GGTGAACC	GCGTTGGA	TCCAACGC
U053	CAACAATG	CTTCACGG	CCGTGAAG
U054	TGGTGGCA	TCCTGTAA	TTACAGGA
U055	AGGCAGAG	AGAATGCC	GGCATTCT
U056	GAATGAGA	GAGGCATT	AATGCCTC
U057	TGCGGCGT	CCTCGGTA	TACCGAGG
U058	CATAATAC	TTCTAACG	CGTTAGAA
U059	GATCTATC	ATGAGGCT	AGCCTCAT
U060	AGCTCGCT	GCAGAATC	GATTCTGC
U061	CGGAACTG	CACTACGA	TCGTAGTG
U062	TAAGGTCA	TGTCGTAG	CTACGACA
U063	TTGCCTAG	ACCACTTA	TAAGTGGT
U064	CCATTCGA	GTTGTCCG	CGGACAAC
U065	ACACTAAG	ATCCATAT	ATATGGAT
U066	GTGTCGGA	GCTTGCGC	GCGCAAGC
U067	TTCCTGTT	AGTATCTT	AAGATACT
U068	CCTTCACC	GACGCTCC	GGAGCGTC
U069	GCCACAGG	CATGCCAT	ATGGCATG
U070	ATTGTGAA	TGCATTGC	GCAATGCA
U071	ACTCGTGT	ATTGGAAC	GTTCCAAT
U072	GTCTACAC	GCCAAGGT	ACCTTGGC
U073	CAATTAAC	CGAGATAT	ATATCTCG
U074	TGGCCGGT	TAGAGCGC	GCGCTCTA
U075	AGTACTCC	AACCTGTT	AACAGGTT
U076	GACGTCTT	GGTTCACC	GGTGAACC
U077	TGCGAGAC	CATTGTTG	CAACAATG
U078	CATAGAGT	TGCCACCA	TGGTGGCA
U079	ACAGGCGC	CTCTGCCT	AGGCAGAG
U080	GTGAATAT	TCTCATTC	GAATGAGA
U081	AACTGTAG	ACGCCGCA	TGCGGCGT
U082	GGTCACGA	GTATTATG	CATAATAC
U083	CTGCTTCC	GATAGATC	GATCTATC
U084	TCATCCTT	AGCGAGCT	AGCTCGCT
U085	AGGTTATA	CAGTTCCG	CGGAACTG
U086	GAACCGCG	TGACCCTA	TAAGGTCA
U087	CTCACCAA	CTAGGCAA	TTGCCTAG
U088	TCTGTTGG	TCGAATGG	CCATTCGA
U089	TATCGCAC	CTTAGTGT	ACACTAAG
U090	CGCTATGT	TCCGACAC	GTGTCGGA

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U091	GTATGTTC	AACAGGAA	TTCCTGTT
U092	ACGCACCT	GGTGAAGG	CCTTCACC
U093	TACTCATA	CCTGTGGC	GCCACAGG
U094	CGTCTGCG	TTCACAAT	ATTGTGAA
U095	TCGATATC	ACACGAGT	ACTCGTGT
U096	CTAGCGCT	GTGTAGAC	GTCTACAC

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