



# Index Adapters

## Pooling Guide

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# Overview

This resource provides recommendations for optimizing color balance across all Illumina systems when pooling indexed libraries. Pooling combines at least two libraries to sequence in one run.

The recommendations are designed to form low-plex, color-balanced pools. Higher plexity pools are inherently color-balanced, so any index adapter combinations are acceptable. To develop alternative pooling strategies, review index adapter sequences in [Illumina Adapter Sequences](#) to ensure color balance for your system.

**i** | Plexity is the number of libraries combined in a reaction or pool. For example, if 12 libraries are combined in one pool, the plexity is 12.

Use this resource as a reference to plan indexing and pooling for library prep. Refer to the library prep kit support pages on the [Illumina support site](#) for additional resources. These resources include software, training, and compatible products. Always check the support pages for the latest versions.

Pooling strategies differ based on the type of chemistry used. For instruments using XLEAP-SBS chemistry, refer to pooling strategies indicated for XLEAP-SBS.

## Dual Indexing Options

Combinatorial dual (CD) indexes have unique dual pairs, but the index adapters share some sequences. In contrast, unique dual (UD) indexes have distinct sequences so each index adapter is unique.

- Single-indexed libraries add six base Index 1 (i7) sequences to generate uniquely tagged libraries.
- Dual-indexed libraries add Index 1 (i7) and Index 2 (i5) sequences to generate uniquely tagged libraries.
- UD indexes have distinct, unrelated index sequences for the i5 and i7 Index Read. Indexes are 8 or 10 bases long.
- CD indexes have a limit of eight unique pairs, resulting in most libraries sharing common indexes on the i5 or i7 end. Indexes are eight bases long.

Plate layouts for Illumina UD Indexes are designed with the proper color balance. Achieving this balance with CD indexes requires additional planning.

# Color Balance

Selecting index adapters with diverse sequences for pooled libraries optimizes color balance for successful sequencing and data analysis.

When index adapter sequences are recorded in Illumina Experiment Manager (IEM), Local Run Manager, Instrument Run Setup, or BaseSpace Sequence Hub Prep tab, the software confirms that libraries in a pool have unique index combinations. However, Local Run Manager, Instrument Run Setup, and BaseSpace Sequence Hub do not check for color balance. IEM checks for color balance, but for HiSeq systems only.

## Example Index Adapter Combinations

The following table provides examples of good and bad index combinations, to demonstrate color balance on a four-channel system. The good examples have signals in both channels (red and green). The bad examples are missing signal in one channel, as indicated with an X.

| Good Examples |                 |          |                 | Bad Examples |                 |             |                 |
|---------------|-----------------|----------|-----------------|--------------|-----------------|-------------|-----------------|
| Index 1       |                 | Index 2  |                 | Index 1      |                 | Index 2     |                 |
| N705          | G G A C T C C T | S503     | T A T C C T C T | N705         | G G A C T C C T | S502        | C T C T C T A T |
| N706          | T A G G C A T G | S503     | T A T C C T C T | N706         | T A G G C A T G | S502        | C T C T C T A T |
| N701          | T A A G G C G A | S504     | A G A G T A G A | N701         | T A A G G C G A | S503        | T A T C C T C T |
| N702          | C G T A C T A G | S504     | A G A G T A G A | N702         | C G T A C T A G | S503        | T A T C C T C T |
| ✓✓✓✓✓✓✓✓      |                 | ✓✓✓✓✓✓✓✓ |                 | ✓✓✓✓✓✓✓✓     |                 | ✓✓✓✓X X X X |                 |

# Sequencing Chemistry

Illumina sequencing systems use one-, two-, or four-channel chemistry to perform base calling. During sequencing, a separate read called the Index Read sequences the index. Dual-indexed sequencing includes Index Read 1 and Index Read 2.

| Chemistry    | Sequencing System                                                                           |
|--------------|---------------------------------------------------------------------------------------------|
| One-channel  | iSeq 100 System                                                                             |
| Two-channel  | NovaSeq X Series<br>NovaSeq 6000<br>NextSeq Systems<br>MiniSeq Systems<br>MiSeq i100 Series |
| Four-channel | MiSeq System<br>HiSeq Systems                                                               |

For more information on sequencing chemistry and base calling, refer to the system guide for your instrument. For information on indexing workflows on Illumina systems, refer to [Indexed Sequencing on Illumina Systems](#).

## One-Channel Chemistry

One-channel chemistry requires one dye and two images to encode data for the four bases. Intensities extracted from one image and compared to a second image result in four distinct populations, each corresponding to a nucleotide. Base calling determines the population that each cluster belongs to.

When sequencing on a one-channel system, the first two cycles of the Index Read cannot start with two G bases. Otherwise, intensity is not generated.

- Make sure that **at least** one index sequence in a library pool does not start with two G bases.
- Select balanced index sequences so that signal is present in at least one image (preferably both) for every cycle.

## Two-Channel Chemistry

Two-channel chemistry requires two dyes and two images to encode data for the four bases. The MiSeq i100 Series, NovaSeq X Series, and NextSeq 1000/2000 use one image from the green channel and one image from the blue channel. Other two-channel systems use one image from the green channel and one image from the red channel. Intensities extracted from an image and compared to another image result in four distinct populations, each corresponding to a base. Base calling determines the population that each cluster belongs to.

Guidelines for index cycle base selection on a two-channel system vary by sequencing system.

For the following sequencing systems, either of the first two cycles of the Index Read must start with at least one base other than G:

- NovaSeq X Series
- NovaSeq 6000
- NextSeq 1000/2000
- NextSeq 550
- MiniSeq
- MiSeq i100 Series

If an Index Read starts with two G bases, signal intensity is not generated. Signal must be present in the first two cycles. Combine index sequences so that signal is present in at least one channel (preferably both) for every cycle:

- **Red or Blue channel**—A or C
- **Green channel**—A or T

When sequencing on a NovaSeq X Series, or a NextSeq 1000/2000 using XLEAP-SBS chemistry, combine index sequences so that signal is present in at least the green channel (preferably both) for every cycle:

- **Blue channel**—A or C
- **Green channel**—C or T

For more example index combinations for two-channel systems, refer to the *Library pooling guidelines for the NextSeq and MiniSeq systems* bulletin on the Illumina website.

## Four-Channel Chemistry

Four-channel chemistry uses four dyes and four images per cycle to observe which dye is incorporated into a cluster. A green laser sequences G and T bases, while a red laser sequences A and C bases. To ensure proper image registration, each cycle must include at least one of two nucleotides per color channel.

When sequencing on a four-channel system, make sure that pooled libraries contain unique and color-compatible index combinations for each Index Read.

# Nextera, Illumina Prep, and Illumina PCR Kits

This section provides guidelines for pooling Nextera, Illumina Prep, and Illumina PCR index kits.

Illumina does not recommend pooling libraries across different UD index kit types. Adapter sequences can be similar or shared across plates. For example, do not pool Illumina UD Indexes with IDT for Illumina UD Indexes.

| Index Adapters              |                                                 | Indexing Scheme          | Format |
|-----------------------------|-------------------------------------------------|--------------------------|--------|
| Illumina UD Indexes         | Illumina DNA/RNA UD Indexes, Tagmentation       | Dual indexing            | Plate  |
|                             | Illumina RNA UD Indexes, Ligation               |                          |        |
|                             | Illumina Unique Dual Indexes, LT                |                          |        |
| IDT for Illumina UD Indexes | IDT for Illumina DNA/RNA UD Indexes             | Dual indexing            | Plate  |
|                             | IDT for Illumina PCR UD Indexes                 |                          |        |
|                             | IDT for Illumina Nextera DNA UD Indexes         |                          |        |
| Nextera DNA Indexes         | Nextera DNA CD Indexes (96 indexes, 96 samples) | Dual indexing            | Plate  |
|                             | Nextera DNA CD Indexes (24 indexes, 24 samples) |                          | Tube   |
|                             | Nextera XT Index Kit v2                         | Single and dual indexing | Tube   |
|                             | Nextera Index Kit                               |                          |        |

## Illumina UD Indexes

The following tables depict plate layouts and pooling strategies for Illumina UD Indexes, which are designed for dual indexing.

Pooling strategies differ based on the type of chemistry used. Refer to [\[XLEAP-SBS Chemistry\] Two-Plex Through Eight-Plex Pooling Strategies on page 9](#) for instruments using XLEAP-SBS chemistry.

These indexes include Illumina DNA/RNA UD Indexes, Tagmentation and Illumina RNA UD Indexes, Ligation.

## Illumina UD Indexes Set A

|          | 1             | 2           | 3           | 4           | 5           | 6             | 7             | 8           | 9             | 10            | 11            | 12          |
|----------|---------------|-------------|-------------|-------------|-------------|---------------|---------------|-------------|---------------|---------------|---------------|-------------|
| <b>A</b> | UDP0001       | UDP000<br>9 | UDP001<br>7 | UDP002<br>5 | UDP003<br>3 | UDP0041       | UDP0049       | UDP005<br>7 | UDP0065       | UDP0073<br>V3 | UDP0081       | UDP008<br>9 |
| <b>B</b> | UDP0002       | UDP001<br>0 | UDP001<br>8 | UDP002<br>6 | UDP003<br>4 | UDP0042       | UDP0050       | UDP005<br>8 | UDP0066       | UDP0074<br>V3 | UDP0082       | UDP009<br>0 |
| <b>C</b> | UDP0003<br>V3 | UDP001<br>1 | UDP001<br>9 | UDP002<br>7 | UDP003<br>5 | UDP0043       | UDP0051       | UDP005<br>9 | UDP0067       | UDP0075<br>V3 | UDP0083       | UDP009<br>1 |
| <b>D</b> | UDP0004       | UDP001<br>2 | UDP002<br>0 | UDP002<br>8 | UDP003<br>6 | UDP0044       | UDP0052       | UDP006<br>0 | UDP0068       | UDP0076<br>V3 | UDP0084<br>V3 | UDP009<br>2 |
| <b>E</b> | UDP0005<br>V3 | UDP001<br>3 | UDP002<br>1 | UDP002<br>9 | UDP003<br>7 | UDP0045       | UDP0053<br>V3 | UDP006<br>1 | UDP0069<br>V3 | UDP0077       | UDP0085       | UDP009<br>3 |
| <b>F</b> | UDP0006       | UDP001<br>4 | UDP002<br>2 | UDP003<br>0 | UDP003<br>8 | UDP0046<br>V3 | UDP0054<br>V3 | UDP006<br>2 | UDP0070<br>V3 | UDP0078       | UDP0086       | UDP009<br>4 |
| <b>G</b> | UDP0007       | UDP001<br>5 | UDP002<br>3 | UDP003<br>1 | UDP003<br>9 | UDP0047       | UDP0055<br>V3 | UDP006<br>3 | UDP0071<br>V3 | UDP0079       | UDP0087       | UDP009<br>5 |
| <b>H</b> | UDP0008       | UDP001<br>6 | UDP002<br>4 | UDP003<br>2 | UDP004<br>0 | UDP0048       | UDP0056<br>V3 | UDP006<br>4 | UDP0072<br>V3 | UDP0080       | UDP0088       | UDP009<br>6 |

## Illumina UD Indexes Set B

|          | 1             | 2           | 3           | 4           | 5           | 6           | 7             | 8             | 9           | 10          | 11            | 12          |
|----------|---------------|-------------|-------------|-------------|-------------|-------------|---------------|---------------|-------------|-------------|---------------|-------------|
| <b>A</b> | UDP0097       | UDP010<br>5 | UDP011<br>3 | UDP0121     | UDP012<br>9 | UDP013<br>7 | UDP0145V<br>3 | UDP0153       | UDP0161     | UDP016<br>9 | UDP0177       | UDP018<br>5 |
| <b>B</b> | UDP0098       | UDP010<br>6 | UDP011<br>4 | UDP012<br>2 | UDP013<br>0 | UDP013<br>8 | UDP0146V<br>3 | UDP0154       | UDP016<br>2 | UDP017<br>0 | UDP0178       | UDP018<br>6 |
| <b>C</b> | UDP0099       | UDP010<br>7 | UDP0115     | UDP012<br>3 | UDP0131     | UDP013<br>9 | UDP0147V<br>3 | UDP0155V<br>3 | UDP016<br>3 | UDP0171     | UDP0179V<br>3 | UDP018<br>7 |
| <b>D</b> | UDP0100       | UDP010<br>8 | UDP011<br>6 | UDP012<br>4 | UDP013<br>2 | UDP014<br>0 | UDP0148V<br>3 | UDP0156       | UDP016<br>4 | UDP017<br>2 | UDP0180       | UDP018<br>8 |
| <b>E</b> | UDP0101       | UDP010<br>9 | UDP0117     | UDP012<br>5 | UDP013<br>3 | UDP0141     | UDP0149       | UDP0157       | UDP016<br>5 | UDP017<br>3 | UDP0181       | UDP018<br>9 |
| <b>F</b> | UDP0102V<br>3 | UDP0110     | UDP0118     | UDP012<br>6 | UDP013<br>4 | UDP014<br>2 | UDP0150       | UDP0158       | UDP016<br>6 | UDP017<br>4 | UDP0182       | UDP019<br>0 |
| <b>G</b> | UDP0103       | UDP0111     | UDP011<br>9 | UDP012<br>7 | UDP013<br>5 | UDP014<br>3 | UDP0151       | UDP0159       | UDP016<br>7 | UDP017<br>5 | UDP0183       | UDP0191     |
| <b>H</b> | UDP0104       | UDP0112     | UDP012<br>0 | UDP012<br>8 | UDP013<br>6 | UDP014<br>4 | UDP0152       | UDP0160       | UDP016<br>8 | UDP017<br>6 | UDP0184       | UDP019<br>2 |

## Illumina UD Indexes Set C

|          | 1             | 2           | 3           | 4             | 5             | 6           | 7             | 8             | 9             | 10            | 11          | 12            |
|----------|---------------|-------------|-------------|---------------|---------------|-------------|---------------|---------------|---------------|---------------|-------------|---------------|
| <b>A</b> | UDP0193<br>V3 | UDP02<br>01 | UDP02<br>09 | UDP0217       | UDP0225       | UDP023<br>3 | UDP0241       | UDP0249       | UDP0257       | UDP0265<br>V3 | UDP02<br>73 | UDP0281       |
| <b>B</b> | UDP0194<br>V3 | UDP02<br>02 | UDP021<br>0 | UDP0218<br>V3 | UDP0226       | UDP023<br>4 | UDP0242<br>V3 | UDP0250       | UDP0258<br>V2 | UDP0266<br>V3 | UDP02<br>74 | UDP0282       |
| <b>C</b> | UDP0195<br>V3 | UDP02<br>03 | UDP021<br>1 | UDP0219       | UDP0227<br>V3 | UDP023<br>5 | UDP0243       | UDP0251       | UDP0259       | UDP0267<br>V3 | UDP02<br>75 | UDP0283       |
| <b>D</b> | UDP0196<br>V3 | UDP02<br>04 | UDP021<br>2 | UDP0220       | UDP0228       | UDP023<br>6 | UDP0244<br>V3 | UDP0252<br>V2 | UDP0260       | UDP0268<br>V3 | UDP02<br>76 | UDP0284       |
| <b>E</b> | UDP0197       | UDP02<br>05 | UDP021<br>3 | UDP0221<br>V3 | UDP0229       | UDP023<br>7 | UDP0245       | UDP0253       | UDP0261       | UDP0269       | UDP02<br>77 | UDP0285<br>V3 |
| <b>F</b> | UDP0198       | UDP02<br>06 | UDP021<br>4 | UDP0222<br>V3 | UDP0230       | UDP023<br>8 | UDP0246       | UDP0254       | UDP0262       | UDP0270       | UDP02<br>78 | UDP0286<br>V3 |
| <b>G</b> | UDP0199       | UDP02<br>07 | UDP021<br>5 | UDP0223<br>V3 | UDP0231       | UDP023<br>9 | UDP0247       | UDP0255       | UDP0263       | UDP0271       | UDP02<br>79 | UDP0287<br>V3 |
| <b>H</b> | UDP0200       | UDP02<br>08 | UDP021<br>6 | UDP0224<br>V3 | UDP0232       | UDP02<br>40 | UDP0248       | UDP0256<br>V3 | UDP0264       | UDP0272       | UDP02<br>80 | UDP0288<br>V3 |

