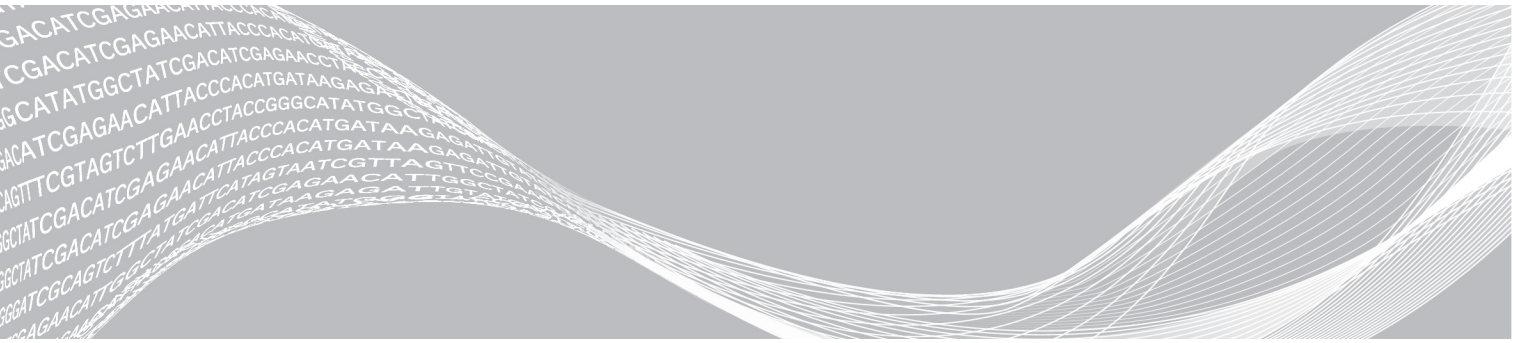
illumina®

Index Adapters

Pooling Guide



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ILLUMINA PROPRIETARY

Introduction

This document provides Illumina® pooling guidelines for performing library prep for sequencing on Illumina sequencing systems.

Visit the support page for your library prep kit on the Illumina website at www.illumina.com.

Additional Resources

This document supplements the library prep kit and workflow reference guides. Review the appropriate reference guide for protocol instructions and product details.

The following documentation is available for download from the Illumina website.

Resource	Description
Custom Protocol Selector	support.illumina.com/custom-protocol-selector.html A wizard for generating customized end-to-end documentation that is tailored to the library prep method, run parameters, and analysis method used for the sequencing run.
Illumina Adapter Sequences (document # 1000000002694)	Provides the nucleotide sequences that comprise Illumina oligonucleotides used in Illumina sequencing technologies.
Illumina Experiment Manager (document # 15031335)	Provides information about creating and editing appropriate sample sheets for Illumina sequencing systems and analysis software and record parameters for your sample plate.
Nextera DNA Flex Library Prep Pooling Guide (document # 1000000031471)	Provides pooling guidelines for performing the Nextera™ DNA Flex library prep for sequencing on Illumina sequencing systems.

Pooling Guidelines

Selecting the correct index combinations avoids Index Read failure due to cluster registration failure and improves accuracy during data analysis. Illumina sequencing uses a two-channel or four-channel method to detect individual bases.

It is important to maintain color balance for each base of the Index Read being sequenced, otherwise Index Read sequencing could fail due to registration failure. This base calling process also ensures accuracy for data analysis.

Follow these low-plex pooling guidelines, depending on your index adapter component.

Not all color-balanced pools are listed. Check the color balance using Illumina Experiment Manager (IEM) for the HiSeq worklfow. For more information, see the *Illumina Experiment Manager Guide (document # 15031335)*.

Four-Channel Sequencing

Always use at least two unique and compatible barcodes for each index sequenced.

Four-channel sequencing systems capture four distinct images, which allows cycle-by-cycle observation of which dye is incorporated into a cluster.

A green laser is used to sequence G and T bases while a red laser sequences A and C bases. To ensure proper image registration, each cycle reads at least one of two nucleotides per color channel. Therefore, all four images are required to build the sequence.

