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KAPA UDI Primer Mixes

Unique Dual-Indexed Primers used with the KAPA Universal Adapter for sample barcoding

Version 01
Content version: February 2020

Store at +2 to+ 8°C

Product Name and Pack Size	Catalog #
KAPA UDI Primer Mixes, 1-96, 96 reactions	09134336001

Contents

Component	Quantity	Amount
Kit for 96 Reactions (Catalog# 09134336001)		
1 x 96 well plate	1	1 reaction per well

Storage and Stability

- Store at +2 to +8°C. The kit components are stable at +2 to +8°C through the expiration date printed on the label.

⚠ Store primer plate in an upright orientation only.

Warnings and Precautions

Employ best laboratory practices to avoid cross contamination of indexed primer pairs.

Application

The KAPA Unique Dual-Indexed (UDI) Primer Mixes are to be used with the KAPA Universal Adapter to generate uniquely labeled libraries from individual biological DNA samples. Sample indexing allows for pooling of libraries prior to target capture or cluster generation, to enable multiplexed sequencing. Each KAPA UDI Primer Mix is a pre-mixed combination of forward and reverse primers. The primers contain a non-redundant (unique), 8-nucleotide index designed to mitigate index misalignment ("index hopping") on Illumina sequencers that employ patterned flow cells and exclusion amplification chemistry.

Number of Reactions

1 kit with 96 indexes for library preparations. Each well contains enough material for one reaction.

How to Use this Product

Guidance on use in the KAPA HyperCap Workflow can be found in the KAPA HyperChoice, KAPA HyperExplore, and KAPA HyperExome Instructions for Use.

	1	2	3	4	5	6	7	8	9	10	11	12
A	UDI-P 01	UDI-P 09	UDI-P 17	UDI-P 25	UDI-P 33	UDI-P 41	UDI-P 49	UDI-P 57	UDI-P 65	UDI-P 73	UDI-P 81	UDI-P 89
B	UDI-P 02	UDI-P 10	UDI-P 18	UDI-P 26	UDI-P 34	UDI-P 42	UDI-P 50	UDI-P 58	UDI-P 66	UDI-P 74	UDI-P 82	UDI-P 90
C	UDI-P 03	UDI-P 11	UDI-P 19	UDI-P 27	UDI-P 35	UDI-P 43	UDI-P 51	UDI-P 59	UDI-P 67	UDI-P 75	UDI-P 83	UDI-P 91
D	UDI-P 04	UDI-P 12	UDI-P 20	UDI-P 28	UDI-P 36	UDI-P 44	UDI-P 52	UDI-P 60	UDI-P 68	UDI-P 76	UDI-P 84	UDI-P 92
E	UDI-P 05	UDI-P 13	UDI-P 21	UDI-P 29	UDI-P 37	UDI-P 45	UDI-P 53	UDI-P 61	UDI-P 69	UDI-P 77	UDI-P 85	UDI-P 93
F	UDI-P 06	UDI-P 14	UDI-P 22	UDI-P 30	UDI-P 38	UDI-P 46	UDI-P 54	UDI-P 62	UDI-P 70	UDI-P 78	UDI-P 86	UDI-P 94
G	UDI-P 07	UDI-P 15	UDI-P 23	UDI-P 31	UDI-P 39	UDI-P 47	UDI-P 55	UDI-P 63	UDI-P 71	UDI-P 79	UDI-P 87	UDI-P 95
H	UDI-P 08	UDI-P 16	UDI-P 24	UDI-P 32	UDI-P 40	UDI-P 48	UDI-P 56	UDI-P 64	UDI-P 72	UDI-P 80	UDI-P 88	UDI-P 96

Fig. 1: Layout of the KAPA UDI Primer Mixes Plate.

Pooling/Multiplexing Guidelines

As a rule, choose primer mixes as highlighted by the boxes in Figure 2 to take advantage of the color-balanced indexes. This will prevent registration failure and laser color complexity issues during sequencing and de-multiplexing. Figure 2 details the recommended two-plexing combinations that are fully color-balanced.