## Illumina UD Indexes Set D

|          | 1             | 2             | 3           | 4           | 5             | 6           | 7           | 8           | 9           | 10          | 11            | 12          |
|----------|---------------|---------------|-------------|-------------|---------------|-------------|-------------|-------------|-------------|-------------|---------------|-------------|
| <b>A</b> | UDP0289V<br>2 | UDP0297       | UDP030<br>5 | UDP031<br>3 | UDP0321V<br>3 | UDP032<br>9 | UDP033<br>7 | UDP034<br>5 | UDP035<br>3 | UDP036<br>1 | UDP0369<br>V3 | UDP037<br>7 |
| <b>B</b> | UDP0290V<br>2 | UDP0298V<br>3 | UDP030<br>6 | UDP031<br>4 | UDP0322       | UDP033<br>0 | UDP033<br>8 | UDP034<br>6 | UDP035<br>4 | UDP036<br>2 | UDP0370V<br>3 | UDP037<br>8 |
| <b>C</b> | UDP0291V<br>2 | UDP0299       | UDP030<br>7 | UDP031<br>5 | UDP0323       | UDP033<br>1 | UDP033<br>9 | UDP034<br>7 | UDP035<br>5 | UDP036<br>3 | UDP0371V<br>3 | UDP037<br>9 |
| <b>D</b> | UDP0292       | UDP0300       | UDP030<br>8 | UDP031<br>6 | UDP0324       | UDP033<br>2 | UDP034<br>0 | UDP034<br>8 | UDP035<br>6 | UDP036<br>4 | UDP0372V<br>3 | UDP038<br>0 |
| <b>E</b> | UDP0293       | UDP0301V<br>2 | UDP030<br>9 | UDP031<br>7 | UDP0325       | UDP033<br>3 | UDP034<br>1 | UDP034<br>9 | UDP035<br>7 | UDP036<br>5 | UDP0373       | UDP038<br>1 |
| <b>F</b> | UDP0294       | UDP0302       | UDP031<br>0 | UDP031<br>8 | UDP0326       | UDP033<br>4 | UDP034<br>2 | UDP035<br>0 | UDP035<br>8 | UDP036<br>6 | UDP0374       | UDP038<br>2 |
| <b>G</b> | UDP0295       | UDP0303       | UDP0311     | UDP031<br>9 | UDP0327       | UDP033<br>5 | UDP034<br>3 | UDP035<br>1 | UDP035<br>9 | UDP036<br>7 | UDP0375       | UDP038<br>3 |
| <b>H</b> | UDP0296       | UDP0304       | UDP031<br>2 | UDP032<br>0 | UDP0328       | UDP033<br>6 | UDP034<br>4 | UDP035<br>2 | UDP036<br>0 | UDP036<br>8 | UDP0376       | UDP038<br>4 |

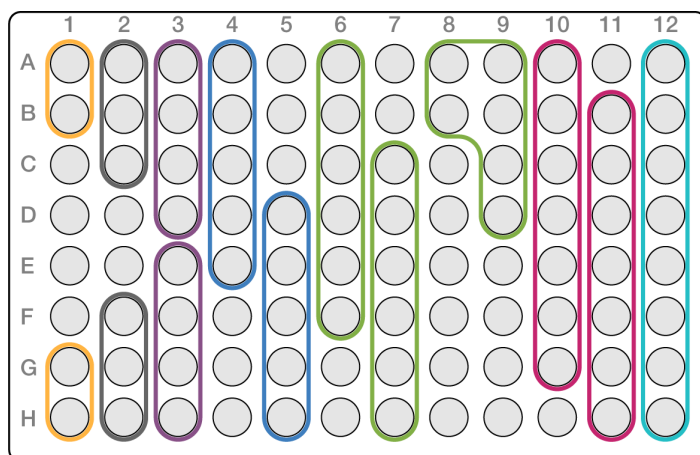
## Two-Plex Through Eight-Plex Pooling Strategies

Two-plex through eight-plex pooling strategies are only recommended for Illumina UD Indexes Set A and Set B. Illumina UD Indexes Set C and Set D are not designed for low-plexity pooling.

The following table shows index adapters (wells) that can be combined in a two-plex through eight-plex pool, while the color-coded figure illustrates each combination.

Pool any plexity  $\geq 2$  from the top or bottom of a column. Do not pool across a row.

| Plexity | Combinations Set A and Set B                                                                                                                                                                                                                                 | Combinations Set C and Set D | Color in Figure |
|---------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------|-----------------|
| 2       | The first two or last two wells in a column: <ul style="list-style-type: none"> <li>• A and B</li> <li>• G and H</li> </ul> Rows C–F are not used.                                                                                                           | Not Recommended              | Orange          |
| 3       | The first three or last three wells in a column: <ul style="list-style-type: none"> <li>• A–C</li> <li>• F–H</li> </ul> Rows D and E are not used.                                                                                                           | Not Recommended              | Gray            |
| 4       | The first four or last four wells in a column: <ul style="list-style-type: none"> <li>• A–D</li> <li>• E–H</li> </ul>                                                                                                                                        | Not Recommended              | Purple          |
| 5       | The first five or last five wells in a column: <ul style="list-style-type: none"> <li>• A–E</li> <li>• D–H</li> </ul>                                                                                                                                        | Not Recommended              | Blue            |
| 6       | [Option 1] The first six or last six wells in a column: <ul style="list-style-type: none"> <li>• A–F</li> <li>• C–H</li> </ul> [Option 2] The first two wells (A and B) or last two wells (G and H) in one column, and any four wells in an adjacent column. | Not Recommended              | Green           |
| 7       | The first seven or last seven wells in a column: <ul style="list-style-type: none"> <li>• A–G</li> <li>• B–H</li> </ul>                                                                                                                                      | Not Recommended              | Pink            |
| 8       | The entire column.                                                                                                                                                                                                                                           | Not Recommended              | Teal            |



### [XLEAP-SBS Chemistry] Two-Plex Through Eight-Plex Pooling Strategies

This section applies to any instruments using XLEAP-SBS Chemistry.

Two-plex through eight-plex pooling strategies are only recommended for Illumina UD Indexes Set A and Set B. Illumina UD Indexes Set C and Set D are not designed for low-plexity pooling.

The following table shows index adapters (wells) that can be combined in a two-plex or three-plex pool.

| Plexity | Combinations Set A                                                                                                                                                                                                                                            | Combinations Set B                                                                                                                                                                                                                         | Combinations Set C and Set D |
|---------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------|
| 2       | <ul style="list-style-type: none"> <li>• C6, D11</li> <li>• C6, H10</li> <li>• E4, A11</li> <li>• F1, H12</li> <li>• F3, G11</li> <li>• H7, E10</li> </ul>                                                                                                    | <ul style="list-style-type: none"> <li>• B9, D11</li> <li>• D11, A12</li> <li>• D11, B12</li> <li>• F3, D11</li> <li>• G3, C10</li> <li>• G3, D11</li> <li>• H5, G9</li> <li>• H7, B10</li> <li>• H8, D11</li> <li>• C8, D11</li> </ul>    | Not Recommended              |
| 3       | <ul style="list-style-type: none"> <li>• A3, C3, D3</li> <li>• E3, G3, H3</li> <li>• B4, C4, D4</li> <li>• F8, G8, H8</li> <li>• B11, C11, G11</li> <li>• B11, F11, G11</li> <li>• A12, G12, H12</li> <li>• E12, G12, H12</li> <li>• D12, G12, H12</li> </ul> | <ul style="list-style-type: none"> <li>• F8, G8, H8</li> <li>• E9, G9, H9</li> <li>• B10, C10, D10</li> <li>• C10, D10, E10</li> <li>• A12, C12, E12</li> <li>• A12, C12, F12</li> <li>• B12, F12, H12</li> <li>• A11, C11, D11</li> </ul> | Not Recommended              |

The following figures display four-plex through eight-plex index pooling recommendations by column for Plates A and B. Numbers represent plate columns, and letters represent the rows to be paired within each column. For example, Plate A, column 2, rows ABCD represent indexes UDP0009, UDP0010, UDP0011, and UDP0012.

The orange wells in the following color-coded figures are not recommended for use.

Figure 1 Illumina UD Plate A Indexes

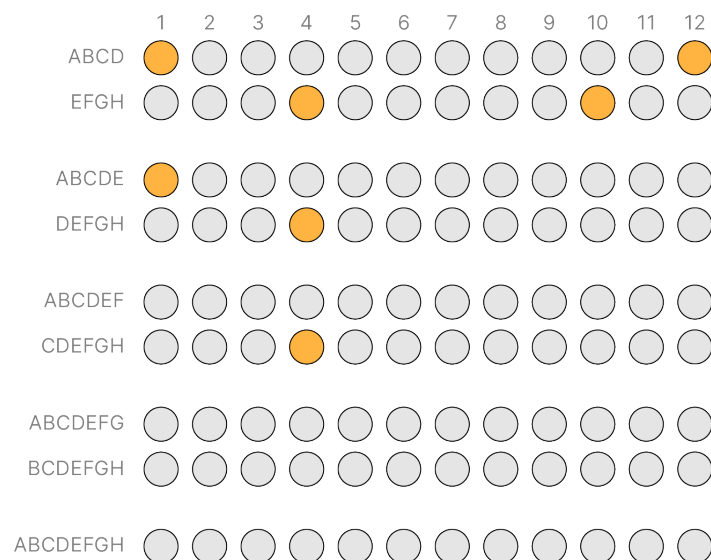
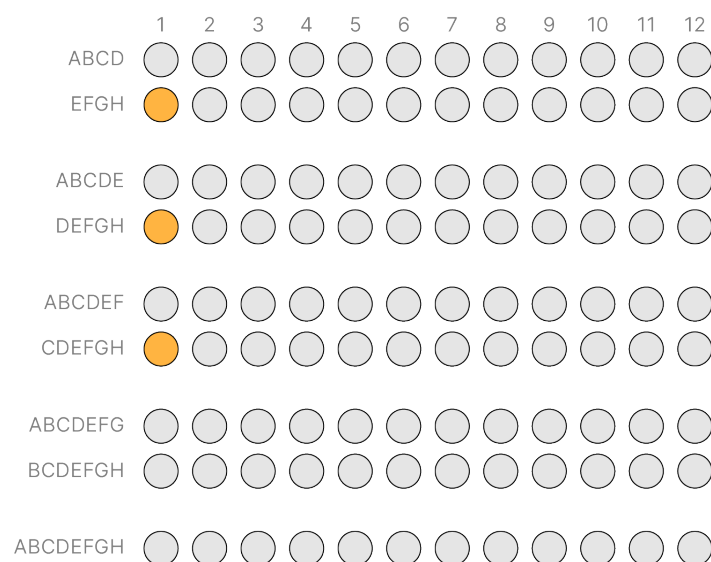


Figure 2 Illumina UD Plate B Indexes



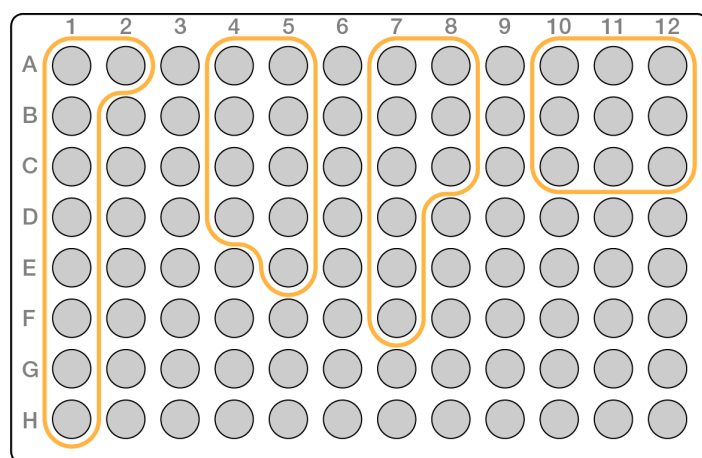
## Nine-Plex Pooling Strategies

Nine-plex pooling strategies are only recommended for Illumina UD Indexes Set A and Set B. Illumina UD Indexes Set C and Set D are not designed for low-plexity pooling.

Use index adapters from any wells that optimize color balance in a sequencing run, for example:

- A1–H1 and A2
- A4–D4 and A5–E5
- A7–F7 and A8–C8
- A10–C10, A11–C11, and A12–C12

The following figure depicts all four examples.



## IDT for Illumina UD Indexes

The following tables depict plate layouts and pooling strategies for IDT for Illumina UD Indexes, which are designed for dual indexing.

The IDT for Illumina UD Indexes include IDT for Illumina Nextera DNA UD Indexes, IDT for Illumina DNA/RNA UD Indexes, and IDT for Illumina PCR UD Indexes.

