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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.

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, NovaSeq 6000, NextSeq Systems, and MiniSeq Systems
Four-channel	The MiSeq System and all 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 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

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 the following instruments:

- NovaSeq X Series (XLEAP-SBS chemistry is the default)
- NextSeq 1000/2000 Series 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
A	UDP0001	UDP000 9	UDP001 7	UDP002 5	UDP003 3	UDP0041	UDP0049	UDP005 7	UDP0065	UDP0073 V3	UDP0081	UDP008 9
B	UDP0002	UDP001 0	UDP001 8	UDP002 6	UDP003 4	UDP0042	UDP0050	UDP005 8	UDP0066	UDP0074 V3	UDP0082	UDP009 0
C	UDP0003 V3	UDP001 1	UDP001 9	UDP002 7	UDP003 5	UDP0043	UDP0051	UDP005 9	UDP0067	UDP0075 V3	UDP0083	UDP009 1
D	UDP0004	UDP001 2	UDP002 0	UDP002 8	UDP003 6	UDP0044	UDP0052	UDP006 0	UDP0068	UDP0076 V3	UDP0084 V3	UDP009 2
E	UDP0005 V3	UDP001 3	UDP002 1	UDP002 9	UDP003 7	UDP0045	UDP0053 V3	UDP006 1	UDP0069 V3	UDP0077	UDP0085	UDP009 3
F	UDP0006	UDP001 4	UDP002 2	UDP003 0	UDP003 8	UDP0046 V3	UDP0054 V3	UDP006 2	UDP0070 V3	UDP0078	UDP0086	UDP009 4
G	UDP0007	UDP001 5	UDP002 3	UDP003 1	UDP003 9	UDP0047	UDP0055 V3	UDP006 3	UDP0071 V3	UDP0079	UDP0087	UDP009 5
H	UDP0008	UDP001 6	UDP002 4	UDP003 2	UDP004 0	UDP0048	UDP0056 V3	UDP006 4	UDP0072 V3	UDP0080	UDP0088	UDP009 6

Illumina UD Indexes Set B

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

Illumina UD Indexes Set C

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

Illumina UD Indexes Set D

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

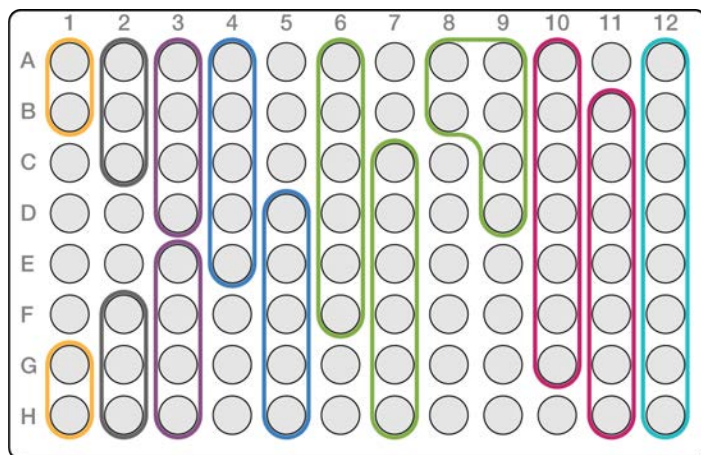
Two-Plex Through Eight-Plex Pooling Strategies

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 ≥ 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"> • A and B • G and H 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"> • A–C • F–H 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"> • A–D • E–H 	Not Recommended	Purple
5	The first five or last five wells in a column: <ul style="list-style-type: none"> • A–E • D–H 	Not Recommended	Blue
6	[Option 1] The first six or last six wells in a column: <ul style="list-style-type: none"> • A–F • C–H [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"> • A–G • B–H 	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	<p>The following well pairs are recommended:</p> <ul style="list-style-type: none"> • C6, D11 • C6, H10 • E4, A11 • F1, H12 • F3, G11 • H7, E10 	<p>The following well pairs are recommended:</p> <ul style="list-style-type: none"> • B9, D11 • D11, A12 • D11, B12 • F3, D11 • G3, C10 • G3, D11 • H5, G9 • H7, B10 • H8, D11 • C8, D11 	Not Recommended

Plexity	Combinations Set A	Combinations Set B	Combinations Set C and Set D
3	<p>The following well pairs are recommended.</p> <ul style="list-style-type: none"> • A3, C3, D3 • E3, G3, H3 • B4, C4, D4 • F8, G8, H8 • B11, C11, G11 • B11, F11, G11 • A12, G12, H12 • E12, G12, H12 • D12, G12, H12 	<p>The following well pairs are recommended.</p> <ul style="list-style-type: none"> • F8, G8, H8 • E9, G9, H9 • B10, C10, D10 • C10, D10, E10 • A12, C12, E12 • A12, C12, F12 • B12, F12, H12 • A11, C11, D11 	<p>Not Recommended</p>

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

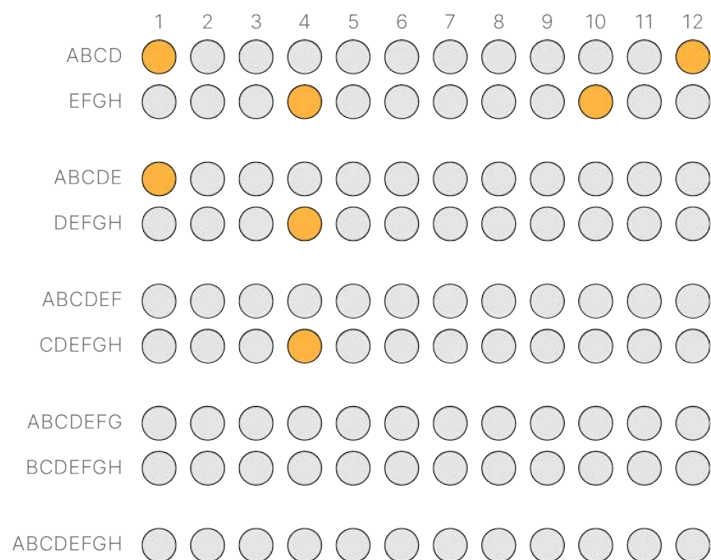
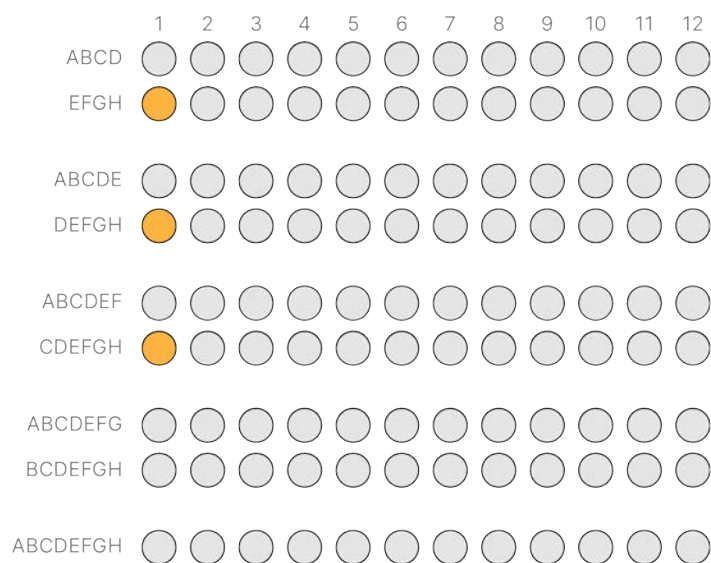