See the detailed suggestions below:

- Pooling two samples (two-plex):
  - Figure 2 demonstrates the recommended two-plex combinations that are fully color-balanced based on the plate layout in Figure 1 (all combinations indicated by the gray boxes, e.g. A1 + B1, C1 + D1, etc.).
- Pooling three to eight samples (three to eight-plex):
  - Use any of the recommended two-plex combinations with any other index in the column (all combinations indicated by colored box e.g. three-plex: A1 + B1 + C1, four-plex: A1 + B1 +C1 +D1, etc.).
- Pooling nine or more samples:
  - Any number of additional libraries may be multiplexed with any of the color-balanced combinations listed below to obtain pools of any plexity.
  - It is recommended to use column groups of indexes (e.g. colored box 1, 2 etc.).

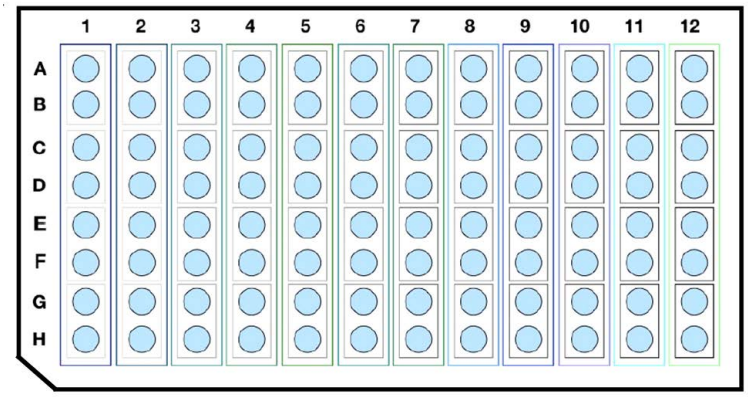

Fig. 2: Recommendations for two-plex (by pairs in grayscale boxes) and eight-plex (by columns in blue and green boxes) pooling. Note that the notched corner is on the bottom left of the plate. This directs the correct orientation of the plate with A1 positioned in the top left of the plate.

Well	KAPA UDI Primer Mix	P7 Index Sequence	P5 Index Sequence	
		All Illumina Instruments	*NovaSeq 6000 MiSeq HiSeq 2000/2500	**NextSeq 500/550 MiniSeq iSeq100 HiSeq 3000/4000/X
A1	UDI-P 01	TGGATCGA	ACCTAGCT	AGCTAGGT
B1	UDI-P 02	CAAGCTAG	GTTCGATC	GATCGAAC
C1	UDI-P 03	GTACCAAG	CATGGTTC	GAACCATG
D1	UDI-P 04	ACGTTGGA	TGCAACCT	AGGTTGCA
E1	UDI-P 05	TCGTGGTA	AGCACCAT	ATGGTGCT
F1	UDI-P 06	CTACAACG	GATGTTGC	GCAACATC
G1	UDI-P 07	GTTCAAGG	CAAGTTCC	GGAACTTG
H1	UDI-P 08	ACCTGGAA	TGGACCTT	AAGGTCCA
A2	UDI-P 09	TCTTAGCG	AGAATCGC	GCGATTCT
B2	UDI-P 10	CTCCGATA	GAGGCTAT	ATAGCCTC
C2	UDI-P 11	GTCCTACT	CAGGATGA	TCATCCTG
D2	UDI-P 12	ACTTCGTC	TGAAGCAG	CTGCTTCA
E2	UDI-P 13	TCTACAGG	AGATGTCC	GGACATCT
F2	UDI-P 14	CTCGTGAA	GAGCACTT	AAGTGCTC
G2	UDI-P 15	GTTCGGAT	CAAGCCTA	TAGGCTTG
H2	UDI-P 16	ACCTAAGC	TGGATTCG	CGAATCCA
A3	UDI-P 17	GTTGACTG	CAACTGAC	GTCAGTTG
B3	UDI-P 18	ACCAGTCA	TGGTCAGT	ACTGACCA
C3	UDI-P 19	TACCGGAA	ATGGCCTT	AAGGCCAT
D3	UDI-P 20	CGTTAAGG	GCAATTCC	GGAATTGC
E3	UDI-P 21	GCTATGTC	CGATACAG	CTGTATCG
F3	UDI-P 22	ATCGCACT	TAGCGTGA	TCACGCTA
G3	UDI-P 23	GCACTTCT	CGTGAAGA	TCTTCACG
H3	UDI-P 24	ATGTCCTC	TACAGGAG	CTCCTGTA