### IDT for Illumina UD Indexes Plate A/Set 1

|   | 1       | 2       | 3       | 4       | 5       | 6       | 7       | 8       | 9       | 10      | 11      | 12      |
|---|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| A | UDP0001 | UDP0009 | UDP0017 | UDP0025 | UDP0033 | UDP0041 | UDP0049 | UDP0057 | UDP0065 | UDP0073 | UDP0081 | UDP0089 |
| B | UDP0002 | UDP0010 | UDP0018 | UDP0026 | UDP0034 | UDP0042 | UDP0050 | UDP0058 | UDP0066 | UDP0074 | UDP0082 | UDP0090 |
| C | UDP0003 | UDP0011 | UDP0019 | UDP0027 | UDP0035 | UDP0043 | UDP0051 | UDP0059 | UDP0067 | UDP0075 | UDP0083 | UDP0091 |
| D | UDP0004 | UDP0012 | UDP0020 | UDP0028 | UDP0036 | UDP0044 | UDP0052 | UDP0060 | UDP0068 | UDP0076 | UDP0084 | UDP0092 |
| E | UDP0005 | UDP0013 | UDP0021 | UDP0029 | UDP0037 | UDP0045 | UDP0053 | UDP0061 | UDP0069 | UDP0077 | UDP0085 | UDP0093 |

|          |         |         |         |         |         |         |         |         |         |         |         |         |
|----------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| <b>F</b> | UDP0006 | UDP0014 | UDP0022 | UDP0030 | UDP0038 | UDP0046 | UDP0054 | UDP0062 | UDP0070 | UDP0078 | UDP0086 | UDP0094 |
| <b>G</b> | UDP0007 | UDP0015 | UDP0023 | UDP0031 | UDP0039 | UDP0047 | UDP0055 | UDP0063 | UDP0071 | UDP0079 | UDP0087 | UDP0095 |
| <b>H</b> | UDP0008 | UDP0016 | UDP0024 | UDP0032 | UDP0040 | UDP0048 | UDP0056 | UDP0064 | UDP0072 | UDP0080 | UDP0088 | UDP0096 |

## IDT for Illumina UD Indexes Plate B/Set 2

|          | 1       | 2       | 3       | 4       | 5       | 6       | 7       | 8       | 9       | 10      | 11      | 12      |
|----------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| <b>A</b> | UDP0097 | UDP0105 | UDP0113 | UDP0121 | UDP0129 | UDP0137 | UDP0145 | UDP0153 | UDP0161 | UDP0169 | UDP0177 | UDP0185 |
| <b>B</b> | UDP0098 | UDP0106 | UDP0114 | UDP0122 | UDP0130 | UDP0138 | UDP0146 | UDP0154 | UDP0162 | UDP0170 | UDP0178 | UDP0186 |
| <b>C</b> | UDP0099 | UDP0107 | UDP0115 | UDP0123 | UDP0131 | UDP0139 | UDP0147 | UDP0155 | UDP0163 | UDP0171 | UDP0179 | UDP0187 |
| <b>D</b> | UDP0100 | UDP0108 | UDP0116 | UDP0124 | UDP0132 | UDP0140 | UDP0148 | UDP0156 | UDP0164 | UDP0172 | UDP0180 | UDP0188 |
| <b>E</b> | UDP0101 | UDP0109 | UDP0117 | UDP0125 | UDP0133 | UDP0141 | UDP0149 | UDP0157 | UDP0165 | UDP0173 | UDP0181 | UDP0189 |
| <b>F</b> | UDP0102 | UDP0110 | UDP0118 | UDP0126 | UDP0134 | UDP0142 | UDP0150 | UDP0158 | UDP0166 | UDP0174 | UDP0182 | UDP0190 |
| <b>G</b> | UDP0103 | UDP0111 | UDP0119 | UDP0127 | UDP0135 | UDP0143 | UDP0151 | UDP0159 | UDP0167 | UDP0175 | UDP0183 | UDP0191 |
| <b>H</b> | UDP0104 | UDP0112 | UDP0120 | UDP0128 | UDP0136 | UDP0144 | UDP0152 | UDP0160 | UDP0168 | UDP0176 | UDP0184 | UDP0192 |

## IDT for Illumina UD Indexes Plate C/Set 3

The V2 indication applies only to IDT for Illumina DNA/RNA and PCR UD Indexes. IDT for Illumina Nextera DNA UD Indexes do not include V2 indexes.

|          | 1           | 2           | 3           | 4           | 5           | 6           | 7           | 8             | 9             | 10          | 11          | 12          |
|----------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|---------------|---------------|-------------|-------------|-------------|
| <b>A</b> | UDP0193     | UDP0201     | UDP020<br>9 | UDP0217     | UDP022<br>5 | UDP023<br>3 | UDP0241     | UDP0249       | UDP0257       | UDP026<br>5 | UDP027<br>3 | UDP0281     |
| <b>B</b> | UDP0194     | UDP020<br>2 | UDP0210     | UDP0218     | UDP022<br>6 | UDP023<br>4 | UDP024<br>2 | UDP0250       | UDP0256V<br>2 | UDP026<br>6 | UDP027<br>4 | UDP028<br>2 |
| <b>C</b> | UDP0195     | UDP020<br>3 | UDP0211     | UDP0219     | UDP022<br>7 | UDP023<br>5 | UDP024<br>3 | UDP0251       | UDP0259       | UDP026<br>7 | UDP027<br>5 | UDP028<br>3 |
| <b>D</b> | UDP0196     | UDP020<br>4 | UDP0212     | UDP022<br>0 | UDP022<br>8 | UDP023<br>6 | UDP024<br>4 | UDP0252V<br>2 | UDP0260       | UDP026<br>8 | UDP027<br>6 | UDP028<br>4 |
| <b>E</b> | UDP0197     | UDP020<br>5 | UDP0213     | UDP0221     | UDP022<br>9 | UDP023<br>7 | UDP024<br>5 | UDP0253       | UDP0261       | UDP026<br>9 | UDP027<br>7 | UDP028<br>5 |
| <b>F</b> | UDP0198     | UDP020<br>6 | UDP0214     | UDP022<br>2 | UDP023<br>0 | UDP023<br>8 | UDP024<br>6 | UDP0254       | UDP0262       | UDP027<br>0 | UDP027<br>8 | UDP028<br>6 |
| <b>G</b> | UDP0199     | UDP020<br>7 | UDP0215     | UDP022<br>3 | UDP0231     | UDP023<br>9 | UDP024<br>7 | UDP0255       | UDP0263       | UDP0271     | UDP027<br>9 | UDP028<br>7 |
| <b>H</b> | UDP020<br>0 | UDP020<br>8 | UDP0216     | UDP022<br>4 | UDP023<br>2 | UDP024<br>0 | UDP024<br>8 | UDP0256       | UDP0264       | UDP027<br>2 | UDP028<br>0 | UDP028<br>8 |

## IDT for Illumina UD Indexes Plate D/Set 4

The V2 indication applies only to IDT for Illumina DNA/RNA and PCR UD Indexes. IDT for Illumina Nextera DNA UD Indexes do not include V2 indexes.

|   | 1             | 2             | 3           | 4           | 5           | 6           | 7           | 8           | 9           | 10          | 11          | 12          |
|---|---------------|---------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| A | UDP0289V<br>2 | UDP0297       | UDP030<br>5 | UDP0313     | UDP0321     | UDP032<br>9 | UDP033<br>7 | UDP034<br>5 | UDP035<br>3 | UDP0361     | UDP036<br>9 | UDP037<br>7 |
| B | UDP0290V<br>2 | UDP0298       | UDP030<br>6 | UDP031<br>4 | UDP032<br>2 | UDP033<br>0 | UDP033<br>8 | UDP034<br>6 | UDP035<br>4 | UDP036<br>2 | UDP037<br>0 | UDP037<br>8 |
| C | UDP0291V<br>2 | UDP0299       | UDP030<br>7 | UDP0315     | UDP032<br>3 | UDP0331     | UDP033<br>9 | UDP034<br>7 | UDP035<br>5 | UDP036<br>3 | UDP0371     | UDP037<br>9 |
| D | UDP0292       | UDP0300       | UDP030<br>8 | UDP0316     | UDP032<br>4 | UDP033<br>2 | UDP034<br>0 | UDP034<br>8 | UDP035<br>6 | UDP036<br>4 | UDP037<br>2 | UDP038<br>0 |
| E | UDP0293       | UDP0301V<br>2 | UDP030<br>9 | UDP0317     | UDP032<br>5 | UDP033<br>3 | UDP0341     | UDP034<br>9 | UDP035<br>7 | UDP036<br>5 | UDP037<br>3 | UDP0381     |
| F | UDP0294       | UDP0302       | UDP0310     | UDP0318     | UDP032<br>6 | UDP033<br>4 | UDP034<br>2 | UDP035<br>0 | UDP035<br>8 | UDP036<br>6 | UDP037<br>4 | UDP038<br>2 |
| G | UDP0295       | UDP0303       | UDP0311     | UDP0319     | UDP032<br>7 | UDP033<br>5 | UDP034<br>3 | UDP0351     | UDP035<br>9 | UDP036<br>7 | UDP037<br>5 | UDP038<br>3 |
| H | UDP0296       | UDP0304       | UDP0312     | UDP032<br>0 | UDP032<br>8 | UDP033<br>6 | UDP034<br>4 | UDP035<br>2 | UDP036<br>0 | UDP036<br>8 | UDP037<br>6 | UDP038<br>4 |

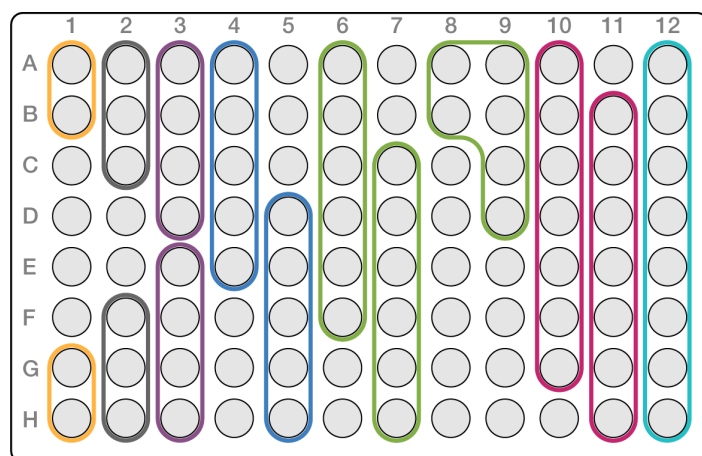
## Two-Plex Through Eight-Plex Pooling Strategies

The following table shows index adapters (wells) that can be combined in a two- to eight-plex pool, while the color-coded figure illustrates each combination.

Pool any plexity  $\geq 2$  from the top or bottom of a column. Do not pool across a row.

| Plexity | Combinations                                                                                                                                       | Color in Figure |
|---------|----------------------------------------------------------------------------------------------------------------------------------------------------|-----------------|
| 2       | The first two or last two wells in a column: <ul style="list-style-type: none"> <li>• A and B</li> <li>• G and H</li> </ul> Rows C–F are not used. | Orange          |
| 3       | The first three or last three wells in a column: <ul style="list-style-type: none"> <li>• A–C</li> <li>• F–H</li> </ul> Rows D and E are not used. | Gray            |
| 4       | The first four or last four wells in a column: <ul style="list-style-type: none"> <li>• A–D</li> <li>• E–H</li> </ul>                              | Purple          |

| Plexity | Combinations                                                                                                                                                                                                                                                | Color in Figure |
|---------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------|
| 5       | The first five or last five wells in a column:<br><ul style="list-style-type: none"> <li>A–E</li> <li>D–H</li> </ul>                                                                                                                                        | Blue            |
| 6       | [Option 1] The first six or last six wells in a column:<br><ul style="list-style-type: none"> <li>A–F</li> <li>C–H</li> </ul> [Option 2] The first two wells (A and B) or last two wells (G and H) in one column, and any four wells in an adjacent column. | Green           |
| 7       | The first seven or last seven wells in a column:<br><ul style="list-style-type: none"> <li>A–G</li> <li>B–H</li> </ul>                                                                                                                                      | Pink            |
| 8       | The entire column.                                                                                                                                                                                                                                          | Teal            |



### [XLEAP-SBS Chemistry] Two-Plex Through Eight-Plex Pooling Strategies

This section applies to any instruments using XLEAP-SBS Chemistry.

Pool any plexity  $\geq 2$  from the top or bottom of a column. Do not pool across a row.

The following table shows index adapters (wells) that can be combined in a two-plex or three-plex pool.

| Plexity | Combinations Set A                                                                                                                                                                                                                   | Combinations Set B                                                                                                                                                                                                   |
|---------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 2       | <ul style="list-style-type: none"> <li>• C6, D11</li> <li>• C6, H10</li> <li>• E4, A11</li> <li>• F1, H12</li> <li>• F3, G11</li> </ul>                                                                                              | <ul style="list-style-type: none"> <li>• B9, D11</li> <li>• D11, A12</li> <li>• D11, B12</li> <li>• F3, D11</li> <li>• G3, C10</li> <li>• G3, D11</li> <li>• H5, G9</li> <li>• H7, B10</li> <li>• H8, D11</li> </ul> |
| 3       | <ul style="list-style-type: none"> <li>• A3, C3, D3</li> <li>• E3, G3, H3</li> <li>• B4, C4, D4</li> <li>• F8, G8, H8</li> <li>• B11, C11, G11</li> <li>• B11, F11, G11</li> <li>• A12, G12, H12</li> <li>• E12, G12, H12</li> </ul> | <ul style="list-style-type: none"> <li>• F8, G8, H8</li> <li>• E9, G9, H9</li> <li>• B10, C10, D10</li> <li>• C10, D10, E10</li> <li>• A12, C12, E12</li> <li>• A12, C12, F12</li> <li>• B12, F12, H12</li> </ul>    |

The following figures display four-plex through eight-plex index pooling recommendations by column for Plates A and B. Numbers represent plate columns, and letters represent the rows to be paired within each column. For instance, Plate A, column 2, rows ABCD represent indexes UDP0009, UDP0010, UDP0011, and UDP0012.

The orange wells in the following color-coded figures are not recommended for use.

Figure 3 IDT for Illumina UD Plate A Indexes

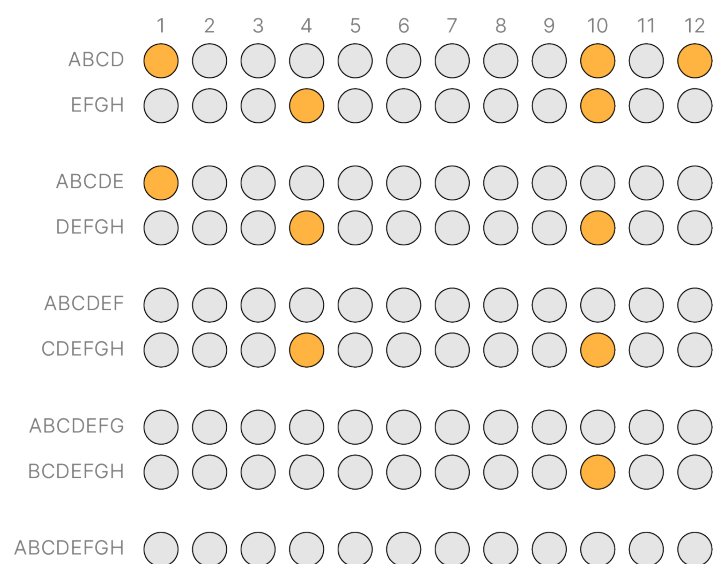
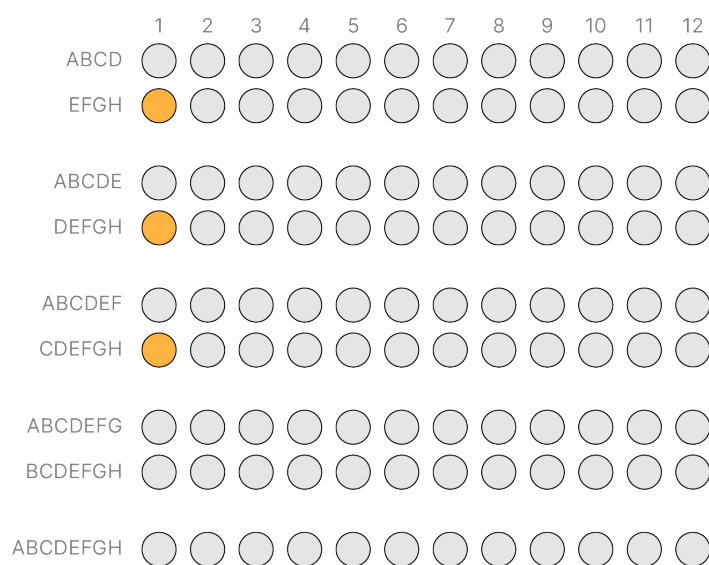


Figure 4 IDT for Illumina UD Plate B Indexes

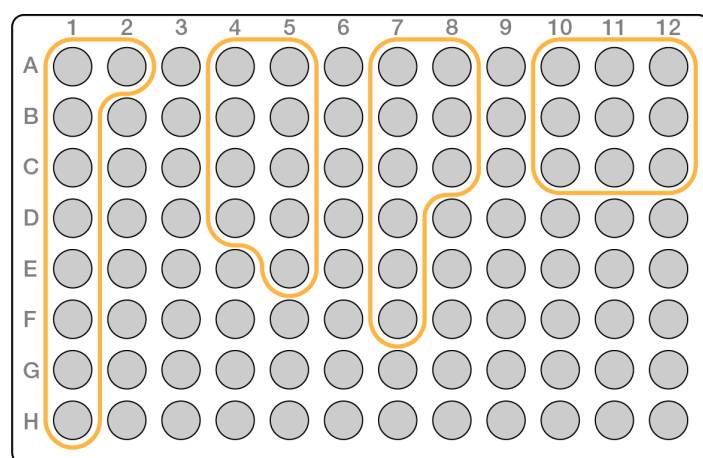


## Nine-Plex Pooling Strategies

Use index adapters from any wells that optimize color balance in a sequencing run, for example:

- A1–H1 and A2
- A4–D4 and A5–E5
- A7–F7 and A8–C8
- A10–C10, A11–C11, and A12–C12

The following figure depicts all four examples.