Figure 2 Illumina UD Plate B Indexes



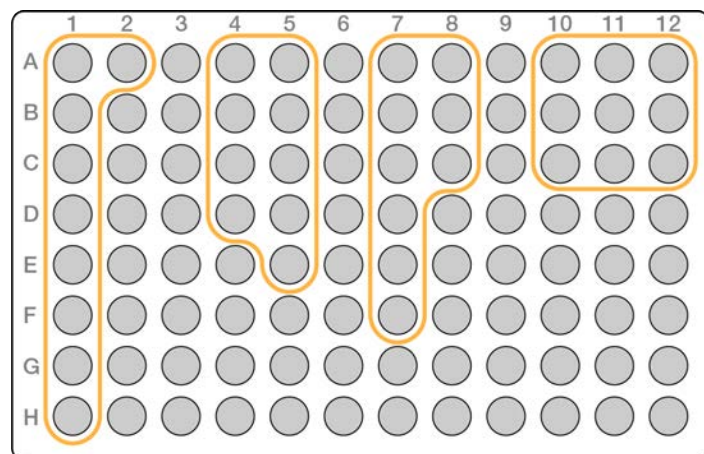
Nine-Plex Pooling Strategies

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
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 UD Indexes Plate B/Set 2

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

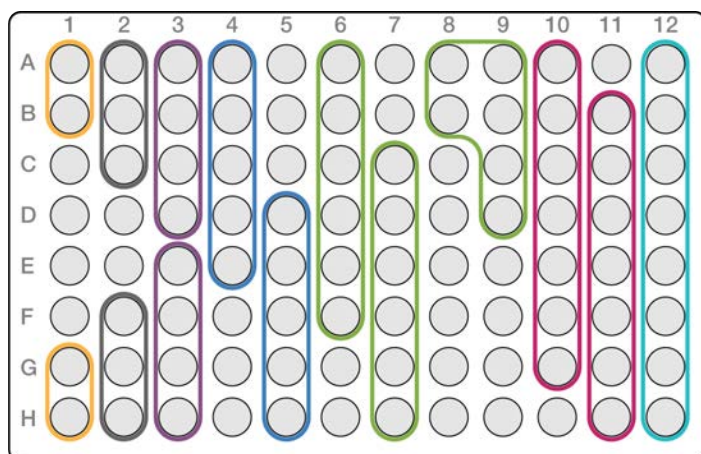
	1	2	3	4	5	6	7	8	9	10	11	12
A	UDP0289V 2	UDP0297	UDP030 5	UDP0313	UDP0321	UDP032 9	UDP033 7	UDP034 5	UDP035 3	UDP0361	UDP036 9	UDP037 7
B	UDP0290V 2	UDP0298	UDP030 6	UDP031 4	UDP032 2	UDP033 0	UDP033 8	UDP034 6	UDP035 4	UDP036 2	UDP037 0	UDP037 8
C	UDP0291V 2	UDP0299	UDP030 7	UDP0315	UDP032 3	UDP0331	UDP033 9	UDP034 7	UDP035 5	UDP036 3	UDP0371	UDP037 9
D	UDP0292	UDP0300	UDP030 8	UDP0316	UDP032 4	UDP033 2	UDP034 0	UDP034 8	UDP035 6	UDP036 4	UDP037 2	UDP038 0
E	UDP0293	UDP0301V 2	UDP030 9	UDP0317	UDP032 5	UDP033 3	UDP0341	UDP034 9	UDP035 7	UDP036 5	UDP037 3	UDP0381
F	UDP0294	UDP0302	UDP0310	UDP0318	UDP032 6	UDP033 4	UDP034 2	UDP035 0	UDP035 8	UDP036 6	UDP037 4	UDP038 2
G	UDP0295	UDP0303	UDP0311	UDP0319	UDP032 7	UDP033 5	UDP034 3	UDP0351	UDP035 9	UDP036 7	UDP037 5	UDP038 3
H	UDP0296	UDP0304	UDP0312	UDP032 0	UDP032 8	UDP033 6	UDP034 4	UDP035 2	UDP036 0	UDP036 8	UDP037 6	UDP038 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 ≥ 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"> • A and B • G and H Rows C–F are not used.	Orange
3	The first three or last three wells in a column: <ul style="list-style-type: none"> • A–C • F–H 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"> • A–D • E–H 	Purple
5	The first five or last five wells in a column: <ul style="list-style-type: none"> • A–E • D–H 	Blue
6	[Option 1] The first six or last six wells in a column: <ul style="list-style-type: none"> • A–F • C–H [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"> • A–G • B–H 	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 ≥ 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	<p>The following well pairs are recommended:</p> <ul style="list-style-type: none"> • C6, D11 • C6, H10 • E4, A11 • F1, H12 • F3, G11 	<p>The following well pairs are recommended:</p> <ul style="list-style-type: none"> • B9, D11 • D11, A12 • D11, B12 • F3, D11 • G3, C10 • G3, D11 • H5, G9 • H7, B10 • H8, D11
3	<p>The following well pairs are recommended:</p> <ul style="list-style-type: none"> • A3, C3, D3 • E3, G3, H3 • B4, C4, D4 • F8, G8, H8 • B11, C11, G11 • B11, F11, G11 • A12, G12, H12 • E12, G12, H12 	<p>The following well pairs are recommended:</p> <ul style="list-style-type: none"> • F8, G8, H8 • E9, G9, H9 • B10, C10, D10 • C10, D10, E10 • A12, C12, E12 • A12, C12, F12 • B12, F12, H12

