

I. Introduction

DNA unique 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 “IDT for Illumina TruSeq® DNA” 8-nt long index sequences and offer a total of 96 dual indexes for multiplexing of up to 96 samples. The indexed PCR primers are supplied pre-dispensed in four different sets of 24 individual tubes, each of which contains one-fourth of the possible dual index combinations (Cat. Nos. R400665–R400668). Each dual index tube (24U Sets A–D) contains sufficient volume for up to two uses.

II. List of Components

Store all components at -20°C.

Product Name	Cat. No.	Dual index set of tubes	Concentration	Volume per well or tube
DNA Unique Dual Index Kit - 24U Set A*	R400665	24U Set A	5 µM	10 µl
DNA Unique Dual Index Kit - 24U Set B*	R400666	24U Set B	5 µM	10 µl
DNA Unique Dual Index Kit - 24U Set C*	R400667	24U Set C	5 µM	10 µl
DNA Unique Dual Index Kit - 24U Set D*	R400668	24U Set D	5 µM	10 µl

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

DNA Unique Dual Index Kit – 24U Set A (Cat. No. R400665; 48 rxns)	
U1 - DNA Unique Dual Index	U13 - DNA Unique Dual Index
U2 - DNA Unique Dual Index	U14 - DNA Unique Dual Index
U3 - DNA Unique Dual Index	U15 - DNA Unique Dual Index
U4 - DNA Unique Dual Index	U16 - DNA Unique Dual Index
U5 - DNA Unique Dual Index	U17 - DNA Unique Dual Index
U6 - DNA Unique Dual Index	U18 - DNA Unique Dual Index
U7 - DNA Unique Dual Index	U19 - DNA Unique Dual Index
U8 - DNA Unique Dual Index	U20 - DNA Unique Dual Index
U9 - DNA Unique Dual Index	U21 - DNA Unique Dual Index
U10 - DNA Unique Dual Index	U22 - DNA Unique Dual Index
U11 - DNA Unique Dual Index	U23 - DNA Unique Dual Index
U12 - DNA Unique Dual Index	U24 - DNA Unique Dual Index

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DNA Unique Dual Index Kit – 24U Set B (Cat. No. R400666; 48 rxns)	
U25 - DNA Unique Dual Index	U37 - DNA Unique Dual Index
U26 - DNA Unique Dual Index	U38 - DNA Unique Dual Index
U27 - DNA Unique Dual Index	U39 - DNA Unique Dual Index
U28 - DNA Unique Dual Index	U40 - DNA Unique Dual Index
U29 - DNA Unique Dual Index	U41 - DNA Unique Dual Index
U30 - DNA Unique Dual Index	U42 - DNA Unique Dual Index
U31 - DNA Unique Dual Index	U43 - DNA Unique Dual Index
U32 - DNA Unique Dual Index	U44 - DNA Unique Dual Index
U33 - DNA Unique Dual Index	U45 - DNA Unique Dual Index
U34 - DNA Unique Dual Index	U46 - DNA Unique Dual Index
U35 - DNA Unique Dual Index	U47 - DNA Unique Dual Index
U36 - DNA Unique Dual Index	U48 - DNA Unique Dual Index

DNA Unique Dual Index Kit – 24U Set C (Cat. No. R400667; 48 rxns)	
U49 - DNA Unique Dual Index	U61 - DNA Unique Dual Index
U50 - DNA Unique Dual Index	U62 - DNA Unique Dual Index
U51 - DNA Unique Dual Index	U63 - DNA Unique Dual Index
U52 - DNA Unique Dual Index	U64 - DNA Unique Dual Index
U53 - DNA Unique Dual Index	U65 - DNA Unique Dual Index
U54 - DNA Unique Dual Index	U66 - DNA Unique Dual Index
U55 - DNA Unique Dual Index	U67 - DNA Unique Dual Index
U56 - DNA Unique Dual Index	U68 - DNA Unique Dual Index
U57 - DNA Unique Dual Index	U69 - DNA Unique Dual Index
U58 - DNA Unique Dual Index	U70 - DNA Unique Dual Index
U59 - DNA Unique Dual Index	U71 - DNA Unique Dual Index
U60 - DNA Unique Dual Index	U72 - DNA Unique Dual Index