		P7 Index Sequence	P5 Index Sequence	
Well	**KAPA UDI Primer Mix**	**All Illumina Instruments**	***NovaSeq 6000 MiSeq HiSeq 2000/2500**	****NextSeq 500/550 MiniSeq iSeq100 HiSeq 3000/4000/X**
A4	UDI-P 25	GGATGATC	CCTACTAG	CTAGTAGG
B4	UDI-P 26	AAGCAGCT	TTCGTCGA	TCGACGAA
C4	UDI-P 27	TGCTATCG	ACGATAGC	GCTATCGT
D4	UDI-P 28	CATCGCTA	GTAGCGAT	ATCGCTAC
E4	UDI-P 29	TCCTGAGT	AGGACTCA	TGAGTCCT
F4	UDI-P 30	CTTCAGAC	GAAGTCTG	CAGACTTC
G4	UDI-P 31	GATCCTTC	CTAGGAAG	CTTCCTAG
H4	UDI-P 32	AGCTTCCT	TCGAAGGA	TCCTTCGA
A5	UDI-P 33	TAGGCAAC	ATCCGTTG	CAACGGAT
B5	UDI-P 34	CGAATGGT	GCTTACCA	TGGTAAGC
C5	UDI-P 35	TCACCAGA	AGTGGTCT	AGACCACT
D5	UDI-P 36	CTGTTGAG	GACAACTC	GAGTTGTC
E5	UDI-P 37	TCAGCTCA	AGTCGAGT	ACTCGACT
F5	UDI-P 38	CTGATCTG	GACTAGAC	GTCTAGTC
G5	UDI-P 39	GATGGAAC	CTACCTTG	CAAGGTAG
H5	UDI-P 40	AGCAAGGT	TCGTTCCA	TGGAACGA
A6	UDI-P 41	TGTCAGCT	ACAGTCGA	TCGACTGT
B6	UDI-P 42	CACTGATC	GTGACTAG	CTAGTCAC
C6	UDI-P 43	GTTACGTG	CAATGCAC	GTGCATTG
D6	UDI-P 44	ACCGTACA	TGGCATGT	ACATGCCA
E6	UDI-P 45	GCAACGTA	CGTTGCAT	ATGCAACG
F6	UDI-P 46	ATGGTACG	TACCATGC	GCATGGTA
G6	UDI-P 47	GTGACAGT	CACTGTCA	TGACAGTG
H6	UDI-P 48	ACAGTGAC	TGTCACTG	CAGTGACA
A7	UDI-P 49	GATTGGTC	CTAACCAG	CTGGTTAG
B7	UDI-P 50	AGCCAACT	TCGGTTGA	TCAACCGA
C7	UDI-P 51	GCATCTTG	CGTAGAAC	GTTCTACG
D7	UDI-P 52	ATGCTCCA	TACGAGGT	ACCTCGTA
E7	UDI-P 53	GTCTTCAG	CAGAAGTC	GACTTCTG
F7	UDI-P 54	ACTCCTGA	TGAGGACT	AGTCCTCA
G7	UDI-P 55	GCATTCGT	CGTAAGCA	TGCTTACG
H7	UDI-P 56	ATGCCTAC	TACGGATG	CATCCGTA
A8	UDI-P 57	GAGTCATG	CTCAGTAC	GTACTGAG
B8	UDI-P 58	AGACTGCA	TCTGACGT	ACGTCAGA
C8	UDI-P 59	GGAACTGA	CCTTGACT	AGTCAAGG
D8	UDI-P 60	AAGGTCAG	TTCCAGTC	GACTGGAA
E8	UDI-P 61	GTGTATCG	CACATAGC	GCTATGTG
F8	UDI-P 62	ACACGCTA	TGTGCGAT	ATCGCACA
G8	UDI-P 63	TCGACGAA	AGCTGCTT	AAGCAGCT
H8	UDI-P 64	CTAGTAGG	GATCATCC	GGATGATC
A9	UDI-P 65	GTGTCTGA	CACAGACT	AGTCTGTG
B9	UDI-P 66	ACACTCAG	TGTGAGTC	GACTCACA
C9	UDI-P 67	GCCTTATC	CGGAATAG	CTATTCCG
D9	UDI-P 68	ATTCCGCT	TAAGGCGA	TCGCCTTA
E9	UDI-P 69	TGATCGGT	ACTAGCCA	TGGCTAGT
F9	UDI-P 70	CAGCTAAC	GTCGATTG	CAATCGAC
G9	UDI-P 71	GAACATGG	CTTGTACC	GGTACAAG
H9	UDI-P 72	AGGTGCAA	TCCACGTT	AACGTGGA
A10	UDI-P 73	GGTGACAA	CCACTGTT	AACAGTGG
B10	UDI-P 74	AACAGTGG	TTGTCACC	GGTGACAA
C10	UDI-P 75	TGCACTTC	ACGTGAAG	CTTCACGT
D10	UDI-P 76	CATGTCCT	GTACAGGA	TCCTGTAC
E10	UDI-P 77	GTACAGCT	CATGTCGA	TCGACATG
F10	UDI-P 78	ACGTGATC	TGCACTAG	CTAGTGCA
G10	UDI-P 79	TGTTGCAG	ACAACGTC	GACGTTGT
H10	UDI-P 80	CACCATGA	GTGGTACT	AGTACCAC