The MiSeq system and all HiSeq systems currently use four-channel chemistry.

Two-Channel Sequencing

- Index Reads must begin with at least one base other than G in either of the first two cycles. If an Index Read begins with two base calls of G, signal intensity is not generated and registration fails. Signal must be present in either of the first two cycles to ensure demultiplexing performance.
- Select index sequences that provide signal in at least one channel, preferably both channels, for every cycle.
  - Red channel—A or C
  - Green channel—A or T

Two-channel sequencing simplifies nucleotide detection because only two images are needed to determine all four base calls. Instead of using a separate dye for each base, two-channel sequencing uses a mix of dyes. Clusters with intensity in the red channel are C bases, and clusters with intensity in the green channel are T base. Clusters with intensity in both red and green are A base. Unlabeled clusters are G base.

The NovaSeq, NextSeq, and MiniSeq platforms use two-channel chemistry.

For more information on pooling for two-channel systems, see the *Library pooling guidelines for the NextSeq and MiniSeq systems* bulletin on the Illumina support site.

One-Channel Sequencing

The first two cycles of an index read cannot start with two G bases, otherwise intensity is not generated. To ensure demultiplexing performance, intensity must be present in either of the first two cycles.

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 images) for every cycle.

The iSeq 100 System uses one-dye sequencing, which 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 which population each cluster belongs to.

TruSeq Pooling Guidelines

This section details pooling strategies for index adapter tubes and index adapter plates for TruSeq based index adapters (indexed by ligation).

Index Adapter Tubes

When using the Index Adapter Tubes, use the following pooling guidelines for single-indexed sequencing.

TruSeq DNA Single Indexes–Sets A and B

TruSeq DNA Single Index–Sets A and B each contain 12 unique indexes. When using these indexes, use the following pooling guidelines for single-indexed sequencing.

The following tables detail pooling strategies for two to four samples generated with the index adapters in each set. For 5–11-plex pools, use the four-plex options with any other available adapters.

Table 1 Single-Index Pooling Strategies for Two-Four Samples (DNA)

Plexity	Option	Set A Only	Set B Only
2	1	AD006 and AD012	Not recommended
	2	AD005 and AD019	
3	1	AD002, AD007, and AD019	AD001, AD010, and AD020
	2	AD005, AD006, and AD015	AD003, AD009, and AD025
	3	Two-plex options with any other available adapter	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 with any other available adapter	Three-plex options with any other available adapter

Table 2 Single-Index Pooling Strategies for Two-Four Samples (RNA)

Plexity	Option	Set A Only	Set B Only
2	1	AR006 and AR012	Not recommended
	2	AR005 and AR019	
3	1	AR002, AR007, and AR019	AR001, AR010, and AR020
	2	AR005, AR006, and AR015	AR003, AR009, and AR025
	3	Two-plex options with any other available adapter	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 with any other available adapter	Three-plex options with any other available adapter

Index Adapter Plate

When using the Index Adapter Plate, use the following pooling guidelines for dual-indexed or single-indexed sequencing.

Dual-Indexed Sequencing

Dual-indexed libraries help ensure quality data and reliable downstream analyses.

IDT for Illumina-TruSeq Unique Dual (UD) Indexes

The IDT for Illumina-TruSeq UD Indexes are compatible with all Illumina sequencing systems. Use dual-indexing when pooling > 12 samples in one pool. IDT for Illumina-TruSeq UD Indexes are intended for dual indexed sequencing. When performing a single indexed sequencing run when using IDT for Illumina-TruSeq UD Indexes, the same pooling guidelines apply.

The following figure provides example dual-index pooling strategies when using the IDT for Illumina-TruSeq UD Indexes. Pool libraries of any plexity > 2 down a column (2-plex, 3-plex, 4-plex, 5-plex, etc) as shown in Figure 1. Do not pool across a row.