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

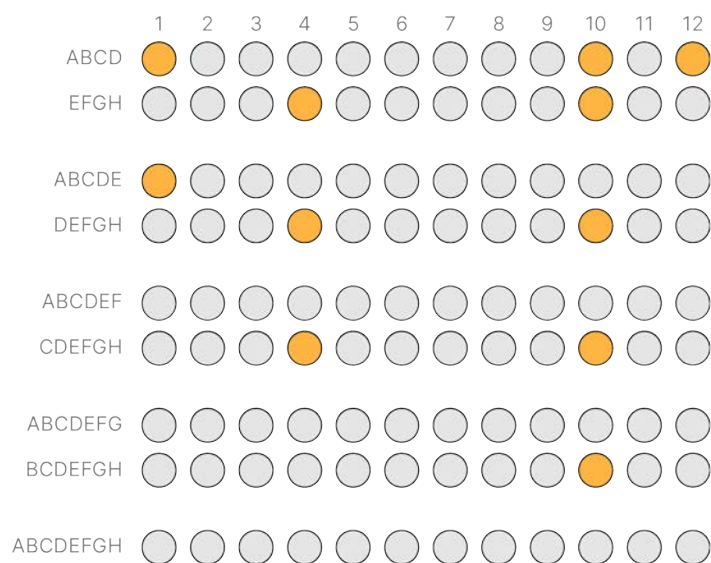
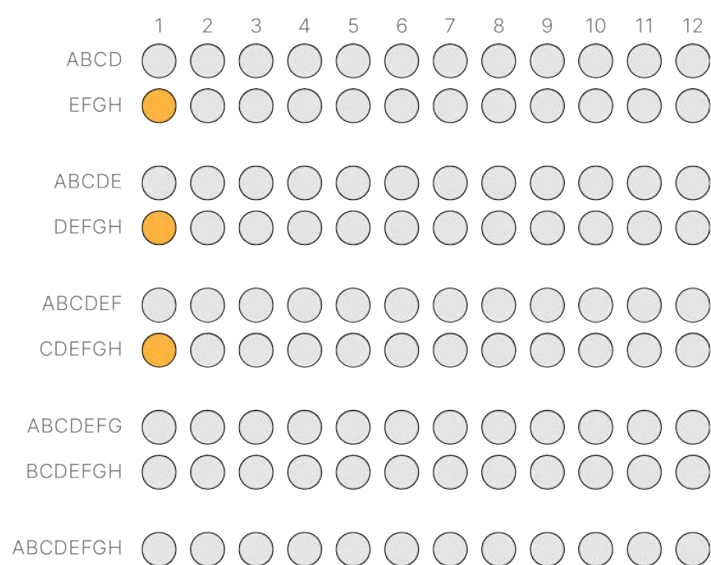


Figure 4 IDT for Illumina UD Plate B Indexes

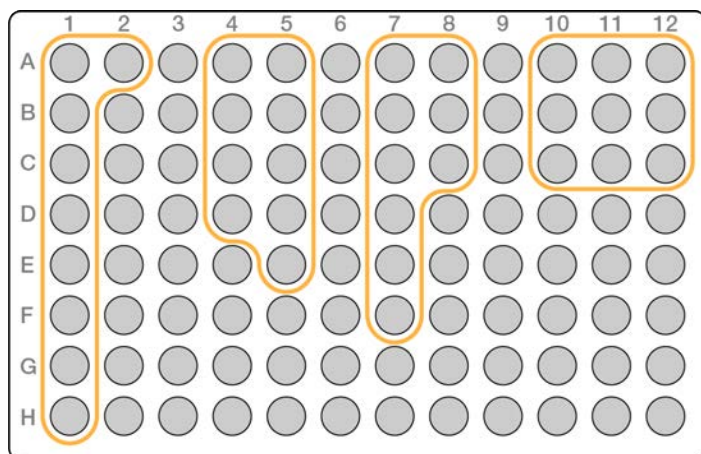


Nine-Plex Pooling Strategies

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.

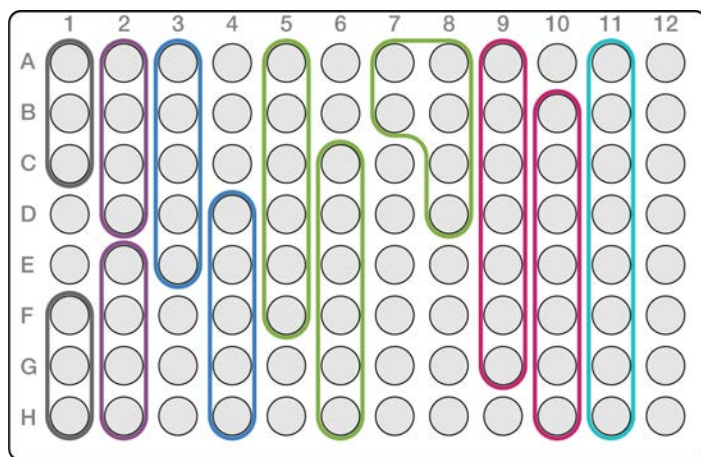
	1	2	3	4	5	6	7	8	9	10	11	12
A	H701	H702	H703	H705	H707	H723	H706	H712	H720	H710	H711	H714
	H505	H506	H517	H505	H506	H517	H505	H506	H517	H505	H506	H517
B	H702	H703	H701	H707	H723	H705	H712	H720	H706	H711	H714	H710
	H517	H505	H506	H517	H505	H506	H517	H505	H506	H517	H505	H506
C	H703	H701	H702	H723	H705	H707	H720	H706	H712	H714	H710	H711
	H506	H517	H505	H506	H517	H505	H506	H517	H505	H506	H517	H505
D	H705	H707	H723	H706	H712	H720	H710	H711	H714	H701	H702	H703
	H503	H503	H503	H503	H503	H503	H503	H503	H503	H503	H503	H503
E	H706	H712	H720	H710	H711	H714	H701	H702	H703	H705	H707	H723
	H516	H516	H516	H516	H516	H516	H516	H516	H516	H516	H516	H516
F	H710	H711	H714	H701	H702	H703	H705	H707	H723	H706	H712	H720
	H522	H510	H513	H522	H510	H513	H522	H510	H513	H522	H510	H513
G	H711	H714	H710	H702	H703	H701	H707	H723	H705	H712	H720	H706
	H513	H522	H510	H513	H522	H510	H513	H522	H510	H513	H522	H510
H	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"> A-C F-H 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"> A-D E-H 	Purple
5	The first five or last five wells in a column: <ul style="list-style-type: none"> A-E D-H 	Blue
6	[Option 1] The first six or last six wells in a column: <ul style="list-style-type: none"> A-F C-H [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"> A-G B-H 	Pink
8	The entire column.	Teal

