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## Introduction

This guide 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 conveniently form low-plex, color-balanced pools. Higher plexity pools are inherently color-balanced so any index adapter combinations are acceptable. To develop alternative pools, review index adapter sequences in *Illumina Adapter Sequences (document # 1000000002694)* to ensure color balance for your system.



### NOTE

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 guide as a reference to plan indexing and pooling for library prep. For library prep instructions, see the reference guide for your kit. All documentation is available on the Illumina website ([support.illumina.com](https://support.illumina.com)).

## 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 8 unique pairs, resulting in the majority of the libraries sharing common indexes on the i5 or i7 end. Indexes are 8 bases long.

Plate layouts for IDT® 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, or BaseSpace Sequence Hub Prep tab, the software confirms that libraries in a pool have unique index combinations. However, Local Run Manager 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 shows good and bad index combinations to demonstrate color balance on a four-channel system. The good examples have signal 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
✓✓✓✓✓✓✓✓		✓✓✓✓✓✓✓✓		✓✓✓✓✓✓✓✓		✓✓✓✓XXXX	

## 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 6000, NextSeq 550, NextSeq 500, and MiniSeq Systems
Four-channel	The MiSeq System and all HiSeq Systems

For more information on sequencing chemistry and base calling, see the system guide for your instrument. See *Indexed Sequencing Overview (document # 15057455)* for indexing workflows 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 to result in four distinct populations, each corresponding to a nucleotide. Base calling determines which population 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, one from the green channel and one from the red channel, to encode data for the four bases. Intensities extracted from an image compared to another image result in four distinct populations, each corresponding to a base. Base calling determines which population each cluster belongs to.

When sequencing on a two-channel system, either of the first two cycles of the Index Read must start with at least one base other than G. 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 channel**—A or C
- ▶ **Green channel**—A or T

For more example index combinations for two-channel systems, see 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.

## Pooling Guidelines for Nextera Kits

This section provides guidelines for pooling indexed Nextera™ libraries. The following index adapters are compatible with PCR extension-based Nextera library prep kits.

Index Adapters	Indexing Scheme	Format
IDT for Illumina Nextera DNA UD Indexes	Dual indexing	Plate
Nextera DNA CD Indexes (96 indexes, 96 samples)	Dual indexing	Plate
Nextera DNA CD Indexes (24 indexes, 24 samples)	Dual indexing	Tube
Nextera XT Index Kit v2	Single and dual indexing	Tube
Nextera Index Kit	Single and dual indexing	Tube

## IDT for Illumina Nextera DNA UD Indexes

The following tables depicts the plate layout for IDT for Illumina Nextera DNA UD Indexes, which are designed for dual-indexing.

### IDT for Illumina Nextera UD Indexes Plate A

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

## IDT for Illumina Nextera UD Indexes Plate B

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

## IDT for Illumina Nextera UD Indexes Plate C

	1	2	3	4	5	6	7	8	9	10	11	12
A	UDP0193	UDP0201	UDP0209	UDP0217	UDP0225	UDP0233	UDP0241	UDP0249	UDP0257	UDP0265	UDP0273	UDP0281
B	UDP0194	UDP0202	UDP0210	UDP0218	UDP0226	UDP0234	UDP0242	UDP0250	UDP0258	UDP0266	UDP0274	UDP0282
C	UDP0195	UDP0203	UDP0211	UDP0219	UDP0227	UDP0235	UDP0243	UDP0251	UDP0259	UDP0267	UDP0275	UDP0283
D	UDP0196	UDP0204	UDP0212	UDP0220	UDP0228	UDP0236	UDP0244	UDP0252	UDP0260	UDP0268	UDP0276	UDP02854
E	UDP0197	UDP0205	UDP0213	UDP0221	UDP0229	UDP0237	UDP0245	UDP0253	UDP0261	UDP0269	UDP0277	UDP0285
F	UDP0198	UDP0206	UDP0214	UDP0222	UDP0230	UDP0238	UDP0246	UDP0254	UDP0262	UDP0270	UDP0278	UDP0286
G	UDP0199	UDP0207	UDP0215	UDP0223	UDP0231	UDP0239	UDP0247	UDP0255	UDP0263	UDP0271	UDP0279	UDP0287
H	UDP0200	UDP0208	UDP0216	UDP0224	UDP0232	UDP0240	UDP0248	UDP0256	UDP0264	UDP0272	UDP0280	UDP0288

## IDT for Illumina Nextera UD Indexes Plate D

	1	2	3	4	5	6	7	8	9	10	11	12
A	UDP0289	UDP0297	UDP0305	UDP0313	UDP0321	UDP0329	UDP0337	UDP0345	UDP0353	UDP0361	UDP0369	UDP0377
B	UDP0290	UDP0298	UDP0306	UDP0314	UDP0322	UDP0330	UDP0338	UDP0346	UDP0354	UDP0362	UDP0370	UDP0378
C	UDP0291	UDP0299	UDP0307	UDP0315	UDP0323	UDP0331	UDP0339	UDP0347	UDP0355	UDP0363	UDP0371	UDP0379
D	UDP0292	UDP0300	UDP0308	UDP0316	UDP0324	UDP0332	UDP0340	UDP0348	UDP0356	UDP0364	UDP0372	UDP0380