## Nextera DNA CD Indexes (96 Indexes, 96 Samples)

The following table depicts the plate layout for Nextera DNA CD Indexes (96 indexes, 96 samples), which are designed for dual indexing.

|          | 1    | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   | 11   | 12   |
|----------|------|------|------|------|------|------|------|------|------|------|------|------|
| <b>A</b> | H701 | H702 | H703 | H705 | H707 | H723 | H706 | H712 | H720 | H710 | H711 | H714 |
|          | H505 | H506 | H517 | H505 | H506 | H517 | H505 | H506 | H517 | H505 | H506 | H517 |
| <b>B</b> | H702 | H703 | H701 | H707 | H723 | H705 | H712 | H720 | H706 | H711 | H714 | H710 |
|          | H517 | H505 | H506 | H517 | H505 | H506 | H517 | H505 | H506 | H517 | H505 | H506 |
| <b>C</b> | H703 | H701 | H702 | H723 | H705 | H707 | H720 | H706 | H712 | H714 | H710 | H711 |
|          | H506 | H517 | H505 | H506 | H517 | H505 | H506 | H517 | H505 | H506 | H517 | H505 |
| <b>D</b> | H705 | H707 | H723 | H706 | H712 | H720 | H710 | H711 | H714 | H701 | H702 | H703 |
|          | H503 | H503 | H503 | H503 | H503 | H503 | H503 | H503 | H503 | H503 | H503 | H503 |
| <b>E</b> | H706 | H712 | H720 | H710 | H711 | H714 | H701 | H702 | H703 | H705 | H707 | H723 |
|          | H516 | H516 | H516 | H516 | H516 | H516 | H516 | H516 | H516 | H516 | H516 | H516 |
| <b>F</b> | H710 | H711 | H714 | H701 | H702 | H703 | H705 | H707 | H723 | H706 | H712 | H720 |
|          | H522 | H510 | H513 | H522 | H510 | H513 | H522 | H510 | H513 | H522 | H510 | H513 |
| <b>G</b> | H711 | H714 | H710 | H702 | H703 | H701 | H707 | H723 | H705 | H712 | H720 | H706 |
|          | H513 | H522 | H510 | H513 | H522 | H510 | H513 | H522 | H510 | H513 | H522 | H510 |
| <b>H</b> | H714 | H710 | H711 | H703 | H701 | H702 | H723 | H705 | H707 | H720 | H706 | H712 |
|          | H510 | H513 | H522 | H510 | H513 | H522 | H510 | H513 | H522 | H510 | H513 | H522 |

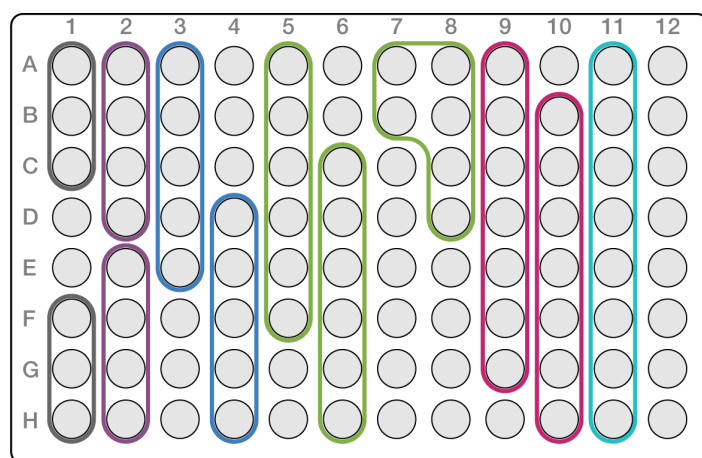
### Three-Plex Through Eight-Plex Pooling Strategies

The following table shows index adapters (wells) that can be combined in a three- to eight-plex pool, while the color-coded figure illustrates each combination.

A minimum plexity of three makes sure that libraries are color balanced for sequencing on any Illumina system. To create a two-plex pool, review the index adapter sequences to ensure color balance on your system.

| Plexity | Combinations                                                                                                                                       | Color in Figure |
|---------|----------------------------------------------------------------------------------------------------------------------------------------------------|-----------------|
| 3       | The first three or last three wells in a column: <ul style="list-style-type: none"> <li>• A–C</li> <li>• F–H</li> </ul> Rows D and E are not used. | Gray            |
| 4       | The first four or last four wells in a column: <ul style="list-style-type: none"> <li>• A–D</li> <li>• E–H</li> </ul>                              | Purple          |
| 5       | The first five or last five wells in a column: <ul style="list-style-type: none"> <li>• A–E</li> <li>• D–H</li> </ul>                              | Blue            |

| Plexity | Combinations                                                                                                                                                                                                                                                 | Color in Figure |
|---------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------|
| 6       | [Option 1] The first six or last six wells in a column: <ul style="list-style-type: none"> <li>• A–F</li> <li>• C–H</li> </ul> [Option 2] The first two wells (A and B) or last two wells (G and H) in one column, and any four wells in an adjacent column. | Green           |
| 7       | The first seven or last seven wells in a column: <ul style="list-style-type: none"> <li>• A–G</li> <li>• B–H</li> </ul>                                                                                                                                      | Pink            |
| 8       | The entire column.                                                                                                                                                                                                                                           | Teal            |

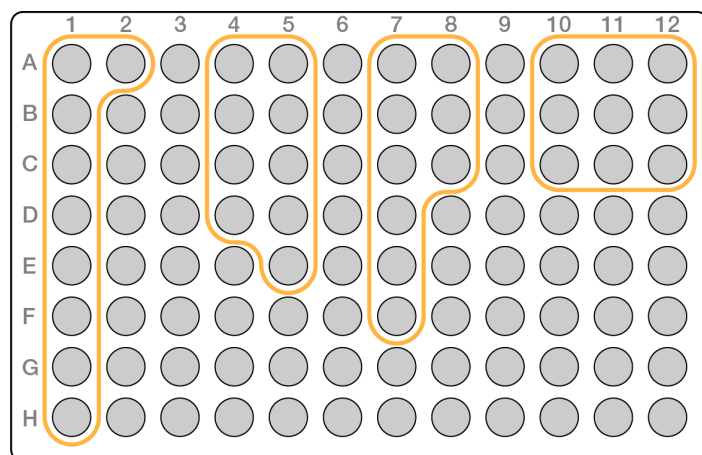


## Nine-Plex Pooling Strategies

Use index adapters from any wells that optimize color balance in a sequencing run, for example:

- A1–H1 and A2
- A4–D4 and A5–E5
- A7–F7 and A8–C8
- A10–C10, A11–C11, and A12–C12

The following figure depicts all four examples.



## Nextera DNA CD Indexes (24 Indexes, 24 Samples)

Nextera DNA CD Indexes (24 indexes, 24 samples) contain six Index 1 (i7) adapters and four Index 2 (i5) adapters packaged in tubes. The following table shows strategies for pooling three to eight dual-indexed libraries prepared with these indexes.

A minimum plexity of three makes sure that libraries are color balanced for sequencing on any Illumina system. To create a two-plex pool, review the index adapter sequences to ensure color balance on your system.

| Plexity | Index 1 (i7) Adapters                                                                                                                                                                   | Index 2 (i5) Adapters                                                                                                                                                         |
|---------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 3       | One of the following combinations: <ul style="list-style-type: none"> <li>• H705, H706, and H707</li> <li>• H710, H711, and H714</li> </ul>                                             | H505, H506, and H517                                                                                                                                                          |
| 4       | One of the following combinations: <ul style="list-style-type: none"> <li>• H705, H706, H707, and any other i7 adapter</li> <li>• H710, H711, H714, and any other i7 adapter</li> </ul> | H503, H505, H506, and H517                                                                                                                                                    |
| 5       | One of the following combinations: <ul style="list-style-type: none"> <li>• H705, H706, H707, and any two i7 adapters</li> <li>• H710, H711, H714, and any two i7 adapters</li> </ul>   | H503, H505, H506, H517, and any other i5 adapter                                                                                                                              |
| 6       | One of the following combinations: <ul style="list-style-type: none"> <li>• H705, H706, H707, H710, H711, H714</li> <li>• Two 3-plex pools</li> </ul>                                   | One of the following combinations: <ul style="list-style-type: none"> <li>• H503, H505, H506, H517, and any two additional i5 adapters</li> <li>• Two 3-plex pools</li> </ul> |

| Plexity | Index 1 (i7) Adapters                                                                                                                                                                                    | Index 2 (i5) Adapters                                                                                                                                                                                    |
|---------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 7       | One of the following combinations: <ul style="list-style-type: none"> <li>• H705, H706, H707, H710, H711, H714, and any other i7 adapter</li> <li>• Two 3-plex pools and any other i7 adapter</li> </ul> | One of the following combinations: <ul style="list-style-type: none"> <li>• H503, H505, H506, H517, and any three additional i5 adapters</li> <li>• Two 3-plex pools and any other i5 adapter</li> </ul> |
| 8       | H705, H706, H707, H710, H711, H714, and two other i7 adapters                                                                                                                                            | H503, H505, H506, and H517, used two times each                                                                                                                                                          |

### Four-Plex Example

Libraries with the following Index 1 and Index 2 adapter pairs can be combined in a four-plex pool:

- H705-H503
- H706-H505
- H707-H506
- H710-H517

### Five-Plex Example

Libraries with the following Index 1 and Index 2 adapter pairs can be combined in a five-plex pool:

- H503-H705
- H505-H706
- H506-H707
- H517-H710
- H503-H711

### Six-Plex Example

Libraries with the following Index 1 and Index 2 adapter pairs can be combined in a six-plex pool:

- H705-H503
- H706-H505
- H707-H506
- H710-H517
- H711-H503
- H714-H505

## Nextera XT Index Kit v2 and Nextera Index Kit

The Nextera XT Index Kit v2 is available in Sets A–D. Each set contains 12 Index 1 (i7) and eight Index 2 (i5) adapters packaged in tubes.

The Nextera Index Kit also packages Index 1 and Index 2 adapters in tubes, and is available in the following sizes:

- 24 indexes, 96 samples—Contains six Index 1 and four Index 2 adapters.
- 96 indexes, 384 samples—Contains 12 Index 1 adapters and eight Index 2 adapters.

### Dual Indexing

The following table shows strategies for pooling dual-indexed libraries for dual-indexed sequencing, which sequences Index 1 and Index 2.

| Plexity | Index 1 (i7) Adapters                                                                                                                                                                   | Index 2 (i5) Adapters                                                                                                                                                       |
|---------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 2–6     | At least two unique i7 adapters                                                                                                                                                         | At least two unique i5 adapters                                                                                                                                             |
| 7–12    | One of the following combinations: <ul style="list-style-type: none"> <li>• N701, N702, N704, and any other i7 adapter</li> <li>• N703, N705, N706, and any other i7 adapter</li> </ul> | One of the following combinations: <ul style="list-style-type: none"> <li>• N503 and N504</li> <li>• N505 and N506</li> </ul>                                               |
| > 12    | N701–N706 and any other i7 adapter                                                                                                                                                      | One of the following combinations: <ul style="list-style-type: none"> <li>• N503, N504, and any other i5 adapter</li> <li>• N505, N506, and any other i5 adapter</li> </ul> |

### Single Indexing With Dual Index Adapters

The following table shows strategies for creating 2–12-plex pools of dual-indexed libraries for single-indexed sequencing, which sequences Index 1 only.

| Plexity | Index 1 (i7) Adapters                                                                                                                       | Index 2 (i5) Adapters |
|---------|---------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| 2       | One of the following combinations: <ul style="list-style-type: none"> <li>• N701 and N702</li> <li>• N702 and N704</li> </ul>               | Any i5 adapters       |
| 3       | One of the following combinations: <ul style="list-style-type: none"> <li>• N701, N702, and N704</li> <li>• N703, N705, and N706</li> </ul> | Any i5 adapters       |

| Plexity | Index 1 (i7) Adapters                                                                                                                                                                | Index 2 (i5) Adapters |
|---------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| 4 or 5  | One of the following combinations: <ul style="list-style-type: none"><li>• N701, N702, N704, and any other i7 adapter</li><li>• N703, N705, N706, and any other i7 adapter</li></ul> | Any i5 adapters       |
| 6       | N701, N702, N703, N704, N705, and N706                                                                                                                                               | Any i5 adapters       |
| 7–12    | N701–N706 and any other i7 adapter                                                                                                                                                   | Any i5 adapters       |

# AmpliSeq for Illumina Panels

This section provides guidelines for pooling two to eight AmpliSeq for Illumina libraries ligated with AmpliSeq CD Indexes for Illumina. These index adapters are designed for dual indexing and used with all AmpliSeq for Illumina panels. Use any column- or row-based pooling strategy with any set to create two- to eight-plex pools.

## AmpliSeq CD Indexes for Illumina

The following tables depict plate layouts for AmpliSeq CD Indexes for Illumina.