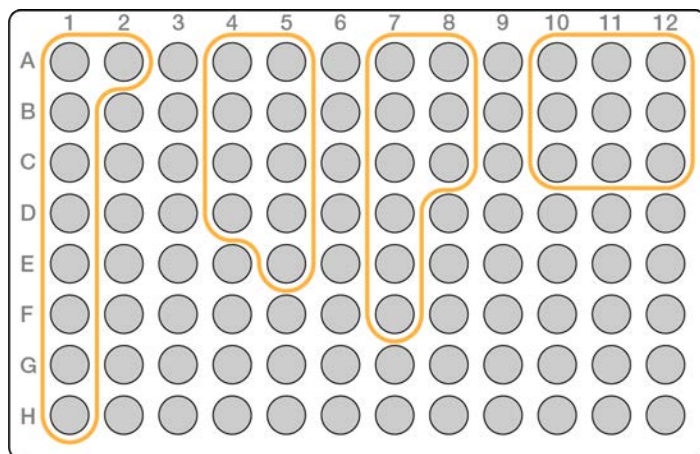


Nine-Plex Pooling Strategies

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"> • H705, H706, and H707 • H710, H711, and H714 	H505, H506, and H517
4	One of the following combinations: <ul style="list-style-type: none"> • H705, H706, H707, and any other i7 adapter • H710, H711, H714, and any other i7 adapter 	H503, H505, H506, and H517

Plexity	Index 1 (i7) Adapters	Index 2 (i5) Adapters
5	One of the following combinations: <ul style="list-style-type: none"> • H705, H706, H707, and any two i7 adapters • H710, H711, H714, and any two i7 adapters 	H503, H505, H506, H517, and any other i5 adapter
6	One of the following combinations: <ul style="list-style-type: none"> • H705, H706, H707, H710, H711, H714 • Two 3-plex pools 	One of the following combinations: <ul style="list-style-type: none"> • H503, H505, H506, H517, and any two additional i5 adapters • Two 3-plex pools
7	One of the following combinations: <ul style="list-style-type: none"> • H705, H706, H707, H710, H711, H714, and any other i7 adapter • Two 3-plex pools and any other i7 adapter 	One of the following combinations: <ul style="list-style-type: none"> • H503, H505, H506, H517, and any three additional i5 adapters • Two 3-plex pools and any other i5 adapter
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"> • N701, N702, N704, and any other i7 adapter • N703, N705, N706, and any other i7 adapter 	One of the following combinations: <ul style="list-style-type: none"> • N503 and N504 • N505 and N506
> 12	N701–N706 and any other i7 adapter	One of the following combinations: <ul style="list-style-type: none"> • N503, N504, and any other i5 adapter • N505, N506, and any other i5 adapter

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">• N701 and N702• N702 and N704	Any i5 adapters
3	One of the following combinations: <ul style="list-style-type: none">• N701, N702, and N704• N703, N705, and N706	Any i5 adapters
4 or 5	One of the following combinations: <ul style="list-style-type: none">• N701, N702, N704, and any other i7 adapter• N703, N705, N706, and any other i7 adapter	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
A	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	Q7006	Q7016	Q7005	Q7008	Q7015	Q7007	Q7017	Q7024	Q7024	Q7026	Q7023	Q7025
	Q5002	Q5001	Q5008	Q5007	Q5010	Q5009	Q5002	Q5001	Q5001	Q5007	Q5010	Q5009
C	Q7016	Q7008	Q7015	Q7006	Q7007	Q7005	Q7024	Q7026	Q7023	Q7017	Q7025	Q7018
	Q5007	Q5008	Q5009	Q5010	Q5013	Q5014	Q5007	Q5008	Q5009	Q5010	Q5013	Q5014
D	Q7015	Q7007	Q7016	Q7005	Q7008	Q7006	Q7023	Q7025	Q7024	Q7018	Q7026	Q7017
	Q5008	Q5007	Q5010	Q5009	Q5014	Q5013	Q5008	Q5007	Q5010	Q5009	Q5014	Q5013
E	Q7017	Q7025	Q7018	Q7023	Q7026	Q7024	Q7006	Q7007	Q7005	Q7015	Q7008	Q7016
	Q5009	Q5010	Q5013	Q5014	Q5001	Q5002	Q5009	Q5010	Q5013	Q5014	Q5001	Q5002
F	Q7018	Q7026	Q7017	Q7024	Q7025	Q7023	Q7005	Q7008	Q7006	Q7016	Q7007	Q7015
	Q5010	Q5009	Q5014	Q5013	Q5002	Q5001	Q5010	Q5009	Q5014	Q5013	Q5002	Q5001
G	Q7026	Q7024	Q7025	Q7018	Q7023	Q7017	Q7008	Q7016	Q7007	Q7005	Q7015	Q7006
	Q5013	Q5014	Q5001	Q5002	Q5007	Q5008	Q5013	Q5014	Q5001	Q5002	Q5007	Q5008
H	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
A	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	Q7028	Q7036	Q7027	Q7030	Q7035	Q7029	Q7039	Q7042	Q7040	Q7048	Q7041	Q7047
	Q5002	Q5001	Q5008	Q5007	Q5010	Q5009	Q5002	Q5001	Q5008	Q5007	Q5010	Q5009