Figure 1 Example Pooling Strategies

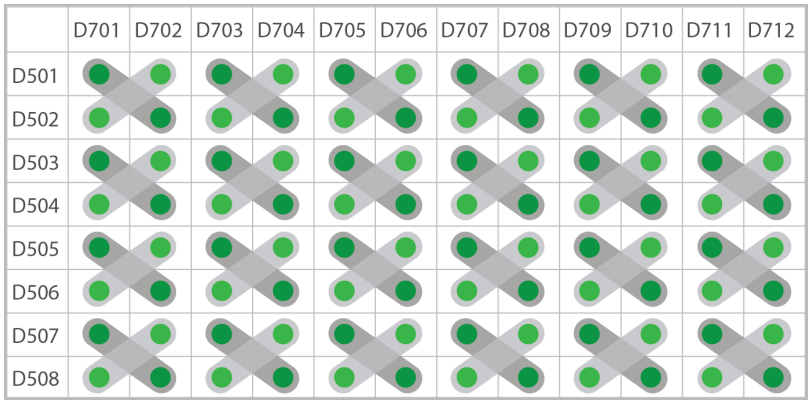
	1	2	3	4	5	6	7	8	9	10	11	12
A	UD0001 UD0001	UD0009 UD0009	UD0017 UD0017	UD0025 UD0025	UD0033 UD0033	UD0041 UD0041	UD0049 UD0049	UD0057 UD0057	UD0065 UD0065	UD0073 UD0073	UD0081 UD0081	UD0089 UD0089
B	UD0002 UD0002	UD0010 UD0010	UD0018 UD0018	UD0026 UD0026	UD0034 UD0034	UD0042 UD0042	UD0050 UD0050	UD0058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

TruSeq DNA and RNA Combinatorial Dual Indexes

The following figures illustrate pooling strategies for 2–16 samples generated with the TruSeq DNA Combinatorial Dual Indexes (96 indexes, 96 samples) or TruSeq RNA Combinatorial Indexes (96 indexes, 96 samples).

- Color-balanced pools are shaded gray and have green wells.
- Two-plex pools are diagonal, shaded, and have green wells.
- Odd-numbered pools have dark gray wells. The gray wells are not used for sequencing pooled libraries, but can be used for sequencing single libraries.

Dual-Indexed, Two-Plex



Dual-Indexed, Three-Plex

	D701	D702	D703	D704	D705	D706	D707	D708	D709	D710	D711	D712
D501	●	●	●	●	●	●	●	●	●	●	●	●
D502	●		●		●		●		●		●	
D503	●	●	●	●	●	●	●	●	●	●	●	●
D504	●		●		●		●		●		●	
D505	●	●	●	●	●	●	●	●	●	●	●	●
D506	●		●		●		●		●		●	
D507	●	●	●	●	●	●	●	●	●	●	●	●
D508	●		●		●		●		●		●	

Dual-Indexed, Four-Plex

	D701	D702	D703	D704	D705	D706	D707	D708	D709	D710	D711	D712
D501	●	●	●	●	●	●	●	●	●	●	●	●
D502	●	●	●	●	●	●	●	●	●	●	●	●
D503	●	●	●	●	●	●	●	●	●	●	●	●
D504	●	●	●	●	●	●	●	●	●	●	●	●
D505	●	●	●	●	●	●	●	●	●	●	●	●
D506	●	●	●	●	●	●	●	●	●	●	●	●
D507	●	●	●	●	●	●	●	●	●	●	●	●
D508	●	●	●	●	●	●	●	●	●	●	●	●

Dual-Indexed, Five-Plex

	D701	D702	D703	D704	D705	D706	D707	D708	D709	D710	D711	D712
D501	●	●	●	●	●	●	●	●	●	●	●	●
D502	●	●		●	●		●	●		●	●	
D503	●	●	●	●	●	●	●	●	●	●	●	●
D504	●	●		●	●		●	●		●	●	
D505	●	●	●	●	●	●	●	●	●	●	●	●
D506	●	●		●	●		●	●		●	●	
D507	●	●	●	●	●	●	●	●	●	●	●	●
D508	●	●		●	●		●	●		●	●	