### AmpliSeq CD Indexes Set A

|          | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    | 11    | 12    |
|----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| <b>A</b> | Q7005 | Q7015 | Q7006 | Q7007 | Q7016 | Q7008 | Q7018 | Q7023 | Q7017 | Q7025 | Q7024 | Q7026 |
|          | Q5001 | Q5002 | Q5007 | Q5008 | Q5009 | Q5010 | Q5001 | Q5002 | Q5007 | Q5008 | Q5009 | Q5010 |
| <b>B</b> | Q7006 | Q7016 | Q7005 | Q7008 | Q7015 | Q7007 | Q7017 | Q7024 | Q7024 | Q7026 | Q7023 | Q7025 |
|          | Q5002 | Q5001 | Q5008 | Q5007 | Q5010 | Q5009 | Q5002 | Q5001 | Q5001 | Q5007 | Q5010 | Q5009 |
| <b>C</b> | Q7016 | Q7008 | Q7015 | Q7006 | Q7007 | Q7005 | Q7024 | Q7026 | Q7023 | Q7017 | Q7025 | Q7018 |
|          | Q5007 | Q5008 | Q5009 | Q5010 | Q5013 | Q5014 | Q5007 | Q5008 | Q5009 | Q5010 | Q5013 | Q5014 |
| <b>D</b> | Q7015 | Q7007 | Q7016 | Q7005 | Q7008 | Q7006 | Q7023 | Q7025 | Q7024 | Q7018 | Q7026 | Q7017 |
|          | Q5008 | Q5007 | Q5010 | Q5009 | Q5014 | Q5013 | Q5008 | Q5007 | Q5010 | Q5009 | Q5014 | Q5013 |
| <b>E</b> | Q7017 | Q7025 | Q7018 | Q7023 | Q7026 | Q7024 | Q7006 | Q7007 | Q7005 | Q7015 | Q7008 | Q7016 |
|          | Q5009 | Q5010 | Q5013 | Q5014 | Q5001 | Q5002 | Q5009 | Q5010 | Q5013 | Q5014 | Q5001 | Q5002 |
| <b>F</b> | Q7018 | Q7026 | Q7017 | Q7024 | Q7025 | Q7023 | Q7005 | Q7008 | Q7006 | Q7016 | Q7007 | Q7015 |
|          | Q5010 | Q5009 | Q5014 | Q5013 | Q5002 | Q5001 | Q5010 | Q5009 | Q5014 | Q5013 | Q5002 | Q5001 |
| <b>G</b> | Q7026 | Q7024 | Q7025 | Q7018 | Q7023 | Q7017 | Q7008 | Q7016 | Q7007 | Q7005 | Q7015 | Q7006 |
|          | Q5013 | Q5014 | Q5001 | Q5002 | Q5007 | Q5008 | Q5013 | Q5014 | Q5001 | Q5002 | Q5007 | Q5008 |
| <b>H</b> | Q7025 | Q7023 | Q7026 | Q7017 | Q7024 | Q7018 | Q7007 | Q7015 | Q7008 | Q7006 | Q7016 | Q7005 |
|          | Q5014 | Q5013 | Q5002 | Q5001 | Q5008 | Q5007 | Q5014 | Q5013 | Q5002 | Q5001 | Q5008 | Q5007 |

### AmpliSeq CD Indexes Set B

|          | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    | 11    | 12    |
|----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| <b>A</b> | Q7027 | Q7035 | Q7028 | Q7029 | Q7036 | Q7030 | Q7040 | Q7041 | Q7039 | Q7047 | Q7042 | Q7048 |
|          | Q5001 | Q5002 | Q5007 | Q5008 | Q5009 | Q5010 | Q5001 | Q5002 | Q5007 | Q5008 | Q5009 | Q5010 |
| <b>B</b> | Q7028 | Q7036 | Q7027 | Q7030 | Q7035 | Q7029 | Q7039 | Q7042 | Q7040 | Q7048 | Q7041 | Q7047 |
|          | Q5002 | Q5001 | Q5008 | Q5007 | Q5010 | Q5009 | Q5002 | Q5001 | Q5008 | Q5007 | Q5010 | Q5009 |

|          |       |       |       |       |       |       |       |       |       |       |       |       |
|----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| <b>C</b> | Q7036 | Q7030 | Q7035 | Q7028 | Q7029 | Q7027 | Q7042 | Q7048 | Q7041 | Q7039 | Q7047 | Q7040 |
|          | Q5007 | Q5008 | Q5009 | Q5010 | Q5013 | Q5014 | Q5007 | Q5008 | Q5009 | Q5010 | Q5013 | Q5014 |
| <b>D</b> | Q7035 | Q7029 | Q7036 | Q7027 | Q7030 | Q7028 | Q7041 | Q7047 | Q7042 | Q7040 | Q7048 | Q7039 |
|          | Q5008 | Q5007 | Q5010 | Q5009 | Q5014 | Q5013 | Q5008 | Q5007 | Q5010 | Q5009 | Q5014 | Q5013 |
| <b>E</b> | Q7039 | Q7047 | Q7040 | Q7041 | Q7048 | Q7042 | Q7028 | Q7029 | Q7027 | Q7035 | Q7030 | Q7036 |
|          | Q5009 | Q5010 | Q5013 | Q5014 | Q5001 | Q5002 | Q5009 | Q5010 | Q5013 | Q5014 | Q5001 | Q5002 |
| <b>F</b> | Q7040 | Q7048 | Q7039 | Q7042 | Q7047 | Q7041 | Q7027 | Q7030 | Q7028 | Q7036 | Q7029 | Q7035 |
|          | Q5010 | Q5009 | Q5014 | Q5013 | Q5002 | Q5001 | Q5010 | Q5009 | Q5014 | Q5013 | Q5002 | Q5001 |
| <b>G</b> | Q7048 | Q7042 | Q7047 | Q7040 | Q7041 | Q7039 | Q7030 | Q7036 | Q7029 | Q7027 | Q7035 | Q7028 |
|          | Q5013 | Q5014 | Q5001 | Q5002 | Q5007 | Q5008 | Q5013 | Q5014 | Q5001 | Q5002 | Q5007 | Q5008 |
| <b>H</b> | Q7047 | Q7041 | Q7048 | Q7039 | Q7042 | Q7040 | Q7029 | Q7035 | Q7030 | Q7028 | Q7036 | Q7027 |
|          | Q5014 | Q5013 | Q5002 | Q5001 | Q5008 | Q5007 | Q5014 | Q5013 | Q5002 | Q5001 | Q5008 | Q5007 |

## AmpliSeq CD Indexes Set C

|          | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    | 11    | 12    |
|----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| <b>A</b> | Q7005 | Q7015 | Q7006 | Q7007 | Q7016 | Q7008 | Q7018 | Q7023 | Q7017 | Q7025 | Q7024 | Q7026 |
|          | Q5017 | Q5018 | Q5025 | Q5026 | Q5027 | Q5028 | Q5017 | Q5018 | Q5025 | Q5026 | Q5027 | Q5028 |
| <b>B</b> | Q7006 | Q7016 | Q7005 | Q7008 | Q7015 | Q7007 | Q7017 | Q7024 | Q7018 | Q7026 | Q7023 | Q7025 |
|          | Q5018 | Q5017 | Q5026 | Q5025 | Q5028 | Q5027 | Q5018 | Q5017 | Q5026 | Q5025 | Q5028 | Q5027 |
| <b>C</b> | Q7016 | Q7008 | Q7015 | Q7006 | Q7007 | Q7005 | Q7024 | Q7026 | Q7023 | Q7017 | Q7025 | Q7018 |
|          | Q5025 | Q5026 | Q5027 | Q5028 | Q5031 | Q5032 | Q5025 | Q5026 | Q5027 | Q5028 | Q5031 | Q5032 |
| <b>D</b> | Q7015 | Q7007 | Q7016 | Q7005 | Q7008 | Q7006 | Q7023 | Q7025 | Q7024 | Q7018 | Q7026 | Q7017 |
|          | Q5026 | Q5025 | Q5028 | Q5027 | Q5032 | Q5031 | Q5026 | Q5025 | Q5028 | Q5027 | Q5032 | Q5031 |
| <b>E</b> | Q7017 | Q7025 | Q7018 | Q7023 | Q7026 | Q7024 | Q7006 | Q7007 | Q7005 | Q7015 | Q7008 | Q7016 |
|          | Q5027 | Q5028 | Q5031 | Q5032 | Q5017 | Q5018 | Q5027 | Q5028 | Q5031 | Q5032 | Q5017 | Q5018 |
| <b>F</b> | Q7018 | Q7026 | Q7017 | Q7024 | Q7025 | Q7023 | Q7005 | Q7008 | Q7006 | Q7016 | Q7007 | Q7015 |
|          | Q5028 | Q5027 | Q5032 | Q5031 | Q5018 | Q5017 | Q5028 | Q5027 | Q5032 | Q5031 | Q5018 | Q5017 |
| <b>G</b> | Q7026 | Q7024 | Q7025 | Q7018 | Q7023 | Q7017 | Q7008 | Q7016 | Q7007 | Q7005 | Q7015 | Q7006 |
|          | Q5031 | Q5032 | Q5017 | Q5018 | Q5025 | Q5026 | Q5031 | Q5032 | Q5017 | Q5018 | Q5025 | Q5026 |
| <b>H</b> | Q7025 | Q7023 | Q7026 | Q7017 | Q7024 | Q7018 | Q7007 | Q7015 | Q7008 | Q7006 | Q7016 | Q7005 |
|          | Q5032 | Q5031 | Q5018 | Q5017 | Q5026 | Q5025 | Q5032 | Q5031 | Q5018 | Q5017 | Q5026 | Q5025 |

## AmpliSeq CD Indexes Set D

|          | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    | 11    | 12    |
|----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| <b>A</b> | Q7027 | Q7035 | Q7028 | Q7029 | Q7036 | Q7030 | Q7040 | Q7041 | Q7039 | Q7047 | Q7042 | Q7048 |
|          | Q5017 | Q5018 | Q5025 | Q5026 | Q5027 | Q5028 | Q5017 | Q5018 | Q5025 | Q5026 | Q5027 | Q5028 |
| <b>B</b> | Q7028 | Q7036 | Q7027 | Q7030 | Q7035 | Q7029 | Q7039 | Q7042 | Q7040 | Q7048 | Q7041 | Q7047 |
|          | Q5018 | Q5017 | Q5026 | Q5025 | Q5028 | Q5027 | Q5018 | Q5017 | Q5026 | Q5025 | Q5028 | Q5027 |

|          | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    | 11    | 12    |
|----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| <b>C</b> | Q7036 | Q7030 | Q7035 | Q7028 | Q7029 | Q7027 | Q7042 | Q7048 | Q7041 | Q7039 | Q7047 | Q7040 |
|          | Q5025 | Q5026 | Q5027 | Q5028 | Q5031 | Q5032 | Q5025 | Q5026 | Q5027 | Q5028 | Q5031 | Q5032 |
| <b>D</b> | Q7035 | Q7029 | Q7036 | Q7027 | Q7030 | Q7028 | Q7041 | Q7047 | Q7042 | Q7040 | Q7048 | Q7039 |
|          | Q5026 | Q5025 | Q5028 | Q5027 | Q5032 | Q5031 | Q5026 | Q5025 | Q5028 | Q5027 | Q5032 | Q5031 |
| <b>E</b> | Q7039 | Q7047 | Q7040 | Q7041 | Q7048 | Q7042 | Q7028 | Q7029 | Q7027 | Q7035 | Q7030 | Q7036 |
|          | Q5027 | Q5028 | Q5031 | Q5032 | Q5017 | Q5018 | Q5027 | Q5028 | Q5031 | Q5032 | Q5017 | Q5018 |
| <b>F</b> | Q7040 | Q7048 | Q7039 | Q7042 | Q7047 | Q7041 | Q7027 | Q7030 | Q7028 | Q7036 | Q7029 | Q7035 |
|          | Q5028 | Q5027 | Q5032 | Q5031 | Q5018 | Q5017 | Q5028 | Q5027 | Q5032 | Q5031 | Q5018 | Q5017 |
| <b>G</b> | Q7048 | Q7042 | Q7047 | Q7040 | Q7041 | Q7039 | Q7030 | Q7036 | Q7029 | Q7027 | Q7035 | Q7028 |
|          | Q5031 | Q5032 | Q5017 | Q5018 | Q5025 | Q5026 | Q5031 | Q5032 | Q5017 | Q5018 | Q5025 | Q5026 |
| <b>H</b> | Q7047 | Q7041 | Q7048 | Q7039 | Q7042 | Q7040 | Q7029 | Q7035 | Q7030 | Q7028 | Q7036 | Q7027 |
|          | Q5032 | Q5031 | Q5018 | Q5017 | Q5026 | Q5025 | Q5032 | Q5031 | Q5018 | Q5017 | Q5026 | Q5025 |

## AmpliSeq UD Indexes for Illumina

The following table depicts the index plate layout for AmpliSeq UD Indexes for Illumina.

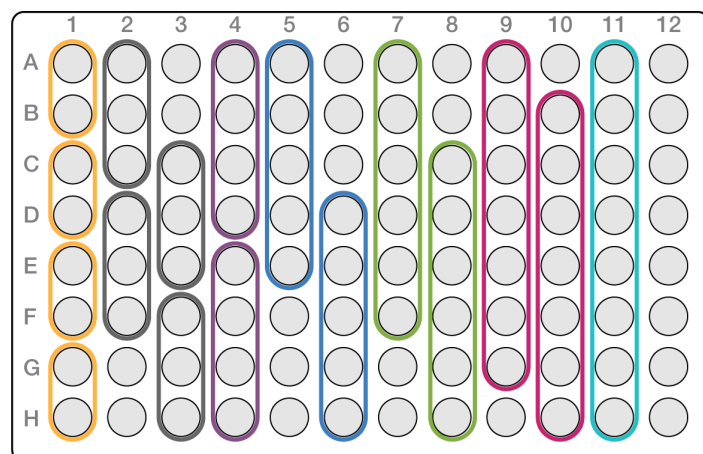
|          | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    | 11    | 12    |
|----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| <b>A</b> | Q7005 | Q7023 | Q7035 | empty | empty | empty | empty | empty | empty | empty | empty | empty |
|          | Q5007 | Q5013 | Q5025 |       |       |       |       |       |       |       |       |       |
| <b>B</b> | Q7006 | Q7024 | Q7036 | empty | empty | empty | empty | empty | empty | empty | empty | empty |
|          | Q5008 | Q5014 | Q5026 |       |       |       |       |       |       |       |       |       |
| <b>C</b> | Q7007 | Q7025 | Q7039 | empty | empty | empty | empty | empty | empty | empty | empty | empty |
|          | Q5039 | Q5035 | Q5031 |       |       |       |       |       |       |       |       |       |
| <b>D</b> | Q7008 | Q7026 | Q7040 | empty | empty | empty | empty | empty | empty | empty | empty | empty |
|          | Q5040 | Q5036 | Q5032 |       |       |       |       |       |       |       |       |       |
| <b>E</b> | Q7015 | Q7027 | Q7041 | empty | empty | empty | empty | empty | empty | empty | empty | empty |
|          | Q5003 | Q5027 | Q5009 |       |       |       |       |       |       |       |       |       |
| <b>F</b> | Q7016 | Q7028 | Q7042 | empty | empty | empty | empty | empty | empty | empty | empty | empty |
|          | Q5004 | Q5028 | Q5010 |       |       |       |       |       |       |       |       |       |
| <b>G</b> | Q7017 | Q7029 | Q7047 | empty | empty | empty | empty | empty | empty | empty | empty | empty |
|          | Q5017 | Q5001 | Q5029 |       |       |       |       |       |       |       |       |       |
| <b>H</b> | Q7018 | Q7030 | Q7048 | empty | empty | empty | empty | empty | empty | empty | empty | empty |
|          | Q5018 | Q5002 | Q5030 |       |       |       |       |       |       |       |       |       |

## Column-Based Strategies

The following table shows index adapters (wells) that can be combined in a two- to eight-plex pool. The color-coded figure illustrates each combination.