C	Q7036	Q7030	Q7035	Q7028	Q7029	Q7027	Q7042	Q7048	Q7041	Q7039	Q7047	Q7040
	Q5007	Q5008	Q5009	Q5010	Q5013	Q5014	Q5007	Q5008	Q5009	Q5010	Q5013	Q5014
D	Q7035	Q7029	Q7036	Q7027	Q7030	Q7028	Q7041	Q7047	Q7042	Q7040	Q7048	Q7039
	Q5008	Q5007	Q5010	Q5009	Q5014	Q5013	Q5008	Q5007	Q5010	Q5009	Q5014	Q5013
E	Q7039	Q7047	Q7040	Q7041	Q7048	Q7042	Q7028	Q7029	Q7027	Q7035	Q7030	Q7036
	Q5009	Q5010	Q5013	Q5014	Q5001	Q5002	Q5009	Q5010	Q5013	Q5014	Q5001	Q5002
F	Q7040	Q7048	Q7039	Q7042	Q7047	Q7041	Q7027	Q7030	Q7028	Q7036	Q7029	Q7035
	Q5010	Q5009	Q5014	Q5013	Q5002	Q5001	Q5010	Q5009	Q5014	Q5013	Q5002	Q5001
G	Q7048	Q7042	Q7047	Q7040	Q7041	Q7039	Q7030	Q7036	Q7029	Q7027	Q7035	Q7028
	Q5013	Q5014	Q5001	Q5002	Q5007	Q5008	Q5013	Q5014	Q5001	Q5002	Q5007	Q5008
H	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
A	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	Q7006	Q7016	Q7005	Q7008	Q7015	Q7007	Q7017	Q7024	Q7018	Q7026	Q7023	Q7025
	Q5018	Q5017	Q5026	Q5025	Q5028	Q5027	Q5018	Q5017	Q5026	Q5025	Q5028	Q5027
C	Q7016	Q7008	Q7015	Q7006	Q7007	Q7005	Q7024	Q7026	Q7023	Q7017	Q7025	Q7018
	Q5025	Q5026	Q5027	Q5028	Q5031	Q5032	Q5025	Q5026	Q5027	Q5028	Q5031	Q5032
D	Q7015	Q7007	Q7016	Q7005	Q7008	Q7006	Q7023	Q7025	Q7024	Q7018	Q7026	Q7017
	Q5026	Q5025	Q5028	Q5027	Q5032	Q5031	Q5026	Q5025	Q5028	Q5027	Q5032	Q5031
E	Q7017	Q7025	Q7018	Q7023	Q7026	Q7024	Q7006	Q7007	Q7005	Q7015	Q7008	Q7016
	Q5027	Q5028	Q5031	Q5032	Q5017	Q5018	Q5027	Q5028	Q5031	Q5032	Q5017	Q5018
F	Q7018	Q7026	Q7017	Q7024	Q7025	Q7023	Q7005	Q7008	Q7006	Q7016	Q7007	Q7015
	Q5028	Q5027	Q5032	Q5031	Q5018	Q5017	Q5028	Q5027	Q5032	Q5031	Q5018	Q5017
G	Q7026	Q7024	Q7025	Q7018	Q7023	Q7017	Q7008	Q7016	Q7007	Q7005	Q7015	Q7006
	Q5031	Q5032	Q5017	Q5018	Q5025	Q5026	Q5031	Q5032	Q5017	Q5018	Q5025	Q5026
H	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
A	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	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
C	Q7036	Q7030	Q7035	Q7028	Q7029	Q7027	Q7042	Q7048	Q7041	Q7039	Q7047	Q7040
	Q5025	Q5026	Q5027	Q5028	Q5031	Q5032	Q5025	Q5026	Q5027	Q5028	Q5031	Q5032
D	Q7035	Q7029	Q7036	Q7027	Q7030	Q7028	Q7041	Q7047	Q7042	Q7040	Q7048	Q7039
	Q5026	Q5025	Q5028	Q5027	Q5032	Q5031	Q5026	Q5025	Q5028	Q5027	Q5032	Q5031
E	Q7039	Q7047	Q7040	Q7041	Q7048	Q7042	Q7028	Q7029	Q7027	Q7035	Q7030	Q7036
	Q5027	Q5028	Q5031	Q5032	Q5017	Q5018	Q5027	Q5028	Q5031	Q5032	Q5017	Q5018
F	Q7040	Q7048	Q7039	Q7042	Q7047	Q7041	Q7027	Q7030	Q7028	Q7036	Q7029	Q7035
	Q5028	Q5027	Q5032	Q5031	Q5018	Q5017	Q5028	Q5027	Q5032	Q5031	Q5018	Q5017
G	Q7048	Q7042	Q7047	Q7040	Q7041	Q7039	Q7030	Q7036	Q7029	Q7027	Q7035	Q7028
	Q5031	Q5032	Q5017	Q5018	Q5025	Q5026	Q5031	Q5032	Q5017	Q5018	Q5025	Q5026
H	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.

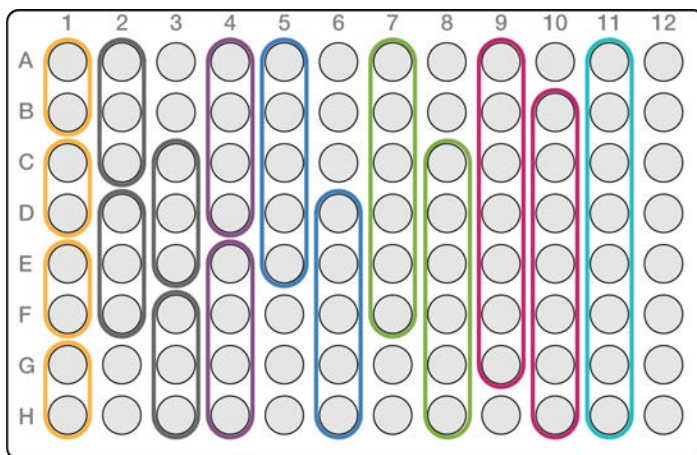
	1	2	3	4	5	6	7	8	9	10	11	12
A	Q7005	Q7023	Q7035	empty	empty	empty	empty	empty	empty	empty	empty	empty
	Q5007	Q5013	Q5025									
B	Q7006	Q7024	Q7036	empty	empty	empty	empty	empty	empty	empty	empty	empty
	Q5008	Q5014	Q5026									
C	Q7007	Q7025	Q7039	empty	empty	empty	empty	empty	empty	empty	empty	empty
	Q5039	Q5035	Q5031									
D	Q7008	Q7026	Q7040	empty	empty	empty	empty	empty	empty	empty	empty	empty
	Q5040	Q5036	Q5032									
E	Q7015	Q7027	Q7041	empty	empty	empty	empty	empty	empty	empty	empty	empty
	Q5003	Q5027	Q5009									
F	Q7016	Q7028	Q7042	empty	empty	empty	empty	empty	empty	empty	empty	empty
	Q5004	Q5028	Q5010									
G	Q7017	Q7029	Q7047	empty	empty	empty	empty	empty	empty	empty	empty	empty
	Q5017	Q5001	Q5029									
H	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: <ul style="list-style-type: none"> • A and B • C and D • E and F • G and H 	Orange
3	Three consecutive wells in a column: <ul style="list-style-type: none"> • A–C • D–F • C–E • F–H 	Gray
4	Four consecutive wells in a column: <ul style="list-style-type: none"> • A–D • E–H 	Purple
5	Five consecutive wells in a column: <ul style="list-style-type: none"> • A–E • D–H 	Blue
6	Six consecutive wells in a column: <ul style="list-style-type: none"> • A–F • C–H 	Green
7	Seven consecutive wells in a column: <ul style="list-style-type: none"> • A–G • B–H 	Pink
8	The entire column. All eight combinations are unique.	Teal

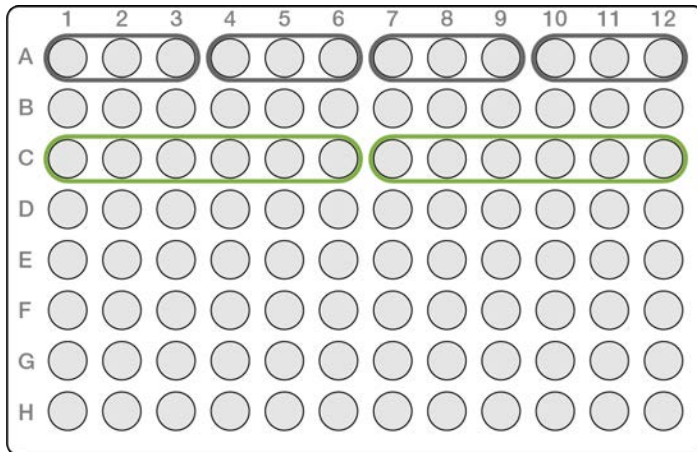


Row-Based Strategies

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. <ul style="list-style-type: none"> • 1–3 • 4–6 • 7–9 • 10–12 	Gray
6	Six consecutive wells in a row: <ul style="list-style-type: none"> • 1–6 • 7–12 	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 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 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 ≥ 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) on the NovaSeq X Series. 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">• A7, E10• A7, F10• A8, C9• A8, D9• B7, E10• B7, F10• B8, C9• B8, D9• C2, A11• C2, A3• C2, A7• C2, B11• C2, B3• C2, B7• C4, A7• C4, B7• C9, G10• C9, H10• D2, A11• D2, A3• D2, A7• D2, B11• D2, B3• D2, B7• D4, A7• D4, B7• D9, G10• D9, H10• G10, E12• G10, F12• H10, E12• H10, F12
3	<ul style="list-style-type: none">• A4, F4, H4• D5, F5, G5• A10, D10, H10• A11, C11, G11• A11, D11, G11• C11, F11, G11