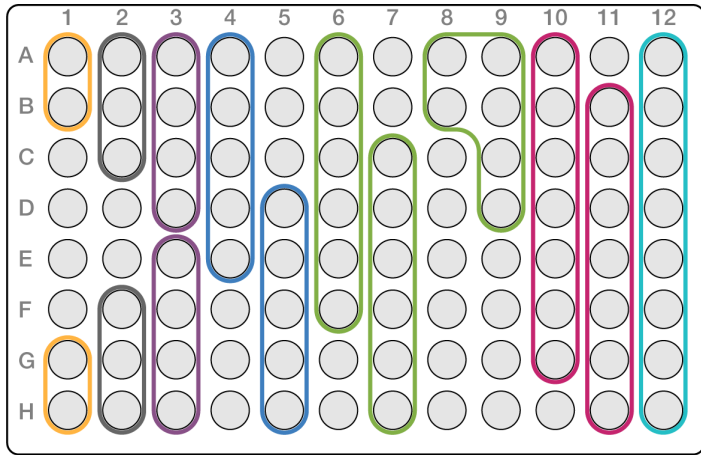
	1	2	3	4	5	6	7	8	9	10	11	12
<b>E</b>	UDP0293	UDP0301	UDP0309	UDP0317	UDP0325	UDP0333	UDP0341	UDP0349	UDP0357	UDP0365	UDP0373	UDP0381
<b>F</b>	UDP0294	UDP0302	UDP0310	UDP0318	UDP0326	UDP0334	UDP0342	UDP0350	UDP0358	UDP0366	UDP0374	UDP0382
<b>G</b>	UDP0295	UDP0303	UDP0311	UDP0319	UDP0327	UDP0335	UDP0343	UDP0351	UDP0359	UDP0367	UDP0375	UDP0383
<b>H</b>	UDP0296	UDP0304	UDP0312	UDP0320	UDP0328	UDP0336	UDP0344	UDP0352	UDP0360	UDP0368	UDP0376	UDP0384

## Two-Plex Through Eight-Plex Pooling Strategies

The following table shows index adapters (wells) that can be combined in a 2–8-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
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
6	<b>[Option 1]</b> The first six or last six wells in a column: <ul style="list-style-type: none"> <li>• A–F</li> <li>• D–H</li> </ul> <b>[Option 2]</b> 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

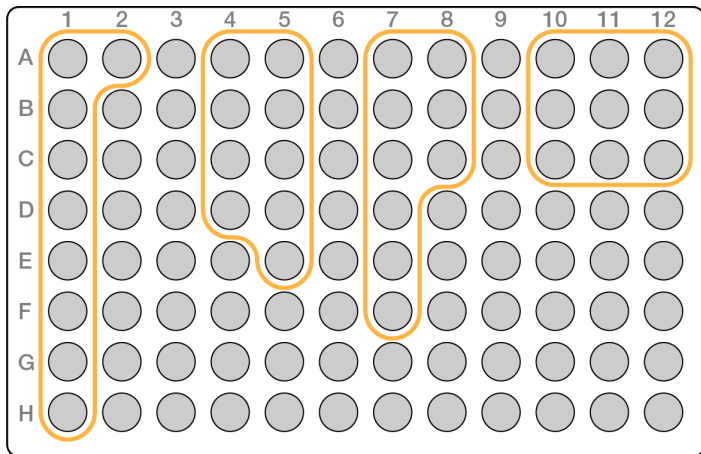


### Nine-Plex Pooling Strategies

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 (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
A	H701 H505	H702 H506	H703 H517	H705 H505	H707 H506	H723 H517	H706 H505	H712 H506	H720 H517	H710 H505	H711 H506	H714 H517



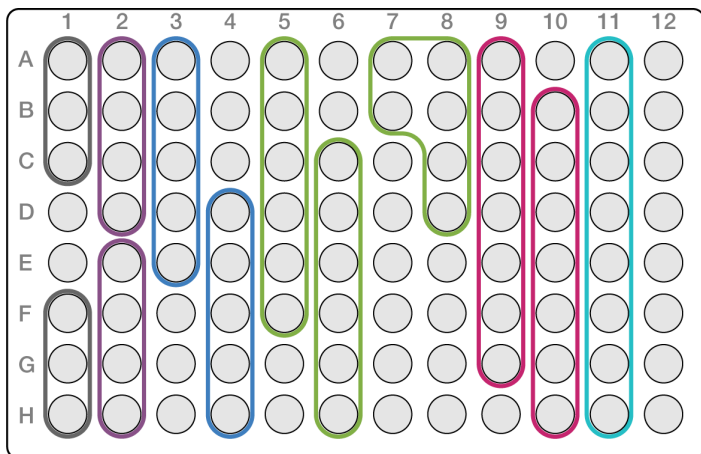
<b>B</b>	H702 H517	H703 H505	H701 H506	H707 H517	H723 H505	H705 H506	H712 H517	H720 H505	H706 H506	H711 H517	H714 H505	H710 H506
<b>C</b>	H703 H506	H701 H517	H702 H505	H723 H506	H705 H517	H707 H505	H720 H506	H706 H517	H712 H505	H714 H506	H710 H517	H711 H505
<b>D</b>	H705 H503	H707 H503	H723 H503	H706 H503	H712 H503	H720 H503	H710 H503	H711 H503	H714 H503	H701 H503	H702 H503	H703 H503
<b>E</b>	H706 H516	H712 H516	H720 H516	H710 H516	H711 H516	H714 H516	H701 H516	H702 H516	H703 H516	H705 H516	H707 H516	H723 H516
<b>F</b>	H710 H522	H711 H510	H714 H513	H701 H522	H702 H510	H703 H513	H705 H522	H707 H510	H723 H513	H706 H522	H712 H510	H720 H513
<b>G</b>	H711 H513	H714 H522	H710 H510	H702 H513	H703 H522	H701 H510	H707 H513	H723 H522	H705 H510	H712 H513	H720 H522	H706 H510
<b>H</b>	H714 H510	H710 H513	H711 H522	H703 H510	H701 H513	H702 H522	H723 H510	H705 H513	H707 H522	H720 H510	H706 H513	H712 H522