All combinations apply to any column on the plate. The minimum plexity for a column is two.

| Plexity | Combinations                                                                                                                                               | Color in Figure |
|---------|------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------|
| 2       | Two consecutive wells in a column:<br><ul style="list-style-type: none"> <li>• A and B</li> <li>• C and D</li> <li>• E and F</li> <li>• G and H</li> </ul> | Orange          |
| 3       | Three consecutive wells in a column:<br><ul style="list-style-type: none"> <li>• A–C</li> <li>• D–F</li> <li>• C–E</li> <li>• F–H</li> </ul>               | Gray            |
| 4       | Four consecutive wells in a column:<br><ul style="list-style-type: none"> <li>• A–D</li> <li>• E–H</li> </ul>                                              | Purple          |
| 5       | Five consecutive wells in a column:<br><ul style="list-style-type: none"> <li>• A–E</li> <li>• D–H</li> </ul>                                              | Blue            |
| 6       | Six consecutive wells in a column:<br><ul style="list-style-type: none"> <li>• A–F</li> <li>• C–H</li> </ul>                                               | Green           |
| 7       | Seven consecutive wells in a column:<br><ul style="list-style-type: none"> <li>• A–G</li> <li>• B–H</li> </ul>                                             | Pink            |
| 8       | The entire column. All eight combinations are unique.                                                                                                      | Teal            |

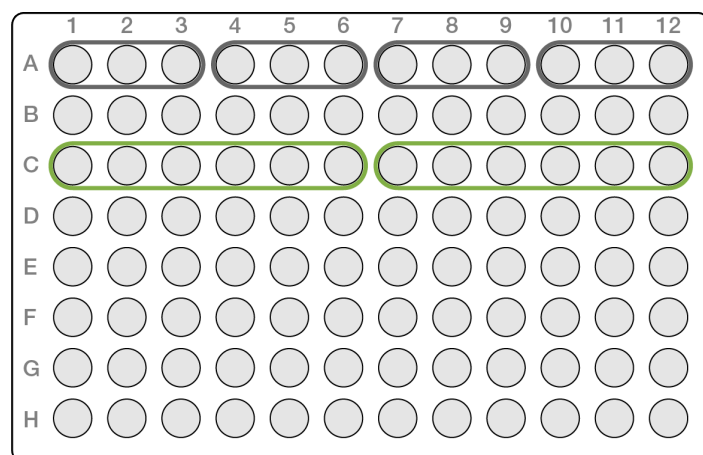


## Row-Based Strategies

The following table shows index adapters (wells) that can be combined in three- or six-plex pool. The color-coded figure illustrates each well combination.

All combinations apply to any row on the plate. A row can contain any set of pools confined to columns 1–6 or columns 7–12. The minimum plexity for a row is three.

| Plexity | Combinations                                                                                                                                | Color in Figure |
|---------|---------------------------------------------------------------------------------------------------------------------------------------------|-----------------|
| 3       | Three consecutive wells in a row.<br><ul style="list-style-type: none"> <li>• 1–3</li> <li>• 4–6</li> <li>• 7–9</li> <li>• 10–12</li> </ul> | Gray            |
| 6       | Six consecutive wells in a row:<br><ul style="list-style-type: none"> <li>• 1–6</li> <li>• 7–12</li> </ul>                                  | Green           |



# TruSeq Kits

This section provides guidelines for pooling indexed TruSeq libraries. The following index adapters are compatible with ligation-based TruSeq library prep kits.

| Index Adapters                                                                   | Indexing Scheme          | Format |
|----------------------------------------------------------------------------------|--------------------------|--------|
| IDT for Illumina–TruSeq DNA UD Indexes<br>IDT for Illumina–TruSeq RNA UD Indexes | Dual indexing            | Plate  |
| TruSeq DNA CD Indexes<br>TruSeq RNA CD Indexes                                   | Single and dual indexing | Plate  |
| TruSeq DNA Single Indexes<br>TruSeq RNA Single Indexes                           | Single indexing          | Tubes  |

## IDT for Illumina–TruSeq UD Indexes

The following tables depict plate layouts for IDT for Illumina–TruSeq UD Indexes.

These indexes include IDT for Illumina–TruSeq DNA UD Indexes and IDT for Illumina–TruSeq RNA UD Indexes. These indexes are designed for select TruSeq libraries.

For NextSeq 2000 two-plex pooling, pool two consecutive wells in a column as follows.

- A and B
- C and D
- E and F
- G and H

For other systems, pool any plexity  $\geq 2$  down a column. Do not pool across a row.

## IDT for Illumina TruSeq UD Indexes (96 Indexes, 96 Samples)

|   | 1       | 2       | 3       | 4       | 5       | 6       | 7       | 8       | 9       | 10      | 11      | 12      |
|---|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| A | UDI0001 | UDI0009 | UDI0017 | UDI0025 | UDI0033 | UDI0041 | UDI0049 | UDI0057 | UDI0065 | UDI0073 | UDI0081 | UDI0089 |
| B | UDI0002 | UDI0010 | UDI0018 | UDI0026 | UDI0034 | UDI0042 | UDI0050 | UDI0058 | UDI0066 | UDI0074 | UDI0082 | UDI0090 |
| C | UDI0003 | UDI0011 | UDI0019 | UDI0027 | UDI0035 | UDI0043 | UDI0051 | UDI0059 | UDI0067 | UDI0075 | UDI0083 | UDI0091 |
| D | UDI0004 | UDI0012 | UDI0020 | UDI0028 | UDI0036 | UDI0044 | UDI0052 | UDI0060 | UDI0068 | UDI0076 | UDI0084 | UDI0092 |
| E | UDI0005 | UDI0013 | UDI0021 | UDI0029 | UDI0037 | UDI0045 | UDI0053 | UDI0061 | UDI0069 | UDI0077 | UDI0085 | UDI0093 |
| F | UDI0006 | UDI0014 | UDI0022 | UDI0030 | UDI0038 | UDI0046 | UDI0054 | UDI0062 | UDI0070 | UDI0078 | UDI0086 | UDI0094 |
| G | UDI0007 | UDI0015 | UDI0023 | UDI0031 | UDI0039 | UDI0047 | UDI0055 | UDI0063 | UDI0071 | UDI0079 | UDI0087 | UDI0095 |
| H | UDI0008 | UDI0016 | UDI0024 | UDI0032 | UDI0040 | UDI0048 | UDI0056 | UDI0064 | UDI0072 | UDI0080 | UDI0088 | UDI0096 |

## IDT for Illumina TruSeq UD Indexes (24 Indexes, 96 Samples)

|   | 1       | 2       | 3       | 4       | 5       | 6       | 7       | 8       | 9       | 10      | 11      | 12      |
|---|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| A | UDI0001 | UDI0009 | UDI0017 | UDI0001 | UDI0009 | UDI0017 | UDI0001 | UDI0009 | UDI0017 | UDI0001 | UDI0009 | UDI0017 |
| B | UDI0002 | UDI0010 | UDI0018 | UDI0002 | UDI0010 | UDI0018 | UDI0002 | UDI0010 | UDI0018 | UDI0002 | UDI0010 | UDI0018 |
| C | UDI0003 | UDI0011 | UDI0019 | UDI0003 | UDI0011 | UDI0019 | UDI0003 | UDI0011 | UDI0019 | UDI0003 | UDI0011 | UDI0019 |
| D | UDI0004 | UDI0012 | UDI0020 | UDI0004 | UDI0012 | UDI0020 | UDI0004 | UDI0012 | UDI0020 | UDI0004 | UDI0012 | UDI0020 |
| E | UDI0005 | UDI0013 | UDI0021 | UDI0005 | UDI0013 | UDI0021 | UDI0005 | UDI0013 | UDI0021 | UDI0005 | UDI0013 | UDI0021 |
| F | UDI0006 | UDI0014 | UDI0022 | UDI0006 | UDI0014 | UDI0022 | UDI0006 | UDI0014 | UDI0022 | UDI0006 | UDI0014 | UDI0022 |
| G | UDI0007 | UDI0015 | UDI0023 | UDI0007 | UDI0015 | UDI0023 | UDI0007 | UDI0015 | UDI0023 | UDI0007 | UDI0015 | UDI0023 |
| H | UDI0008 | UDI0016 | UDI0024 | UDI0008 | UDI0016 | UDI0024 | UDI0008 | UDI0016 | UDI0024 | UDI0008 | UDI0016 | UDI0024 |

**[XLEAP-SBS Chemistry] Two-Plex Through Eight-Plex Pooling Strategies**

This section applies to any instruments using XLEAP-SBS Chemistry.

Two-plex through eight-plex pooling strategies are provided for use of IDT for Illumina TruSeq UD Indexes (96 Indexes, 96 Samples). For more information, refer to [Color Balance on page 2](#) or contact Illumina Technical Support.

The following table shows index adapters (wells) that can be combined in two-plex through eight-plex pools. For two-plex pools, the listed index pools make sure that green signal is present in every index cycle. For three-plex through eight-plex pools, the following index pools are examples that include two-channel representation in each cycle. Additional options are available that combine samples from multiple columns (three- to six-plex), within the same column (four- to six-plex), or that meet less stringent requirement of at least green signal every index cycle.

| Plexity | Recommended Index Combinations                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|---------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 2       | <ul style="list-style-type: none"> <li>• A7, E10</li> <li>• A7, F10</li> <li>• A8, C9</li> <li>• A8, D9</li> <li>• B7, E10</li> <li>• B7, F10</li> <li>• B8, C9</li> <li>• B8, D9</li> <li>• C2, A11</li> <li>• C2, A3</li> <li>• C2, A7</li> <li>• C2, B11</li> <li>• C2, B3</li> <li>• C2, B7</li> <li>• C4, A7</li> <li>• C4, B7</li> <li>• C9, G10</li> <li>• C9, H10</li> <li>• D2, A11</li> <li>• D2, A3</li> <li>• D2, A7</li> <li>• D2, B11</li> <li>• D2, B3</li> <li>• D2, B7</li> <li>• D4, A7</li> <li>• D4, B7</li> <li>• D9, G10</li> <li>• D9, H10</li> <li>• G10, E12</li> <li>• G10, F12</li> <li>• H10, E12</li> <li>• H10, F12</li> </ul> |
| 3       | <ul style="list-style-type: none"> <li>• A4, F4, H4</li> <li>• D5, F5, G5</li> <li>• A10, D10, H10</li> <li>• A11, C11, G11</li> <li>• A11, D11, G11</li> <li>• C11, F11, G11</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |

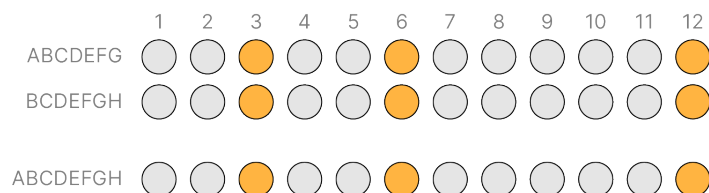
| Plexity | Recommended Index Combinations                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|---------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 4       | <ul style="list-style-type: none"> <li>• A1, D1, E1, H1</li> <li>• B1, C1, E1, G1</li> <li>• A2, C2, E2, G2</li> <li>• B2, D2, F2, H2</li> <li>• A4, B4, E4, G4</li> <li>• B4, C4, F4, H4</li> <li>• A5, B5, C5, E5</li> <li>• C5, D5, F5, H5</li> <li>• A7, C7, F7, G7</li> <li>• B7, D7, E7, H7</li> <li>• A8, B8, C8, G8</li> <li>• D8, E8, F8, H8</li> <li>• A9, C9, F9, H9</li> <li>• B9, D9, E9, H9</li> <li>• A10, C10, F10, H10</li> <li>• B10, D10, E10, G10</li> <li>• A11, B11, C11, E11</li> <li>• D11, F11, G11, H11</li> </ul>                                                                             |
| 5       | <ul style="list-style-type: none"> <li>• A1, B1, C1, E1, G1</li> <li>• B1, D1, E1, F1, H1</li> <li>• A2, B2, C2, E2, G2</li> <li>• B2, C2, D2, F2, H2</li> <li>• A4, B4, C4, E4, G4</li> <li>• B4, C4, D4, F4, H4</li> <li>• A5, B5, C5, D5, E5</li> <li>• D5, E5, F5, G5, H5</li> <li>• A7, B7, C7, E7, G7</li> <li>• B7, C7, D7, F7, H7</li> <li>• A8, B8, C8, D8, G8</li> <li>• B8, C8, E8, F8, H8</li> <li>• A9, B9, C9, E9, G9</li> <li>• B9, D9, F9, G9, H9</li> <li>• A10, B10, C10, D10, G10</li> <li>• B10, D10, E10, F10, H10</li> <li>• A11, B11, C11, D11, E11</li> <li>• D11, E11, F11, G11, H11</li> </ul> |

| Plexity | Recommended Index Combinations                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|---------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 6       | <ul style="list-style-type: none"> <li>• A1, B1, C1, D1, E1, G1</li> <li>• B2, D2, E2, F2, G2, H2</li> <li>• A4, B4, C4, D4, E4, G4</li> <li>• B4, D4, E4, F4, G4, H4</li> <li>• A5, B5, C5, D5, E5, F5</li> <li>• C5, D5, E5, F5, G5, H5</li> <li>• A7, B7, C7, D7, E7, G7</li> <li>• B7, D7, E7, F7, G7, H7</li> <li>• A8, B8, C8, D8, E8, G8</li> <li>• C8, D8, E8, F8, G8, H8</li> <li>• A9, B9, C9, D9, E9, G9</li> <li>• B9, D9, E9, F9, G9, H9</li> <li>• A10, B10, C10, D10, E10, G10</li> <li>• B10, D10, E10, F10, G10, H10</li> <li>• A11, B11, C11, D11, E11, F11</li> <li>• C11, D11, E11, F11, G11, H11</li> </ul> |

The following figure displays seven-plex and eight-plex index pooling recommendations by column. Numbers represent plate columns, and letters represent the rows to be paired within each column.

The orange wells in the following color-coded figures are not recommended for use.

Figure 5 IDT for Illumina TruSeq UD Indexes



## TruSeq CD Indexes (Dual Indexing)

The following table depicts the plate layout for TruSeq DNA CD Indexes and TruSeq RNA CD Indexes (formerly TruSeq HT Indexes).