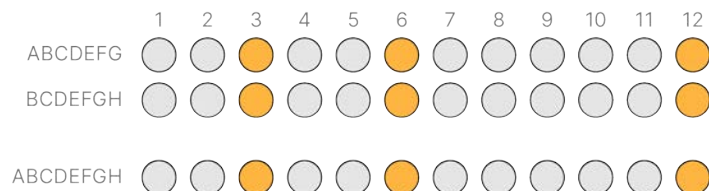
Plexity	Recommended Index Combinations
4	<ul style="list-style-type: none">• A1, D1, E1, H1• B1, C1, E1, G1• A2, C2, E2, G2• B2, D2, F2, H2• A4, B4, E4, G4• B4, C4, F4, H4• A5, B5, C5, E5• C5, D5, F5, H5• A7, C7, F7, G7• B7, D7, E7, H7• A8, B8, C8, G8• D8, E8, F8, H8• A9, C9, F9, H9• B9, D9, E9, H9•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

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

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).

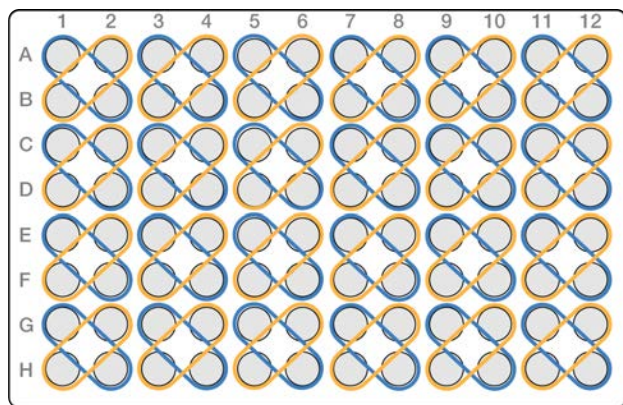
	1	2	3	4	5	6	7	8	9	10	11	12
A	D701	D702	D703	D704	D705	D706	D707	D708	D709	D710	D711	D712
	D501	D501	D501	D501	D501	D501	D501	D501	D501	D501	D501	D501
B	D701	D702	D703	D704	D705	D706	D707	D708	D709	D710	D711	D712
	D502	D502	D502	D502	D502	D502	D502	D502	D502	D502	D502	D502
C	D701	D702	D703	D704	D705	D706	D707	D708	D709	D710	D711	D712
	D503	D503	D503	D503	D503	D503	D503	D503	D503	D503	D503	D503
D	D701	D702	D703	D704	D705	D706	D707	D708	D709	D710	D711	D712
	D504	D504	D504	D504	D504	D504	D504	D504	D504	D504	D504	D504
E	D701	D702	D703	D704	D705	D706	D707	D708	D709	D710	D711	D712
	D505	D505	D505	D505	D505	D505	D505	D505	D505	D505	D505	D505
F	D701	D702	D703	D704	D705	D706	D707	D708	D709	D710	D711	D712
	D506	D506	D506	D506	D506	D506	D506	D506	D506	D506	D506	D506
G	D701	D702	D703	D704	D705	D706	D707	D708	D709	D710	D711	D712
	D507	D507	D507	D507	D507	D507	D507	D507	D507	D507	D507	D507
H	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 ≥ 2 , but can be used for one-plex pools (single libraries).

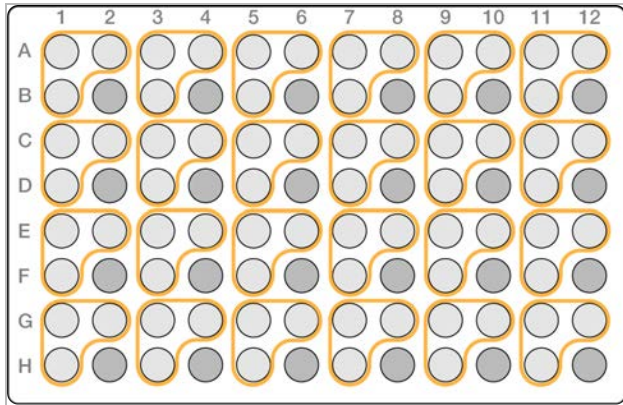
Two-Plex, Dual-Index

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



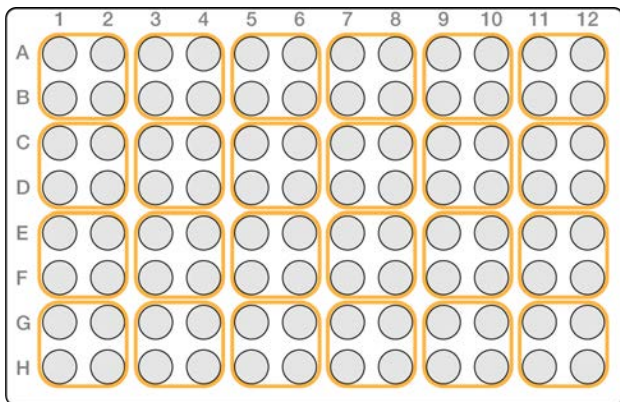
Three-Plex, Dual-Index

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



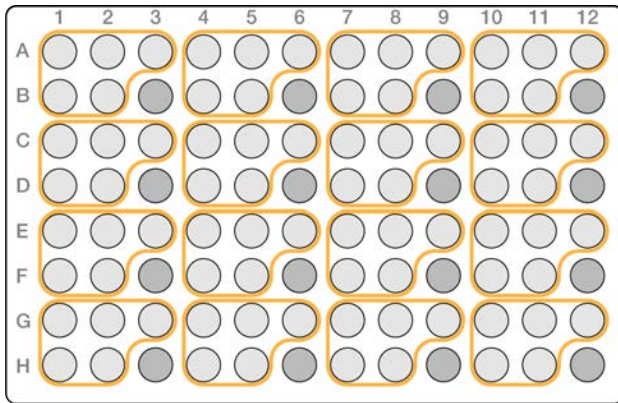
Four-Plex, Dual-Index

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



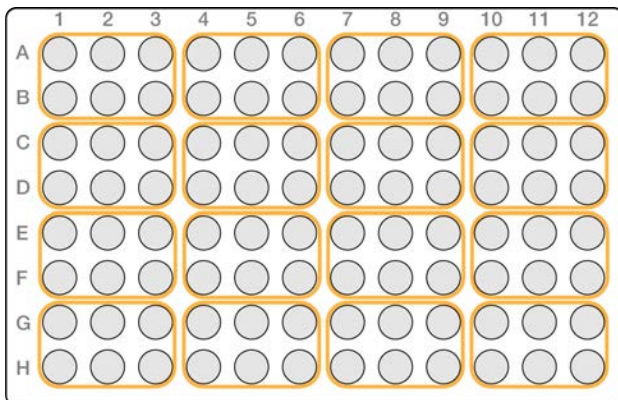
Five-Plex, Dual-Index

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.