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

The following table shows index adapters (wells) that can be combined in a 3–8-plex pool, while the color-coded figure illustrates each combination.

A minimum plexity of three ensures 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
6	<b>[Option 1]</b> The first six or last six wells in a column: <ul style="list-style-type: none"> <li>• A–F</li> <li>• D–H</li> </ul> <b>[Option 2]</b> 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

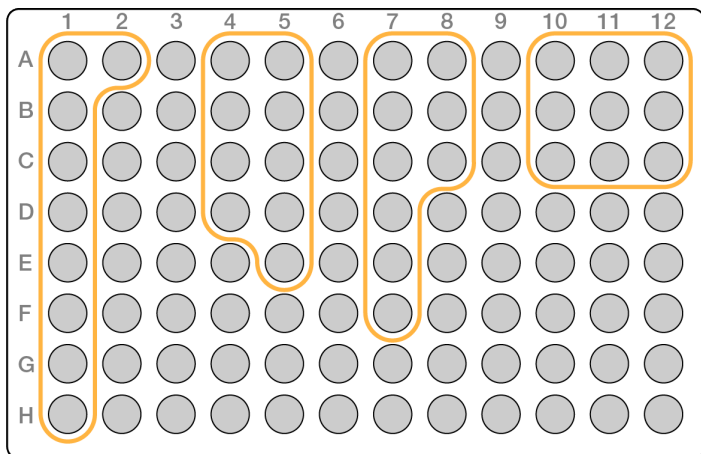


### Nine-Plex Pooling Strategies

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 3–8 dual-indexed libraries prepared with these indexes.

A minimum plexity of three ensures 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>
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 two sizes:

- ▶ The 24 indexes, 96 samples size contains six Index 1 and four Index 2 adapters.
- ▶ The 96 indexes, 384 samples size 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
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

## Pooling Guidelines for AmpliSeq for Illumina Kits

This section provides guidelines for pooling 2–8 AmpliSeq for Illumina libraries ligated with AmpliSeq CD Indexes for Illumina. These index adapters are designed for dual indexing and use with all AmpliSeq for Illumina panels. Use any column- or row-based pooling strategy with any set to create 2–8-plex pools.

## AmpliSeq CD Indexes Set A for Illumina

The following table depicts the index plate layout for AmpliSeq CD Indexes Set A for Illumina.

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

## AmpliSeq CD Indexes Set B for Illumina

The following table depicts the index plate layout for AmpliSeq CD Indexes Set B for Illumina.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Q7027 Q5001	Q7035 Q5002	Q7028 Q5007	Q7029 Q5008	Q7036 Q5009	Q7030 Q5010	Q7040 Q5001	Q7041 Q5002	Q7039 Q5007	Q7047 Q5008	Q7042 Q5009	Q7048 Q5010
B	Q7028 Q5002	Q7036 Q5001	Q7027 Q5008	Q7030 Q5007	Q7035 Q5010	Q7029 Q5009	Q7039 Q5002	Q7042 Q5001	Q7040 Q5008	Q7048 Q5007	Q7041 Q5010	Q7047 Q5009
C	Q7036 Q5007	Q7030 Q5008	Q7035 Q5009	Q7028 Q5010	Q7029 Q5013	Q7027 Q5014	Q7042 Q5007	Q7048 Q5008	Q7041 Q5009	Q7039 Q5010	Q7047 Q5013	Q7040 Q5014
D	Q7035 Q5008	Q7029 Q5007	Q7036 Q5010	Q7027 Q5009	Q7030 Q5014	Q7028 Q5013	Q7041 Q5008	Q7047 Q5007	Q7042 Q5010	Q7040 Q5009	Q7048 Q5014	Q7039 Q5013
E	Q7039 Q5009	Q7047 Q5010	Q7040 Q5013	Q7041 Q5014	Q7048 Q5001	Q7042 Q5002	Q7028 Q5009	Q7029 Q5010	Q7027 Q5013	Q7035 Q5014	Q7030 Q5001	Q7036 Q5002
F	Q7040 Q5010	Q7048 Q5009	Q7039 Q5014	Q7042 Q5013	Q7047 Q5002	Q7041 Q5001	Q7027 Q5010	Q7030 Q5009	Q7028 Q5014	Q7036 Q5013	Q7029 Q5002	Q7035 Q5001
G	Q7048 Q5013	Q7042 Q5014	Q7047 Q5001	Q7040 Q5002	Q7041 Q5007	Q7039 Q5008	Q7030 Q5013	Q7036 Q5014	Q7029 Q5001	Q7027 Q5002	Q7035 Q5007	Q7028 Q5008
H	Q7047 Q5014	Q7041 Q5013	Q7048 Q5002	Q7039 Q5001	Q7042 Q5008	Q7040 Q5007	Q7029 Q5014	Q7035 Q5013	Q7030 Q5002	Q7028 Q5001	Q7036 Q5008	Q7027 Q5007