		P7 Index Sequence	P5 Index Sequence	
Well	**KAPA UDI Primer Mix**	**All Illumina Instruments**	***NovaSeq 6000 MiSeq HiSeq 2000/2500**	****NextSeq 500/550 MiniSeq iSeq100 HiSeq 3000/4000/X**
A11	UDI-P 81	TATGCGTG	ATACGCAC	GTGCGTAT
B11	UDI-P 82	CGCATACA	GCGTATGT	ACATACGC
C11	UDI-P 83	TCCTCTGA	AGGAGACT	AGTCTCCT
D11	UDI-P 84	CTTCTCAG	GAAGAGTC	GACTCTTC
E11	UDI-P 85	TCAGGCAA	AGTCCGTT	AACGGACT
F11	UDI-P 86	CTGAATGG	GACTTACC	GGTAAGTC
G11	UDI-P 87	GGTAGCTA	CCATCGAT	ATCGATGG
H11	UDI-P 88	AACGATCG	TTGCTAGC	GCTAGCAA
A12	UDI-P 89	TGTATGGC	ACATACCG	CGGTATGT
B12	UDI-P 90	CACGCAAT	GTGCGTTA	TAACGCAC
C12	UDI-P 91	GTTCGTGA	CAAGCACT	AGTGCTTG
D12	UDI-P 92	ACCTACAG	TGGATGTC	GACATCCA
E12	UDI-P 93	GACCTAAG	CTGGATTC	GAATCCAG
F12	UDI-P 94	AGTTCGGA	TCAAGCCT	AGGCTTGA
G12	UDI-P 95	GTGCTTAG	CACGAATC	GATTCGTG
H12	UDI-P 96	ACATCCGA	TGTAGGCT	AGCCTACA

Tab. 1: Sequencing indexes (barcodes) included in the KAPA UDI Primer Mix. For convenience, all 96 index sequences in a comma-separated values file (delimited text file), as well as instructions for installation of KAPA UDI Primer Mix indexes for use with Illumina Experiment Manager are available from the Technical Documents at *sequencing.roche.com/support.*

*The sequence of the P5 index in the orientation required when completing the sample sheet for Illumina HiSeq 2000/2500, MiSeq, and NovaSeq instruments.

**The reverse complement sequence of the P5 index in the orientation required when completing the sample sheet for Illumina iSeq, MiniSeq, NextSeq, HiSeq 3000/4000, and HiSeq X instruments.

Changes to Previous Version

First version.

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In this document, the following symbol is used to highlight important information:

Symbol	Description
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