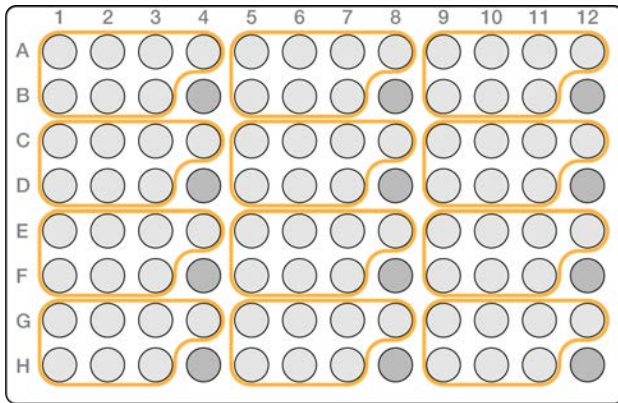
Six-Plex, Dual-Index

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



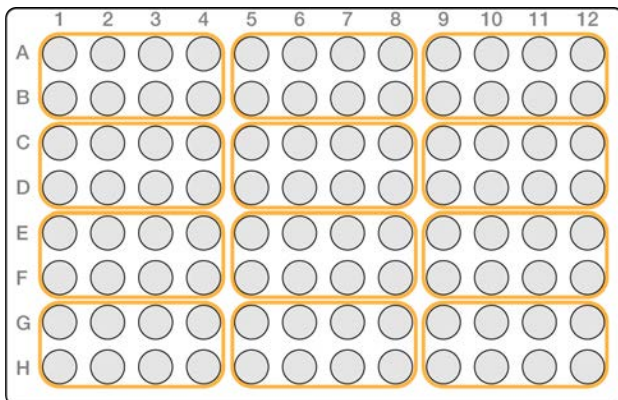
Seven-Plex, Dual-Index

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.



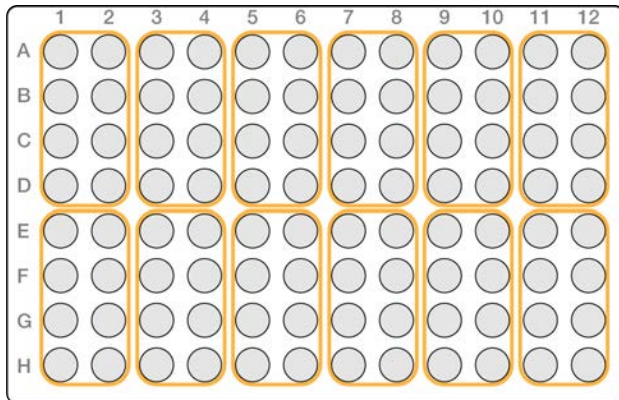
Eight-Plex, Dual-Index (Option 1)

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



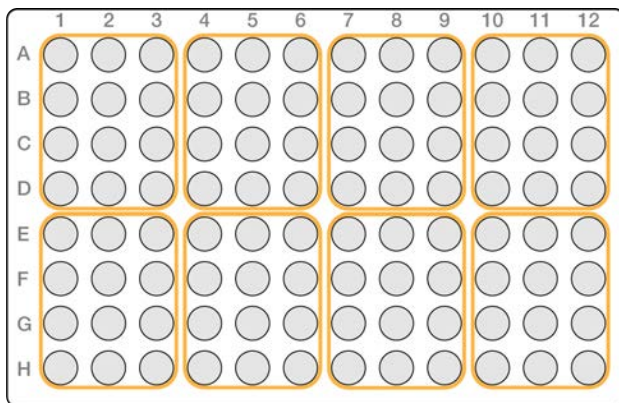
Eight-Plex, Dual-Index (Option 2)

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



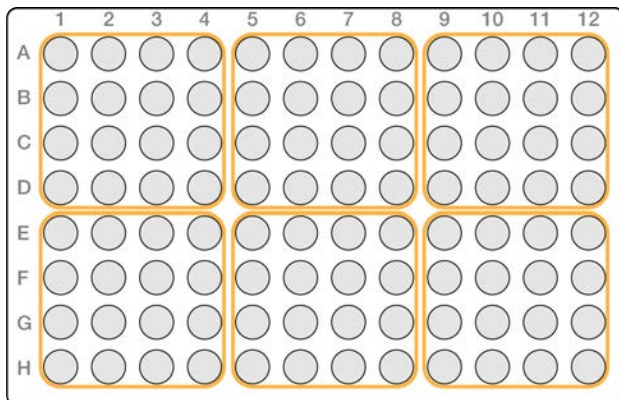
Twelve-Plex, Dual-Index

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).

	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

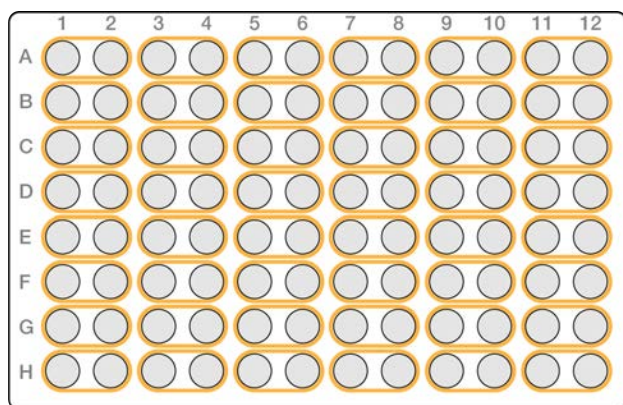
The following figures illustrate pooling strategies for libraries ligated with the Index 1 (i7) and Index 2 (i5) adapters, where only Index 1 is sequenced.

- Circles indicate index adapter combinations that can be combined in a pool.
- Dark gray indicates wells that are not used for pool plexity ≥ 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.