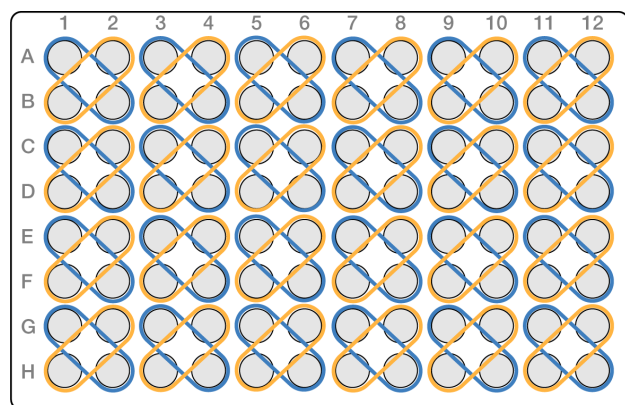
|          | 1    | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   | 11   | 12   |
|----------|------|------|------|------|------|------|------|------|------|------|------|------|
| <b>A</b> | D701 | D702 | D703 | D704 | D705 | D706 | D707 | D708 | D709 | D710 | D711 | D712 |
|          | D501 | D501 | D501 | D501 | D501 | D501 | D501 | D501 | D501 | D501 | D501 | D501 |
| <b>B</b> | D701 | D702 | D703 | D704 | D705 | D706 | D707 | D708 | D709 | D710 | D711 | D712 |
|          | D502 | D502 | D502 | D502 | D502 | D502 | D502 | D502 | D502 | D502 | D502 | D502 |
| <b>C</b> | D701 | D702 | D703 | D704 | D705 | D706 | D707 | D708 | D709 | D710 | D711 | D712 |
|          | D503 | D503 | D503 | D503 | D503 | D503 | D503 | D503 | D503 | D503 | D503 | D503 |
| <b>D</b> | D701 | D702 | D703 | D704 | D705 | D706 | D707 | D708 | D709 | D710 | D711 | D712 |
|          | D504 | D504 | D504 | D504 | D504 | D504 | D504 | D504 | D504 | D504 | D504 | D504 |
| <b>E</b> | D701 | D702 | D703 | D704 | D705 | D706 | D707 | D708 | D709 | D710 | D711 | D712 |
|          | D505 | D505 | D505 | D505 | D505 | D505 | D505 | D505 | D505 | D505 | D505 | D505 |
| <b>F</b> | D701 | D702 | D703 | D704 | D705 | D706 | D707 | D708 | D709 | D710 | D711 | D712 |
|          | D506 | D506 | D506 | D506 | D506 | D506 | D506 | D506 | D506 | D506 | D506 | D506 |
| <b>G</b> | D701 | D702 | D703 | D704 | D705 | D706 | D707 | D708 | D709 | D710 | D711 | D712 |
|          | D507 | D507 | D507 | D507 | D507 | D507 | D507 | D507 | D507 | D507 | D507 | D507 |
| <b>H</b> | D701 | D702 | D703 | D704 | D705 | D706 | D707 | D708 | D709 | D710 | D711 | D712 |
|          | D508 | D508 | D508 | D508 | D508 | D508 | D508 | D508 | D508 | D508 | D508 | D508 |

The following figures illustrate dual indexing strategies for combining these indexes in 2–16-plex pools.

- Circles indicate index adapter combinations that can be combined in a pool.
- Dark gray indicates wells that are not used for pool plexity  $\geq 2$ , but can be used for one-plex pools (single libraries).

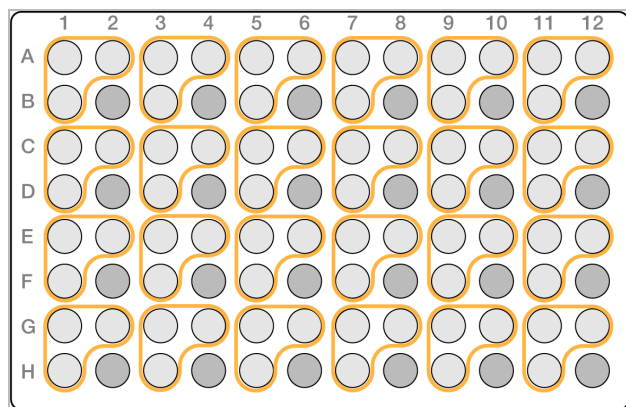
## Two-Plex, Dual-Index

Pool diagonally across adjacent columns and rows. For example: A1 and B2 or A2 and B1.



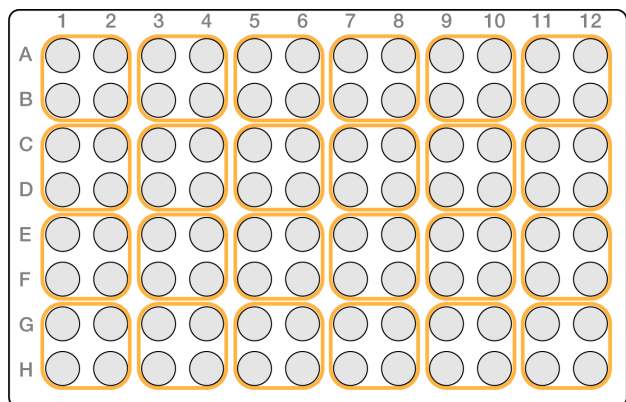
### Three-Plex, Dual-Index

Start at an odd-numbered column and rows A, C, E, and G. Use all wells in a  $2 \times 2$  square except the lower-right well, which is depicted in dark gray.



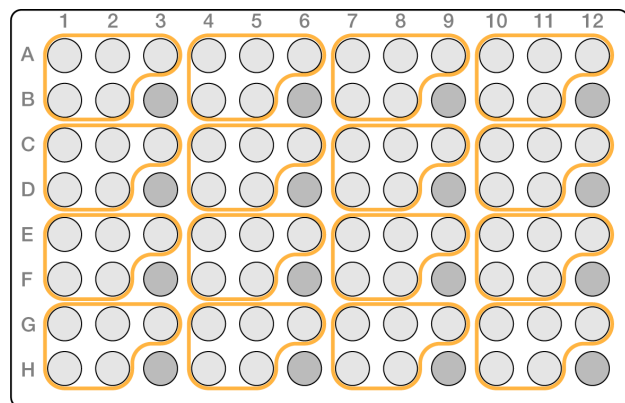
### Four-Plex, Dual-Index

Start at odd-numbered columns and rows A, C, E, and G. Use all four wells in a  $2 \times 2$  square.



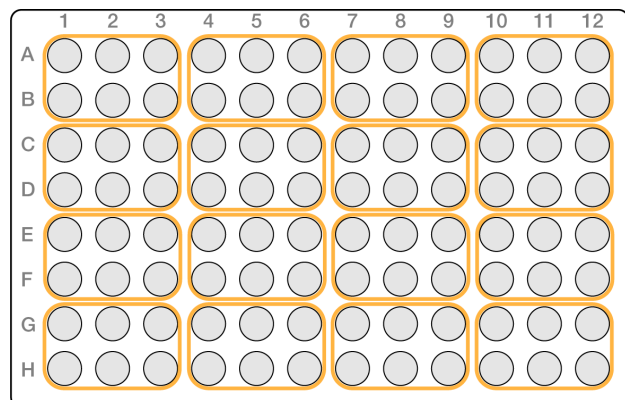
### Five-Plex, Dual-Index

Start at columns 1, 4, 7, and 10 and rows A, C, E, and G. Use all wells in a  $2 \times 3$  rectangle except the lower-right well, which is depicted in dark gray.



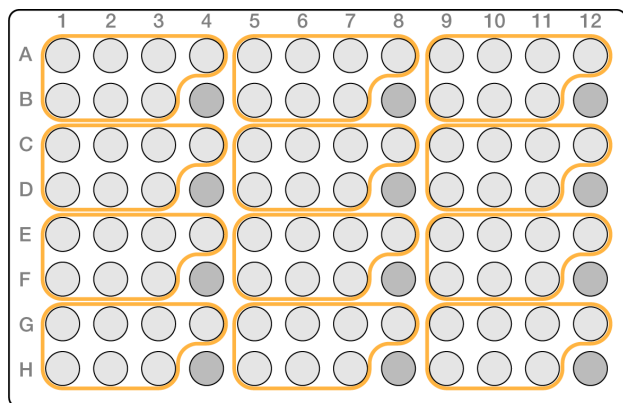
### Six-Plex, Dual-Index

Start at columns 1, 4, 7, and 10 and rows A, C, E, and G. Use all six wells in a  $2 \times 3$  rectangle.



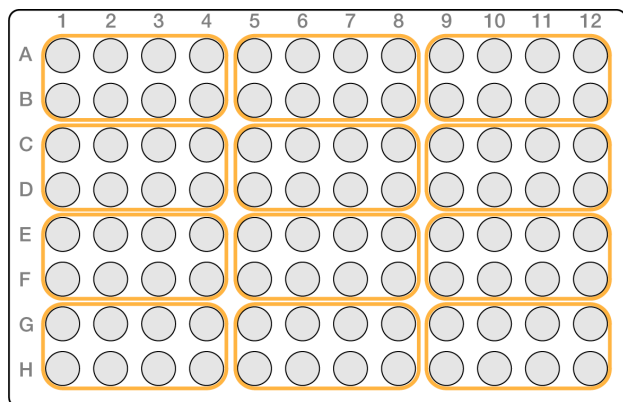
### Seven-Plex, Dual-Index

Start at columns 1, 5, and 9 and rows A, C, E, and G. Use all wells in a  $2 \times 4$  rectangle except the lower-right well, which is depicted in dark gray.



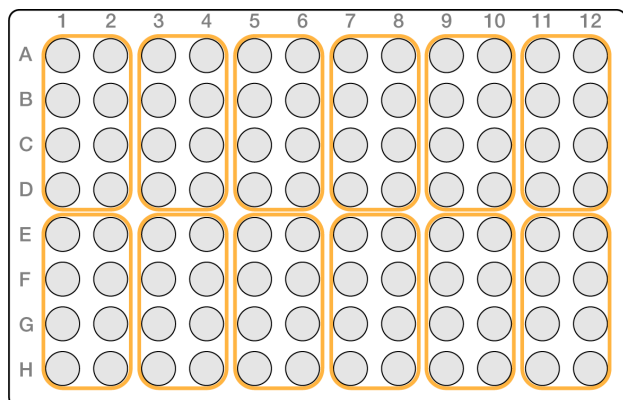
### Eight-Plex, Dual-Index (Option 1)

Start at columns 1, 5, or 9 and rows A, C, E, and G. Use all eight wells in a  $2 \times 4$  rectangle.



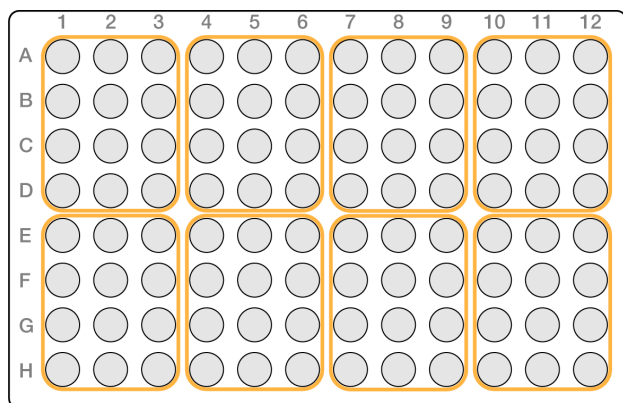
### Eight-Plex, Dual-Index (Option 2)

Start at odd-numbered columns and rows A and E. Use all eight wells in a  $4 \times 2$  rectangle.



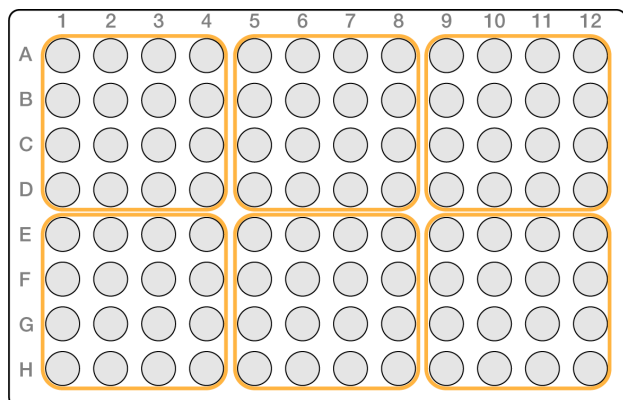
### Twelve-Plex, Dual-Index

Start at columns 1, 4, 7, and 10 and rows A and E. Use all 12 wells in a  $4 \times 3$  rectangle.



### Sixteen-Plex, Dual-Index

Start at columns 1, 5, and 9 and rows A and E. Use all 16 wells in a  $4 \times 4$  square.



## TruSeq CD Indexes (Single Indexing With Dual Index Adapters)

The following table depicts the Index 1 portion of the plate layout for TruSeq DNA CD Indexes and TruSeq RNA CD Indexes (formerly TruSeq HT Indexes).

|   | 1    | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   | 11   | 12   |
|---|------|------|------|------|------|------|------|------|------|------|------|------|
| A | D701 | D702 | D703 | D704 | D705 | D706 | D707 | D708 | D709 | D710 | D711 | D712 |
| B | D701 | D702 | D703 | D704 | D705 | D706 | D707 | D708 | D709 | D710 | D711 | D712 |
| C | D701 | D702 | D703 | D704 | D705 | D706 | D707 | D708 | D709 | D710 | D711 | D712 |
| D | D701 | D702 | D703 | D704 | D705 | D706 | D707 | D708 | D709 | D710 | D711 | D712 |
| E | D701 | D702 | D703 | D704 | D705 | D706 | D707 | D708 | D709 | D710 | D711 | D712 |
| F | D701 | D702 | D703 | D704 | D705 | D706 | D707 | D708 | D709 | D710 | D711 | D712 |
| G | D701 | D702 | D703 | D704 | D705 | D706 | D707 | D708 | D709 | D710 | D711 | D712 |
| H | D701 | D702 | D703 | D704 | D705 | D706 | D707 | D708 | D709 | D710 | D711 | D712 |

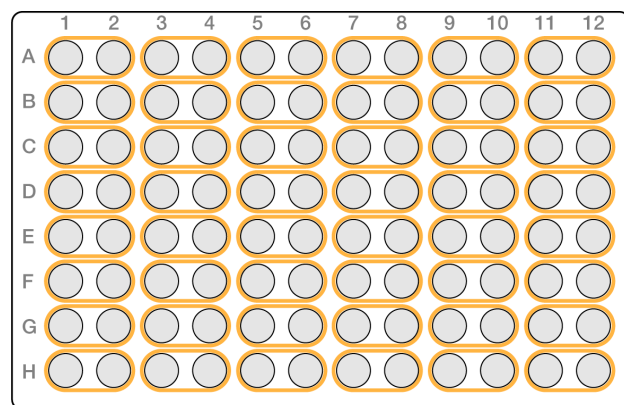
The following figures illustrate pooling strategies for libraries ligated with the Index 1 (i7) and Index 2 (i5) adapters, where only Index 1 is sequenced.

- Circles indicate index adapter combinations that can be combined in a pool.
- Dark gray indicates wells that are not used for pool plexity  $\geq 2$ , but can be used for one-plex pools (single libraries).

These strategies are designed for 2–12-plex pools. For 7–11-plex pools, combine any of the 2–6-plex pools.

