I. Introduction

ThruPLEX HV UDI kits contain unique dual-indexed PCR primers for amplification of indexed Illumina®-compatible NGS libraries. These primers can be used in ThruPLEX HV products. These kits contain indexed PCR primers and together offer up to 96 unique dual indexes for multiplexing up to 96 samples. The indexed PCR primers are supplied pre-dispensed in 96 well plates and are available in two formats: one set of 96 unique dual indexes (Cat. Nos. R400738) and a set of 24 unique dual indexes (Cat. No. R400739) that represents a subset of Cat. No. R400738. Each dual index is provided in sufficient amounts for single uses.

All indexes have been functionally validated to work with Illumina sequencing systems (e.g., MiSeq®, NovaSeq™, MiniSeq™, NextSeq®, and HiSeq® platforms) 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°C.

Name	Cat. No.	Concentration	Volume/well	UDIs
ThruPLEX HV UDI 1-24*	R400739	6.25 µM/oligo 12.5 µM/oligo pair	12 µl	UDI 001–024
ThruPLEX HV UDI Set A	R400738	6.25 µM/oligo 12.5 µM/oligo pair	12 µl	UDI 001–096

*The indexes in the ThruPLEX HV UDI 1-24 are a subset of the ThruPLEX HV UDI Set A.

III. General considerations

A. Best Practices

- We do not recommend subjecting the ThruPLEX HV UDI kits to more than four freeze/thaw cycles.
- Prior to use, remove PCR plates containing the desired unique dual indexes from the freezer and bring to the benchtop. Let thaw for 10 min at room temperature, then spin in a tabletop centrifuge to bring the contents to the bottom of the wells.

B. Product Compatibility

The ThruPLEX UDI kits are designed for use with ThruPLEX HV library preparation kits. Please refer to the kit-specific user manual for instructions on using the indexed PCR primers provided in the ThruPLEX HV UDI kits. Compatible library preparation kits are listed below:

- ThruPLEX DNA-Seq HV (Cat. Nos. R400740 and R400741)
- ThruPLEX Tag-Seq HV (Cat. Nos. R400742 and R400743)

NOTE: The ThruPLEX HV UDI kits are **NOT** compatible with previous versions of ThruPLEX kits, including the ThruPLEX DNA-Seq, ThruPLEX Tag-Seq, and ThruPLEX Plasma-Seq kits. They are ONLY compatible with the ThruPLEX "HV" kits. If you have additional questions about compatibility, please contact technical support.

A. 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 ThruPLEX HV UDI Set A (Cat. No. R400738). For multiplexing libraries of plexity ≥ 2, we recommend pooling indexes down a column (as shown in the example given in the colored boxes). Do not pool libraries across rows if processing less than 8 samples per sequencing run.

IV. ThruPLEX HV UDI Sequences

The ThruPLEX HV UDI are 8-nt long and employ the "IDT for Illumina UD indexes" i5 and i7 dual index sequences (Table I). An .xlsx file containing a full list of these indexes (ThruPLEX HV UDI Index Map) can be downloaded from our website.

Table I. ThruPLEX HV UDI sequences.

Index	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)
U001	CCGCGGTT	AGCGCTAG	CTAGCGCT
U002	TTATAACC	GATATCGA	TCGATATC
U003	GGACTTGG	CGCAGACG	CGTCTGCG
U004	AAGTCCAA	TATGAGTA	TACTCATA
U005	ATCCACTG	AGGTGCGT	ACGCACCT
U006	GCTTGTCA	GAACATAC	GTATGTTC
U007	CAAGCTAG	ACATAGCG	CGCTATGT
U008	TGGATCGA	GTGCGATA	TATCGCAC
U009	AGTTCAGG	CCAACAGA	TCTGTTGG
U010	GACCTGAA	TTGGTGAG	CTCACCAA
U011	TCTCTACT	CGCGGTTC	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	GCTTGTCA
U031	GGCATTCT	CTAGCTTG	CAAGCTAG
U032	AATGCCTC	TCGATCCA	TGGATCGA
U033	TACCGAGG	CCTGAACT	AGTTCAGG
U034	CGTTAGAA	TTCAGGTC	GACCTGAA
U035	AGCCTCAT	AGTAGAGA	TCTCTACT

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)
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
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

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)
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	TGACCTTA	TAAGGTCA
U087	CTCACCAA	CTAGGCAA	TTGCCTAG
U088	TCTGTTGG	TCGAATGG	CCATTCGA
U089	TATCGCAC	CTTAGTGT	ACACTAAG
U090	CGCTATGT	TCCGACAC	GTGTCGGA
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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