## AmpliSeq CD Indexes Set C for Illumina

The following table depicts the index plate layout for AmpliSeq CD Indexes Set C for Illumina.

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

## AmpliSeq CD Indexes Set D for Illumina

The following table depicts the index plate layout for AmpliSeq CD Indexes Set D for Illumina.

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

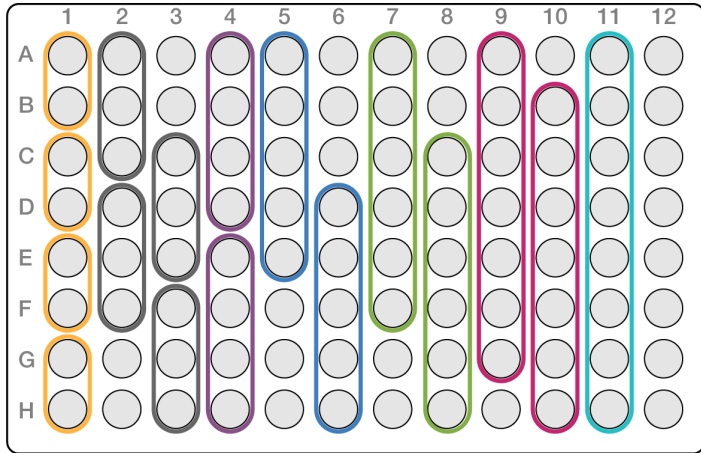
## Column-Based Strategies

The following table shows index adapters (wells) that can be combined in a 2–8-plex pool, while 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: <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: <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: <ul style="list-style-type: none"> <li>• A–D</li> <li>• E–H</li> </ul>	Purple
5	Five consecutive wells in a column: <ul style="list-style-type: none"> <li>• A–E</li> <li>• D–H</li> </ul>	Blue
6	Six consecutive wells in a column: <ul style="list-style-type: none"> <li>• A–F</li> <li>• C–H</li> </ul>	Green

Plexity	Combinations	Color in Figure
7	Seven consecutive wells in a column: <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



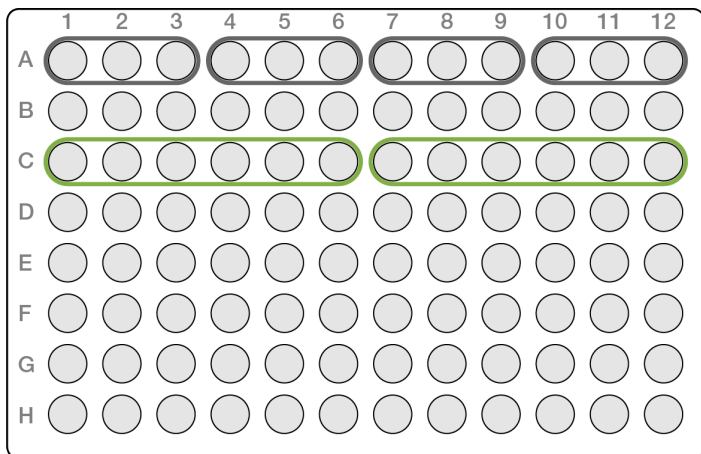
### Row-Based Strategies

The following table shows index adapters (wells) that can be combined in 3- or 6-plex pool, while 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. <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: <ul style="list-style-type: none"> <li>• 1-6</li> <li>• 7-12</li> </ul>	Green





### Pooling Guidelines for 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 IDT for Illumina–TruSeq RNA UD Indexes	Dual indexing	Plate
TruSeq DNA CD Indexes TruSeq RNA CD Indexes	Single and dual indexing	Plate
TruSeq DNA Single Indexes TruSeq RNA Single Indexes	Single indexing	Tubes

### IDT for Illumina–TruSeq UD Indexes

The following table depicts the plate layout for IDT® for Illumina®–TruSeq™ DNA UD Indexes and IDT® for Illumina®–TruSeq™ RNA UD Indexes. These indexes are designed for select TruSeq libraries.

Pool any plexity ≥ 2 down a column. Do not pool across a row.