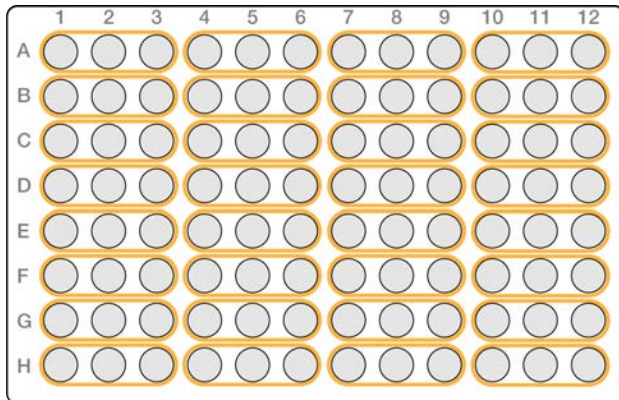
Two-Plex, Single-Index

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



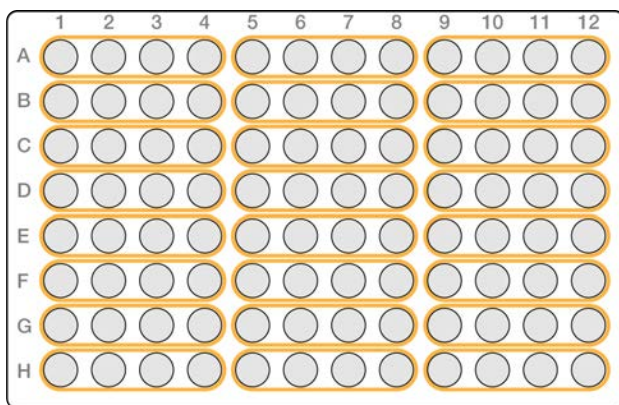
Three-Plex, Single-Index

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



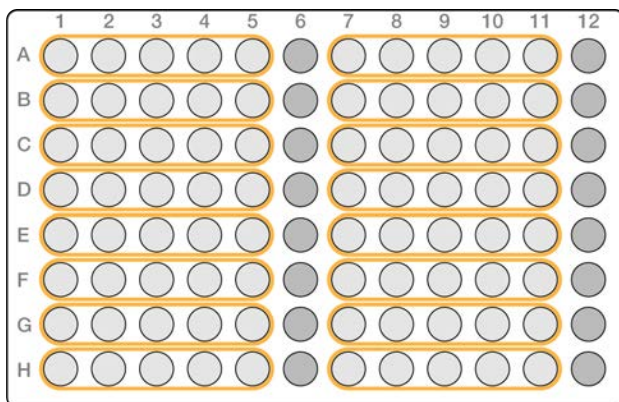
Four-Plex, Single-Index

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



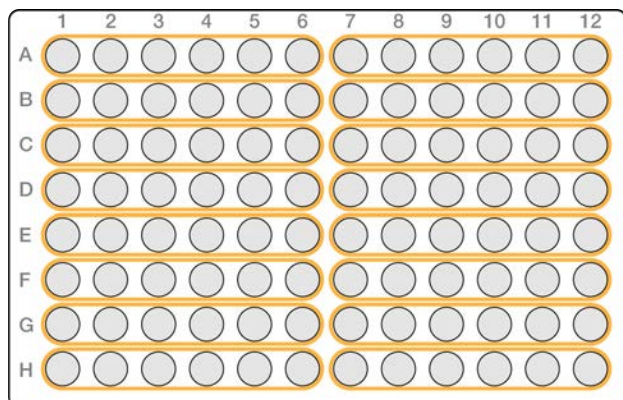
Five-Plex, Single-Index

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



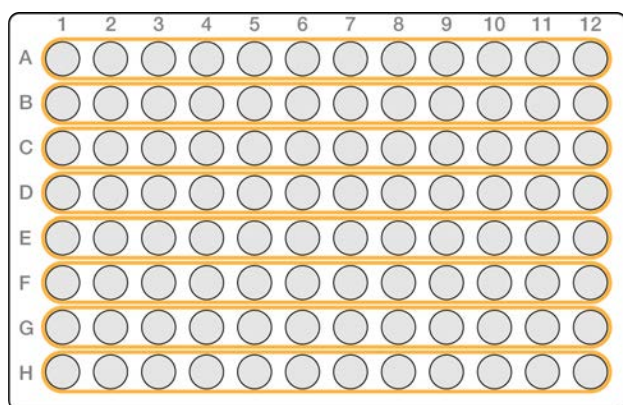
Six-Plex, Single-Index

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 v13	February 2024	Added NextSeq 1000/2000 XLEAP-SBS chemistry information.
1000000041074 v12	September 2023	<p>Added NovaSeq X Series information.</p> <p>Added plate layout and pooling strategies for Illumina UD Indexes, Set A-D.</p> <p>Added clarification for V2 indexes used with IDT for Illumina UD Indexes Set C and Set D.</p> <p>Reorganized the following content to improve continuity and navigation:</p> <ul style="list-style-type: none"> • Color balance and index adapter combinations • AmpliSeq CD Indexes • IDT for Illumina TruSeq UD Indexes
1000000041074 v11	April 2021	<p>Added HTML format.</p> <p>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).</p>
1000000041074 v10	July 2020	<p>Replaced UDI0015, UDI0016, UDI0055, UDI0056, UDP0252, UDP0258, UDP0289, UDP0290, UDP0291, and UDP0301 with V2 versions.</p> <p>Added IDT for Illumina-PCR UD Indexes Set 1, 2, 3, 4.</p>
1000000041074 v09	March 2020	<p>Generalized references to NextSeq Systems to include all versions.</p> <p>Added NextSeq 2000 information to Two-Channel Chemistry section.</p> <p>Added Instrument Run Setup to Color Balance section.</p>

Document #	Date	Description of Change
1000000041074 v08	October 2019	Corrected the third option for TruSeq RNA Single Indexes Set B four-plex pooling strategy. Added index plate layouts for IDT for Illumina TruSeq-UD Indexes (24 Indexes, 96 Samples).
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 indexes to improve clarity and accuracy.
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.
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 #	Date	Description of Change
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). 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). 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"> • TruSeq CD Indexes replaced TruSeq HT Indexes. • For two-plex, dual-indexing with TruSeq CD Indexes, pool diagonally. • Single-indexing with TruSeq CD Indexes requires dual index adapters. <p>Removed the following sequences, which are available in <i>Illumina Adapter Sequences</i> (document # 100000002694):</p> <ul style="list-style-type: none"> • Index adapter sequences for Nextera DNA CD Indexes (for Nextera DNA Flex). • Index adapter and adapter trimming sequences for AmpliSeq for Illumina. <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"> • IDT for Illumina kits. • Index adapters UDP0001–UDP0009 to include four digits.

Document #	Date	Description of Change
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">• Low plexity sequencing on the NovaSeq 6000 System.• Low throughput for Nextera DNA Flex combinatorial dual indexes.
1000000041074 v02	May 2018	Added information on one-channel sequencing for the iSeq 100 System.
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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