

# **KAPA UDI Primer Mixes**

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

**Version 01**Content version: February 2020

**Product Name and Pack Size** 

Catalog #

Store at +2 to+8°C

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       |
|---|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| Α | 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 |
| В | 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 |
| С | 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 |
| Н | 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 |

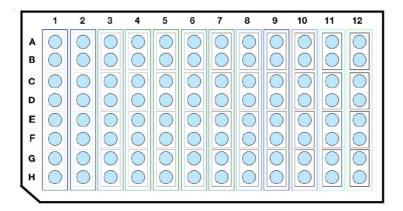
Fig. 1: Layout of the KAPA UDI Primer Mixes Plate.

# **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.

|      |                        | P7 Index Sequence           | P5 Index Sequence                         |  |  |  |
|------|------------------------|-----------------------------|---|--|--|--|
| Well | KAPA UDI<br>Primer Mix | All Illumina<br>Instruments | *NovaSeq 6000<br>MiSeq<br>HiSeq 2000/2500 | **NextSeq 500/550<br>MiniSeq<br>iSeq100<br>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   |  |  |
| А3   | UDI-P 17               | GTTGACTG                    | CAACTGAC                                  | GTCAGTTG   |  |  |
| В3   | UDI-P 18               | ACCAGTCA                    | TGGTCAGT                                  | ACTGACCA   |  |  |
| СЗ   | 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   |  |  |
| НЗ   | UDI-P 24               | ATGTCCTC                    | TACAGGAG                                  | CTCCTGTA   |  |  |

| Well KAPA UDI<br>Primer Mix |          | P7 Index Sequence           | P5 Index Sequence                         |  |  |  |
|-----------------------------|----------|-----------------------------|---|--|--|--|
|                             |          | All Illumina<br>Instruments | *NovaSeq 6000<br>MiSeq<br>HiSeq 2000/2500 | **NextSeq 500/550<br>MiniSeq<br>iSeq100<br>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<br>Primer Mix | All Illumina<br>Instruments | *NovaSeq 6000<br>MiSeq<br>HiSeq 2000/2500 | **NextSeq 500/550<br>MiniSeq<br>iSeq100<br>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.

# **Changes to Previous Version**

First version.

## **Ordering Information**

For a complete overview of Roche Sequencing products, including those used in sequence capture workflows, go to sequencing.roche.com/products.

# Conventions

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

| Symbol | Description   |
|--------|---|
|        | Important Note: Information critical to the success of the procedure or use of the product. |

<sup>\*</sup>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.

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