	1	2	3	4	5	6	7	8	9	10	11	12
A	UD0001	UD0009	UD0017	UD0025	UD0033	UD0041	UD0049	UD0057	UD0065	UD0073	UD0081	UD0089
B	UD0002	UD0010	UD0018	UD0026	UD0034	UD0042	UD0050	UD0058	UD0066	UD0074	UD0082	UD0090
C	UD0003	UD0011	UD0019	UD0027	UD0035	UD0043	UD0051	UD0059	UD0067	UD0075	UD0083	UD0091
D	UD0004	UD0012	UD0020	UD0028	UD0036	UD0044	UD0052	UD0060	UD0068	UD0076	UD0084	UD0092
E	UD0005	UD0013	UD0021	UD0029	UD0037	UD0045	UD0053	UD0061	UD0069	UD0077	UD0085	UD0093
F	UD0006	UD0014	UD0022	UD0030	UD0038	UD0046	UD0054	UD0062	UD0070	UD0078	UD0086	UD0094
G	UD0007	UD0015	UD0023	UD0031	UD0039	UD0047	UD0055	UD0063	UD0071	UD0079	UD0087	UD0095
H	UD0008	UD0016	UD0024	UD0032	UD0040	UD0048	UD0056	UD0064	UD0072	UD0080	UD0088	UD0096

## TruSeq CD Indexes (Dual Indexing)

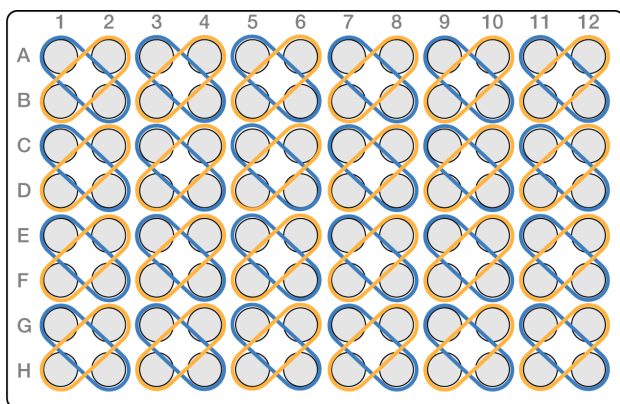
The following table depicts the plate layout for TruSeq DNA CD Indexes and TruSeq RNA CD Indexes (formerly TruSeq HT Indexes). Subsequent figures illustrate dual indexing strategies for combining these indexes in 2–16-plex pools:

- ▶ 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).