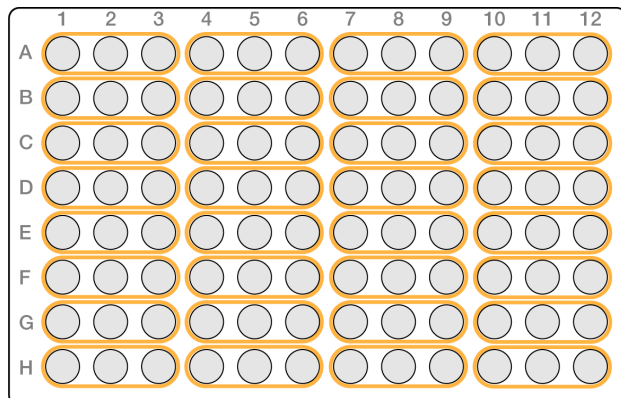
### Two-Plex, Single-Index

Starting at an odd-numbered column, use two consecutive wells across a row.



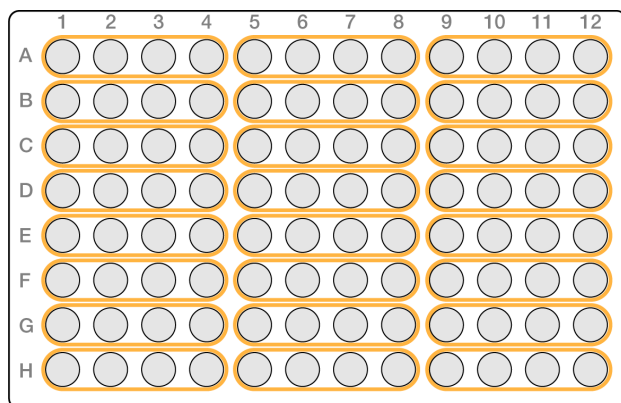
### Three-Plex, Single-Index

Starting at columns 1, 4, 7, or 10, use three consecutive wells across a row.



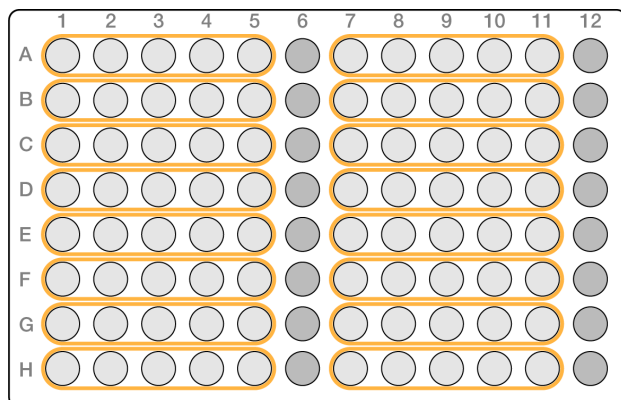
### Four-Plex, Single-Index

Starting a column 1, 5, or 9, use four consecutive wells across a row.



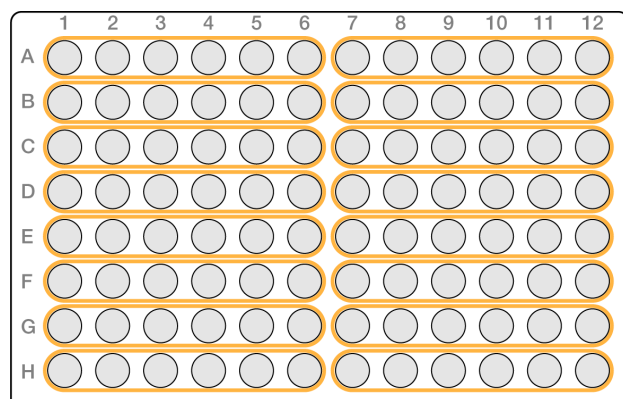
### Five-Plex, Single-Index

Starting at column 1 or 7, use five consecutive wells across a row.



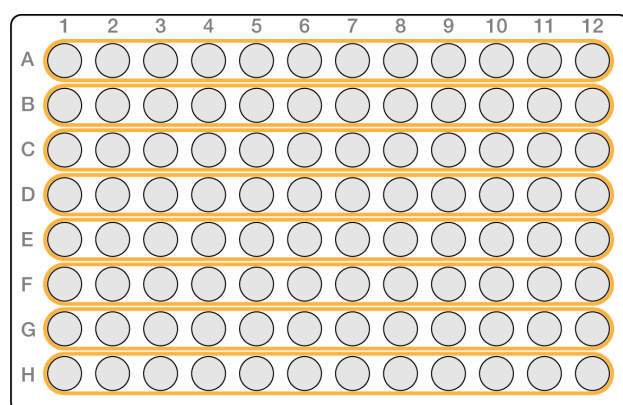
## Six-Plex, Single-Index

Starting at column 1 or 7, use six consecutive wells across a row.



## Twelve-Plex, Single-Index

Use an entire row.



# TruSeq Single Indexes

TruSeq Single Indexes are available in Set A and Set B. Each set contains 12 index adapters packaged in tubes.

The following tables show strategies for 2–12-plex pooling when using either set. For 5–11-plex pools, start with a 4-plex option and add any other index adapters from the same set.

## TruSeq DNA Single Indexes

| Plexity | Option | Set A Index Adapters Only | Set B Index Adapters Only |
|---------|--------|---------------------------|---------------------------|
| 2       | 1      | AD006 and AD012           | Not recommended           |
|         | 2      | AD005 and AD019           | Not recommended           |

| Plexity | Option | Set A Index Adapters Only                              | Set B Index Adapters Only                                |
|---------|--------|--------------------------------------------------------|----------------------------------------------------------|
| 3       | 1      | AD002, AD007, and AD019                                | AD001, AD010, and AD020                                  |
|         | 2      | AD005, AD006, and AD015                                | AD003, AD009, and AD025                                  |
|         | 3      | Two-plex options plus another index adapter from Set A | AD008, AD011, and AD022                                  |
| 4       | 1      | AD005, AD006, AD012, and AD019                         | AD001, AD008, AD010, and AD011                           |
|         | 2      | AD002, AD004, AD007, and AD016                         | AD003, AD009, AD022, and AD027                           |
|         | 3      | Three-plex options plus another adapter from Set A     | Three-plex options plus another index adapter from Set B |

### TruSeq RNA Single Indexes

| Plexity | Option | Set A Index Adapters Only                                | Set B Index Adapters Only                                |
|---------|--------|----------------------------------------------------------|----------------------------------------------------------|
| 2       | 1      | AR006 and AR012                                          | Not recommended                                          |
|         | 2      | AR005 and AR019                                          | Not recommended                                          |
| 3       | 1      | AR002, AR007, and AR019                                  | AR001, AR010, and AR020                                  |
|         | 2      | AR005, AR006, and AR015                                  | AR003, AR009, and AR025                                  |
|         | 3      | Two-plex options plus another index adapter from Set A   | AR008, AR011, and AR022                                  |
| 4       | 1      | AR005, AR006, AR012, and AR019                           | AR001, AR008, AR010, and AR011                           |
|         | 2      | AR002, AR004, AR007, and AR016                           | AR003, AR009, AR022, and AR027                           |
|         | 3      | Three-plex options plus another index adapter from Set A | Three-plex options plus another index adapter from Set B |

## TruSeq Small RNA Indexes

TruSeq Small RNA Indexes A, B, C, and D each contain 12 unique indexes for single indexing.

The following table shows pooling strategies for two to four libraries generated with index adapters from each set. For 5–11-plex pools, use the 4-plex options with any adapters.

| Plexity | Option | Set A Only     | Set B Only      | Set C Only*     | Set D Only      |
|---------|--------|----------------|-----------------|-----------------|-----------------|
| 2       | 1      | RPI6 and RPI12 | Not recommended | Not recommended | RPI37 and RPI45 |

| Plexity | Option | Set A Only                               | Set B Only                               | Set C Only*     | Set D Only                               |
|---------|--------|------------------------------------------|------------------------------------------|-----------------|------------------------------------------|
| 3       | 1      | RPI1, RPI3, and RPI7                     | RPI16, RPI17, and RPI18                  | Not recommended | RPI38, RPI44, and RPI46                  |
|         | 2      | RPI2, RPI4, and RPI8                     | RPI13, RPI17, and RPI23                  |                 | RPI40, RPI47, and RPI48                  |
|         | 3      | Two-plex option with any other adapter   | Not recommended                          |                 | Two-plex option with any other adapter   |
| 4       | 1      | RPI2, RPI9, RPI10, and RPI11             | Three-plex option with any other adapter | Not recommended | RPI37, RPI39, RPI42, and RPI43           |
|         | 2      | RPI4, RPI5, RPI6, and RPI7               |                                          |                 | RPI37, RPI38, RPI45, and RPI46           |
|         | 3      | Three-plex option with any other adapter |                                          |                 | Three-plex option with any other adapter |

\* Always combine TruSeq Small RNA Indexes C with another TruSeq Small RNA Indexes set.

# Revision History

| Document #        | Date           | Description of Change                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|-------------------|----------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1000000041074 v14 | January 2025   | Clarified pooling strategies for instruments using XLEAP-SBS chemistry.<br>Added MiSeq i100 Series information.                                                                                                                                                                                                                                                                                                                                                          |
| 1000000041074 v13 | February 2024  | Added NextSeq 1000/2000 XLEAP-SBS chemistry information.                                                                                                                                                                                                                                                                                                                                                                                                                 |
| 1000000041074 v12 | September 2023 | Added NovaSeq X Series information.<br>Added plate layout and pooling strategies for Illumina UD Indexes, Set A-D.<br>Added clarification for V2 indexes used with IDT for Illumina UD Indexes Set C and Set D.<br>Reorganized the following content to improve continuity and navigation: <ul style="list-style-type: none"> <li>• Color balance and index adapter combinations</li> <li>• AmpliSeq CD Indexes</li> <li>• IDT for Illumina TruSeq UD Indexes</li> </ul> |
| 1000000041074 v11 | April 2021     | Added HTML format.<br>Corrected Plexity 6 [Option 1] details in pooling strategy tables for IDT for Illumina UD Indexes and Nextera DNA CD Indexes (96 Indexes, 96 Samples).                                                                                                                                                                                                                                                                                             |
| 1000000041074 v10 | July 2020      | Replaced UDI0015, UDI0016, UDI0055, UDI0056, UDP0252, UDP0258, UDP0289, UDP0290, UDP0291, and UDP0301 with V2 versions.<br>Added IDT for Illumina-PCR UD Indexes Set 1, 2, 3, 4.                                                                                                                                                                                                                                                                                         |
| 1000000041074 v09 | March 2020     | Generalized references to NextSeq Systems to include all versions.<br>Added NextSeq 2000 information to Two-Channel Chemistry section.<br>Added Instrument Run Setup to Color Balance section.                                                                                                                                                                                                                                                                           |
| 1000000041074 v08 | October 2019   | Corrected the third option for TruSeq RNA Single Indexes Set B four-plex pooling strategy.<br>Added index plate layouts for IDT for Illumina TruSeq-UD Indexes (24 Indexes, 96 Samples).                                                                                                                                                                                                                                                                                 |

| Document #        | Date          | Description of Change                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|-------------------|---------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1000000041074 v07 | May 2019      | <p>Added index plate layouts for IDT for Illumina Nextera DNA UD Indexes Sets B–D.</p> <p>Identified the IDT for Illumina Nextera DNA UD index plate layout as IDT for Illumina Nextera DNA UD Indexes Sets A.</p> <p>Updated section on TruSeq Small RNA indexes to improve clarity and accuracy.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| 1000000041074 v06 | February 2019 | <p>Added index plate layouts for AmpliSeq CD Indexes Sets B–D for Illumina.</p> <p>Identified the AmpliSeq for Illumina index plate layout as AmpliSeq CD Indexes Set A for Illumina.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| 1000000041074 v05 | December 2018 | <p>Corrected the Index 1 (i7) index adapter in well C2 of the AmpliSeq CD Indexes for Illumina plate to Q7008.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| 1000000041074 v04 | November 2018 | <p>Combined, renamed, and reorganized all sections to improve continuity, facilitate navigation, and eliminate redundancy.</p> <p>Consolidated graphics depicting pooling strategies for IDT for Illumina Nextera DNA UD Indexes and Nextera DNA CD Indexes (96 Indexes, 96 Samples).</p> <p>Added pooling guidelines for Nextera XT Index Kit v2 and Nextera Index Kit.</p> <p>Added information on Illumina Experiment Manager, Local Run Manager, and BaseSpace Sequence Hub.</p> <p>Added good and bad examples of color balance on a four-channel system.</p> <p>Identified all Index 1 (i7) and all Index 2 (i5) adapters for Nextera DNA CD Indexes (24 Indexes, 24 Samples).</p> <p>Updated plate layouts to remove duplicate index adapter names and show the Index 1 (i7) adapters first.</p> <p>Updated descriptions of one-, two-, and four-dye chemistry.</p> <p>Described dual indexing options: combinatorial dual (CD) and unique dual (UD) indexes.</p> |

| Document #        | Date         | Description of Change                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|-------------------|--------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                   |              | <p>Described pooling strategies for TruSeq CD Indexes and updated style of the graphics (the strategies have not changed).</p> <p>Referenced <i>Indexed Sequencing Overview</i> (document # 15057455) for indexing workflows on Illumina sequencing systems.</p> <p>Clarified the following points:</p> <ul style="list-style-type: none"> <li>• TruSeq CD Indexes replaced TruSeq HT Indexes.</li> <li>• For two-plex, dual-indexing with TruSeq CD Indexes, pool diagonally.</li> <li>• Single-indexing with TruSeq CD Indexes requires dual index adapters.</li> </ul> <p>Removed the following sequences, which are available in <i>Illumina Adapter Sequences</i> (document # 1000000002694):</p> <ul style="list-style-type: none"> <li>• Index adapter sequences for Nextera DNA CD Indexes (for Nextera DNA Flex).</li> <li>• Index adapter and adapter trimming sequences for AmpliSeq for Illumina.</li> </ul> <p>Corrected column-based, three-plex pooling strategy for AmpliSeq for Illumina.</p> <p>Corrected names of the following items:</p> <ul style="list-style-type: none"> <li>• IDT for Illumina kits.</li> <li>• Index adapters UDP0001–UDP0009 to include four digits.</li> </ul> |
| 1000000041074 v03 | October 2018 | <p>Added information on IDT for Illumina Nextera DNA UD Indexes.</p> <p>Consolidated low-plexity guidelines.</p> <p>Corrected tables for Nextera DNA Flex 96 CD Indexes.</p> <p>Removed single indexing information for IDT for Illumina TruSeq DNA UD Indexes, which is compatible with dual indexing only.</p> <p>Removed incorrect guidelines for:</p> <ul style="list-style-type: none"> <li>• Low plexity sequencing on the NovaSeq 6000 System.</li> <li>• Low throughput for Nextera DNA Flex combinatorial dual indexes.</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| 1000000041074 v02 | May 2018     | <p>Added information on one-channel sequencing for the iSeq 100 System.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |

| Document #        | Date         | Description of Change                                                              |
|-------------------|--------------|------------------------------------------------------------------------------------|
| 1000000041074 v01 | January 2018 | Added pooling guidelines for AmpliSeq for Illumina and Nextera DNA Flex libraries. |
| 1000000041074 v00 | October 2017 | Initial release.                                                                   |



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