DNA Unique Dual Index Kit – 24U Set D (Cat. No. R400668; 48 rxns)	
U73 - DNA Unique Dual Index	U85 - DNA Unique Dual Index
U74 - DNA Unique Dual Index	U86 - DNA Unique Dual Index
U75 - DNA Unique Dual Index	U87 - DNA Unique Dual Index
U76 - DNA Unique Dual Index	U88 - DNA Unique Dual Index
U77 - DNA Unique Dual Index	U89 - DNA Unique Dual Index
U78 - DNA Unique Dual Index	U90 - DNA Unique Dual Index
U79 - DNA Unique Dual Index	U91 - DNA Unique Dual Index
U80 - DNA Unique Dual Index	U92 - DNA Unique Dual Index
U81 - DNA Unique Dual Index	U93 - DNA Unique Dual Index
U82 - DNA Unique Dual Index	U94 - DNA Unique Dual Index
U83 - DNA Unique Dual Index	U95 - DNA Unique Dual Index
U84 - DNA Unique Dual Index	U96 - DNA Unique Dual Index

III. General Considerations

A. Best Practices

- No more than four freeze/thaw cycles are recommended for the DNA unique 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. Thoroughly wipe the tubes with 70% ethanol and allow them to dry.

B. Product Compatibility

The DNA unique 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 unique 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. 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 pooling guidelines, please refer to Illumina's Index Adapters Pooling Guide (Illumina, Publication Document #1000000041074 v00).

IV. DNA Unique Dual Index Sequences

- The DNA unique dual indexes are 8-nt long and employ the “IDT for Illumina TruSeq DNA” i5 and i7 dual index sequences (Table 1; continues over three pages).

Table 1. DNA Unique Dual Index Sequences

i7 Index Name	i7 Bases for Sample Sheet	i5 Bases for Sample Sheet (MiSeq®, NovaSeq™, HiSeq® 2000/2500)	i5 Bases for Sample Sheet (MiniSeq™, NextSeq®, HiSeq 3000/4000)
U1	CCGCGGTT	AGCGCTAG	CTAGCGCT
U2	TTATAACC	GATATCGA	TCGATATC
U3	GGACTTGG	CGCAGACG	CGTCTGCG
U4	AAGTCCAA	TATGAGTA	TACTCATA
U5	ATCCACTG	AGGTGCGT	ACGCACCT
U6	GCTTGTCA	GAACATAC	GTATGTTC
U7	CAAGCTAG	ACATAGCG	CGCTATGT
U8	TGGATCGA	GTGCGATA	TATCGCAC
U9	AGTTCAGG	CCAACAGA	TCTGTTGG
U10	GACCTGAA	TTGGTGAG	CTCACCAA
U11	TCTCTACT	CGCGGTTC	GAACCGCG
U12	CTCTCGTC	TATAACCT	AGGTTATA
U13	CCAAGTCT	AAGGATGA	TCATCCTT
U14	TTGGACTC	GGAAGCAG	CTGCTTCC
U15	GGCTTAAG	TCGTGACC	GGTCACGA
U16	AATCCGGA	CTACAGTT	AACTGTAG
U17	TAATACAG	ATATTCAC	GTGAATAT
U18	CGGCGTGA	GCGCCTGT	ACAGGCGC
U19	ATGTAAGT	ACTCTATG	CATAGAGT
U20	GCACGGAC	GTCTCGCA	TGCGAGAC
U21	GGTACCTT	AAGACGTC	GACGTCTT
U22	AACGTTCC	GGAGTACT	AGTACTCC
U23	GCAGAATT	ACCGGCCA	TGGCCGGT
U24	ATGAGGCC	GTTAATTG	CAATTAAC
U25	ACTAAGAT	AACCGCGG	CCGCGGTT
U26	GTCGGAGC	GGTTATAA	TTATAACC
U27	CTTGGTAT	CCAAGTCC	GGACTTGG
U28	TCCAACGC	TTGGACTT	AAGTCCAA
U29	CCGTGAAG	CAGTGGAT	ATCCACTG
U30	TTACAGGA	TGACAAGC	GCTTGTCA
U31	GGCATTCT	CTAGCTTG	CAAGCTAG
U32	AATGCCTC	TCGATCCA	TGGATCGA