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

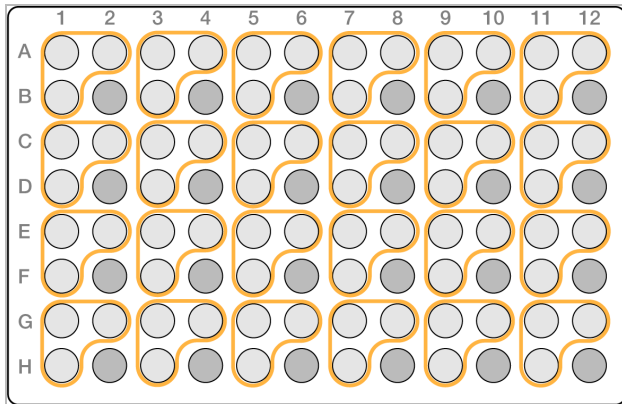
## Two-Plex, Dual-Index

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



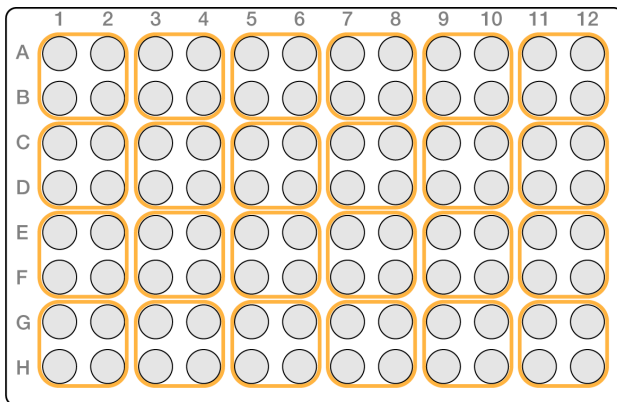
### Three-Plex, Dual-Index

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



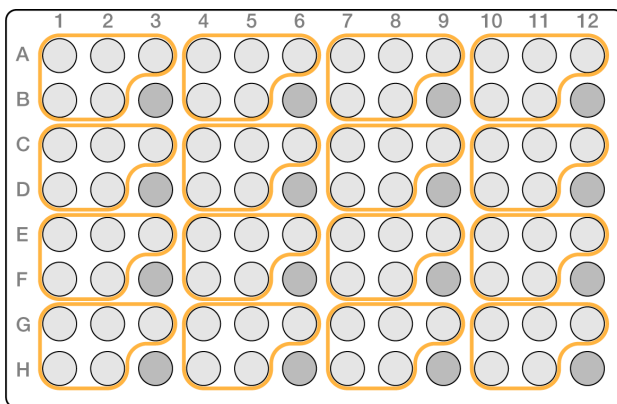
### Four-Plex, Dual-Index

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



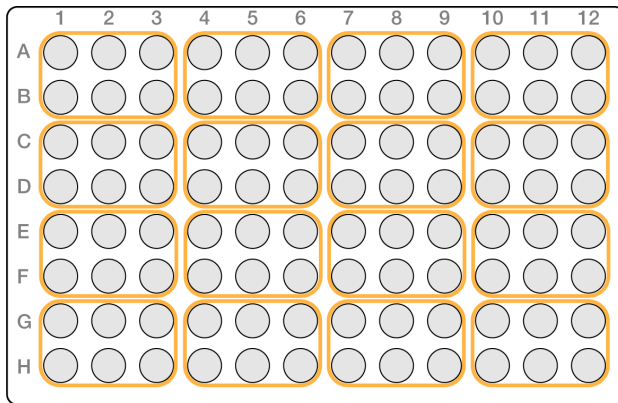
### Five-Plex, Dual-Index

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



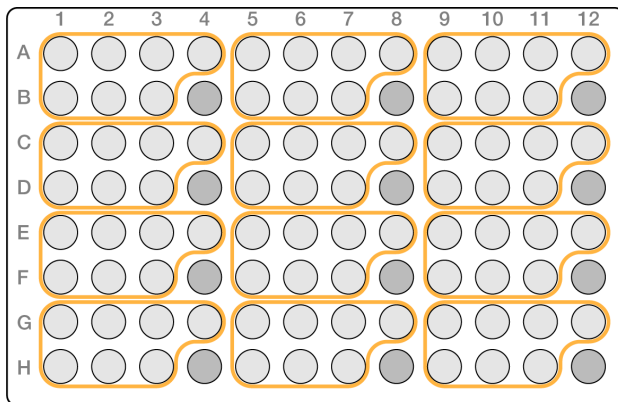
### Six-Plex, Dual-Index

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



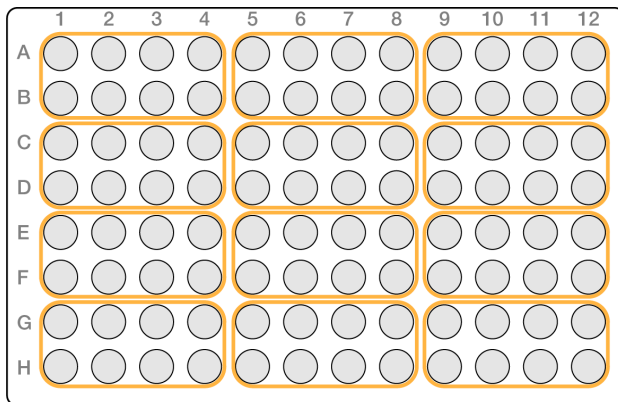
### Seven-Plex, Dual-Index

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



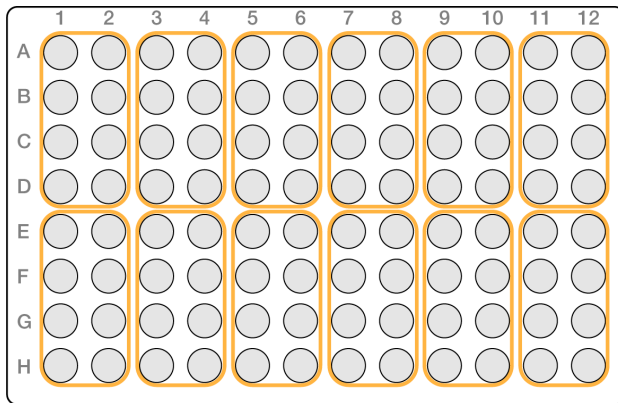
### Eight-Plex, Dual-Index (Option 1)

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



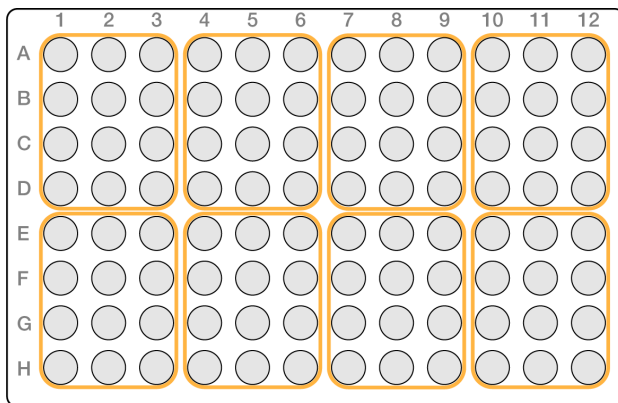
### Eight-Plex, Dual-Index (Option 2)

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



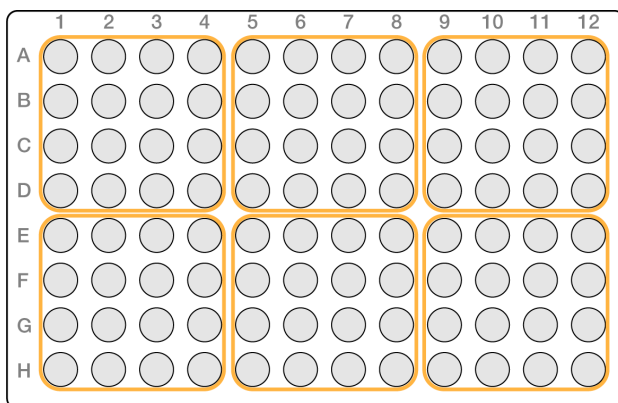
### Twelve-Plex, Dual-Index

Start at columns 1, 4, 7, and 10 and rows A and E. Use all 12 wells in a 4 × 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 × 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). Subsequent figures illustrate pooling strategies for libraries ligated with the Index 1 (i7) and Index 2 (i5) adapters, but only Index 1 is sequenced:

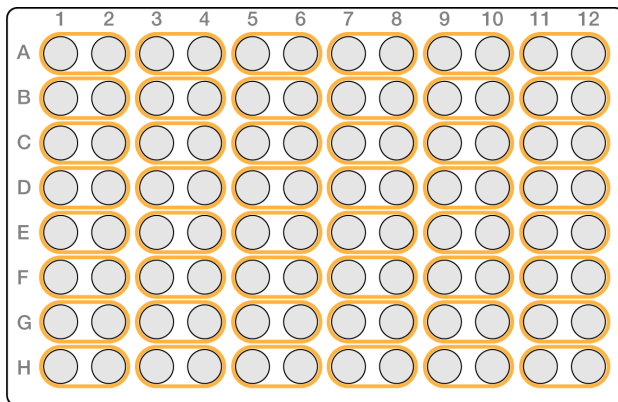
- ▶ Circles indicate index adapter combinations that can be combined in a pool.
- ▶ Dark gray indicates wells that are not used for pool plexity  $\geq 2$ , but can be used for one-plex pools (single libraries).

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

1	2	3	4	5	6	7	8	9	10	11	12
D701	D702	D703	D704	D705	D706	D707	D708	D709	D710	D711	D712
D701	D702	D703	D704	D705	D706	D707	D708	D709	D710	D711	D712
D701	D702	D703	D704	D705	D706	D707	D708	D709	D710	D711	D712
D701	D702	D703	D704	D705	D706	D707	D708	D709	D710	D711	D712
D701	D702	D703	D704	D705	D706	D707	D708	D709	D710	D711	D712
D701	D702	D703	D704	D705	D706	D707	D708	D709	D710	D711	D712
D701	D702	D703	D704	D705	D706	D707	D708	D709	D710	D711	D712
D701	D702	D703	D704	D705	D706	D707	D708	D709	D710	D711	D712