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i7 Index Name	i7 Bases for Sample Sheet	i5 Bases for Sample Sheet (MiSeq, NovaSeq, HiSeq 2000/2500)	i5 Bases for Sample Sheet (MiniSeq, NextSeq, HiSeq 3000/4000)
U33	TACCGAGG	CCTGAACT	AGTTCAGG
U34	CGTTAGAA	TTCAGGTC	GACCTGAA
U35	AGCCTCAT	AGTAGAGA	TCTCTACT
U36	GATTCTGC	GACGAGAG	CTCTCGTC
U37	TCGTAGTG	AGACTTGG	CCAAGTCT
U38	CTACGACA	GAGTCCAA	TTGGACTC
U39	TAAGTGGT	CTTAAGCC	GGCTTAAG
U40	CGGACAAC	TCCGGATT	AATCCGGA
U41	ATATGGAT	CTGTATTA	TAATACAG
U42	GCGCAAGC	TCACGCCG	CGGCGTGA
U43	AAGATACT	ACTTACAT	ATGTAAGT
U44	GGAGCGTC	GTCCGTGC	GCACGGAC
U45	ATGGCATG	AAGGTACC	GGTACCTT
U46	GCAATGCA	GGAACGTT	AACGTTCC
U47	GTTCCAAT	AATTCTGC	GCAGAATT
U48	ACCTTGGC	GGCCTCAT	ATGAGGCC
U49	ATATCTCG	ATCTTAGT	ACTAAGAT
U50	GCGCTCTA	GCTCCGAC	GTCGGAGC
U51	AACAGGTT	ATACCAAG	CTTGGTAT
U52	GGTGAACC	GCGTTGGA	TCCAACGC
U53	CAACAATG	CTTCACGG	CCGTGAAG
U54	TGGTGGCA	TCCTGTAA	TTACAGGA
U55	AGGCAGAG	AGAATGCC	GGCATTCT
U56	GAATGAGA	GAGGCATT	AATGCCTC
U57	TGCGGCGT	CCTCGGTA	TACCGAGG
U58	CATAATAC	TTCTAACG	CGTTAGAA
U59	GATCTATC	ATGAGGCT	AGCCTCAT
U60	AGCTCGCT	GCAGAATC	GATTCTGC
U61	CGGAACTG	CACTACGA	TCGTAGTG
U62	TAAGGTCA	TGTCGTAG	CTACGACA
U63	TTGCCTAG	ACCACTTA	TAAGTGGT
U64	CCATTCGA	GTTGTCCG	CGGACAAC

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

i7 Index Name	i7 Bases for Sample Sheet	i5 Bases for Sample Sheet (MiSeq, NovaSeq, HiSeq 2000/2500)	i5 Bases for Sample Sheet (MiniSeq, NextSeq, HiSeq 3000/4000)
U65	ACACTAAG	ATCCATAT	ATATGGAT
U66	GTGTGCGA	GCTTGCGC	GCGCAAGC
U67	TTCCTGTT	AGTATCTT	AAGATACT
U68	CCTTCACC	GACGCTCC	GGAGCGTC
U69	GCCACAGG	CATGCCAT	ATGGCATG
U70	ATTGTGAA	TGCATTGC	GCAATGCA
U71	ACTCGTGT	ATTGGAAC	GTTCCAAT
U72	GTCTACAC	GCCAAGGT	ACCTTGGC
U73	CAATTAAC	CGAGATAT	ATATCTCG
U74	TGGCCGGT	TAGAGCGC	GCGCTCTA
U75	AGTACTCC	AACCTGTT	AACAGGTT
U76	GACGTCTT	GGTTCACC	GGTGAACC
U77	TGCGAGAC	CATTGTTG	CAACAATG
U78	CATAGAGT	TGCCACCA	TGGTGGCA
U79	ACAGGCGC	CTCTGCCT	AGGCAGAG
U80	GTGAATAT	TCTCATTC	GAATGAGA
U81	AACTGTAG	ACGCCGCA	TGCGGCGT
U82	GGTCACGA	GTATTATG	CATAATAC
U83	CTGCTTCC	GATAGATC	GATCTATC
U84	TCATCCTT	AGCGAGCT	AGCTCGCT
U85	AGGTTATA	CAGTCCG	CGGAACTG
U86	GAACCGCG	TGACCTTA	TAAGGTCA
U87	CTCACCAA	CTAGGCAA	TTGCCTAG
U88	TCTGTTGG	TCGAATGG	CCATTCGA
U89	TATCGCAC	CTTAGTGT	ACACTAAG
U90	CGCTATGT	TCCGACAC	GTGTGCGA
U91	GTATGTTC	AACAGGAA	TTCCTGTT
U92	ACGCACCT	GGTGAAGG	CCTTCACC
U93	TACTCATA	CCTGTGGC	GCCACAGG
U94	CGTCTGCG	TTCACAAT	ATTGTGAA
U95	TCGATATC	ACACGAGT	ACTCGTGT
U96	CTAGCGCT	GTGTAGAC	GTCTACAC

IMPORTANT: When using the indexed PCR primers provided in the DNA unique 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 III.B).

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