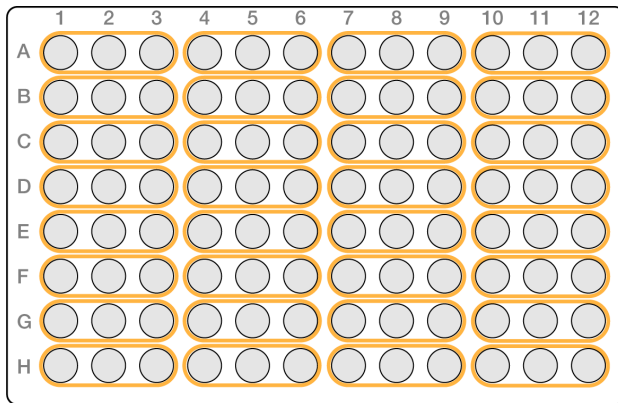
## Two-Plex, Single-Index

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



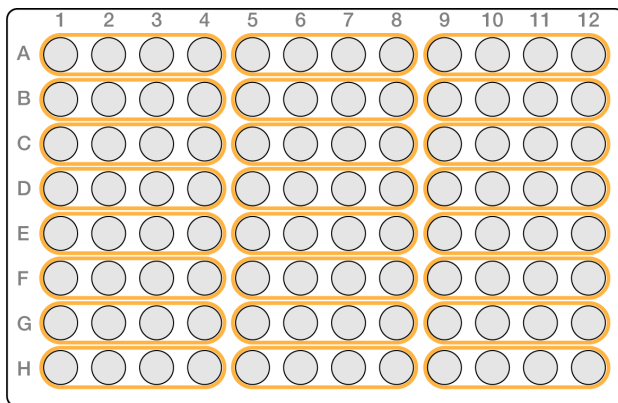
### Three-Plex, Single-Index

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



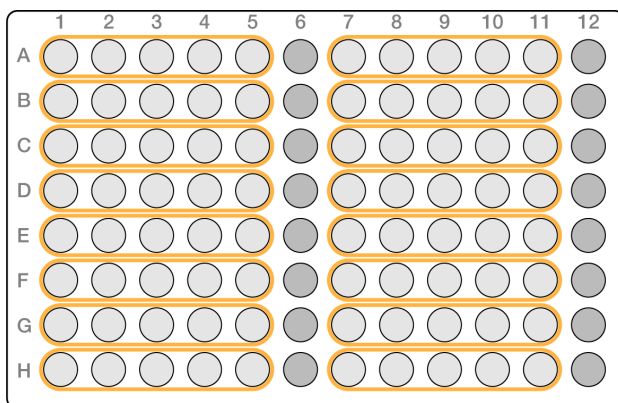
### Four-Plex, Single-Index

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



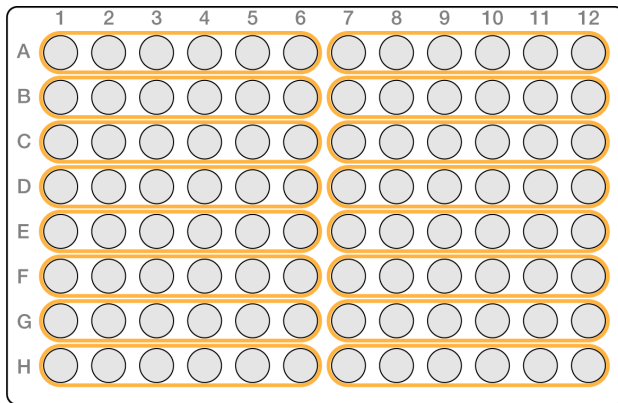
### Five-Plex, Single-Index

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



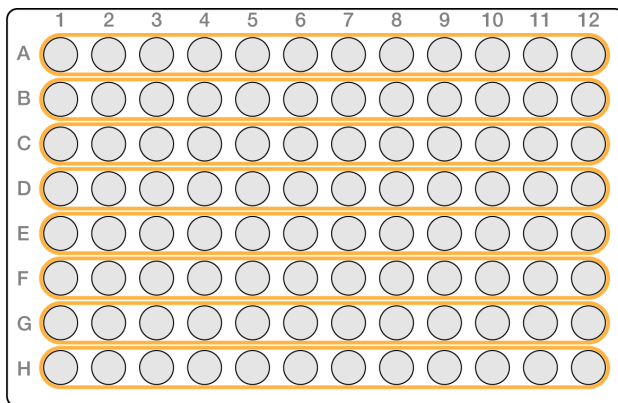
### Six-Plex, Single-Index

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.

**Table 1 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
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



Plexity	Option	Set A Index Adapters Only	Set B Index Adapters Only
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

Table 2 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 A

## TruSeq Small RNA Indices

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

The following table details pooling strategies for 2–4 libraries generated with index adapters from each set. For 5–11-plex pools, use the four-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
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 Indices C with another TruSeq Small RNA Indices set.

## Revision History

Document	Date	Description of Change
Document #1000000041074 v07	May 2019	Added index plate layouts for IDT for Illumina Nextera DNA UD Indexes Sets B–D. Identified the IDT for Illumina Nextera DNA UD index plate layout as IDT for Illumina Nextera DNA UD Indexes Sets A. Updated section on TruSeq Small RNA Indices to improve clarity and accuracy.
Document #1000000041074 v06	February 2019	Added index plate layouts for AmpliSeq CD Indexes Sets B–D for Illumina. Identified the AmpliSeq for Illumina index plate layout as AmpliSeq CD Indexes Set A for Illumina.
Document #1000000041074 v05	December 2018	Corrected the Index 1 (i7) index adapter in well C2 of the AmpliSeq CD Indexes for Illumina plate to Q7008.
Document #1000000041074 v04	November 2018	Combined, renamed, and reorganized all sections to improve continuity, facilitate navigation, and eliminate redundancy. Consolidated graphics depicting pooling strategies for IDT for Illumina Nextera DNA UD Indexes and Nextera DNA CD Indexes (96 Indexes, 96 Samples). Added pooling guidelines for Nextera XT Index Kit v2 and Nextera Index Kit. Added information on Illumina Experiment Manager, Local Run Manager, and BaseSpace Sequence Hub. Added good and bad examples of color balance on a four-channel system. Identified all Index 1 (i7) and all Index 2 (i5) adapters for Nextera DNA CD Indexes (24 Indexes, 24 Samples). Updated plate layouts to remove duplicate index adapter names and show the Index 1 (i7) adapters first. Updated descriptions of one-, two-, and four-dye chemistry. Described dual indexing options: combinatorial dual (CD) and unique dual (UD) indexes. Described pooling strategies for TruSeq CD Indexes and updated style of the graphics (the strategies have not changed). Referenced <i>Indexed Sequencing Overview (document # 15057455)</i> for indexing workflows on Illumina sequencing systems. Clarified the following points: <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> Removed the following sequences, which are available in <i>Illumina Adapter Sequences (document # 1000000002694)</i> : <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> Corrected column-based, three-plex pooling strategy for AmpliSeq for Illumina. Corrected names of the following items: <ul style="list-style-type: none"> <li>• IDT for Illumina kits.</li> <li>• Index adapters UDP0001–UDP0009 to include four digits.</li> </ul>
Document #1000000041074 v03	October 2018	Added information on IDT for Illumina Nextera DNA UD Indexes. Consolidated low-plexity guidelines. Corrected tables for Nextera DNA Flex 96 CD Indexes. Removed single indexing information for IDT for Illumina TruSeq DNA UD Indexes, which is compatible with dual indexing only. Removed incorrect guidelines for: <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>

Document	Date	Description of Change
Document #1000000041074 v02	May 2018	Added information on one-channel sequencing for the iSeq 100 System.
Document #1000000041074 v01	January 2018	Added pooling guidelines for AmpliSeq for Illumina and Nextera DNA Flex libraries.
Document #1000000041074 v00	October 2017	Initial release.

## Technical Assistance

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**Safety data sheets (SDSs)**—Available on the Illumina website at [support.illumina.com/sds.html](http://support.illumina.com/sds.html).

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