Dual-Indexed, Six-Plex

	D701	D702	D703	D704	D705	D706	D707	D708	D709	D710	D711	D712
D501	●	●	●	●	●	●	●	●	●	●	●	●
D502	●	●	●	●	●	●	●	●	●	●	●	●
D503	●	●	●	●	●	●	●	●	●	●	●	●
D504	●	●	●	●	●	●	●	●	●	●	●	●
D505	●	●	●	●	●	●	●	●	●	●	●	●
D506	●	●	●	●	●	●	●	●	●	●	●	●
D507	●	●	●	●	●	●	●	●	●	●	●	●
D508	●	●	●	●	●	●	●	●	●	●	●	●

Dual-Indexed, Seven-Plex

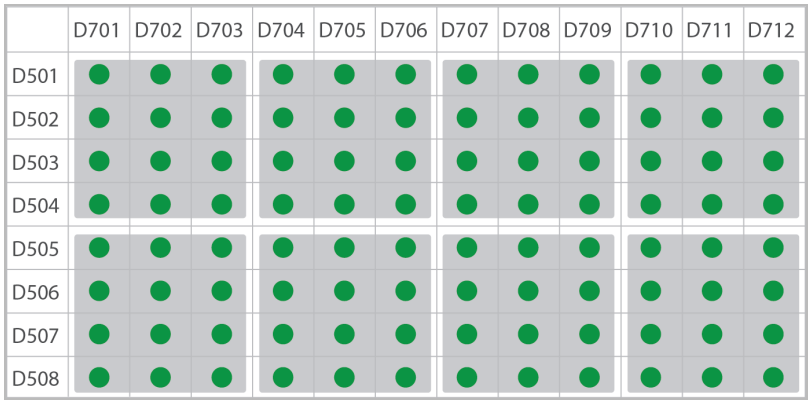
	D701	D702	D703	D704	D705	D706	D707	D708	D709	D710	D711	D712
D501	●	●	●	●	●	●	●	●	●	●	●	●
D502	●	●	●	●	●	●	●	●	●	●	●	●
D503	●	●	●	●	●	●	●	●	●	●	●	●
D504	●	●	●	●	●	●	●	●	●	●	●	●
D505	●	●	●	●	●	●	●	●	●	●	●	●
D506	●	●	●	●	●	●	●	●	●	●	●	●
D507	●	●	●	●	●	●	●	●	●	●	●	●
D508	●	●	●	●	●	●	●	●	●	●	●	●

Dual-Indexed, Eight-Plex (Option 1)

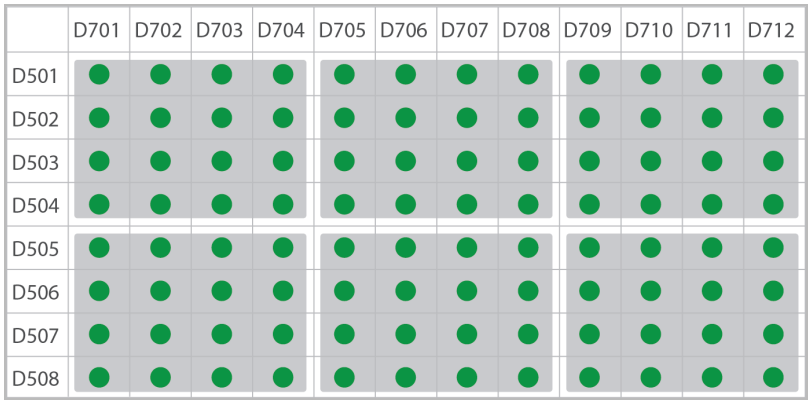
	D701	D702	D703	D704	D705	D706	D707	D708	D709	D710	D711	D712
D501	●	●	●	●	●	●	●	●	●	●	●	●
D502	●	●	●	●	●	●	●	●	●	●	●	●
D503	●	●	●	●	●	●	●	●	●	●	●	●
D504	●	●	●	●	●	●	●	●	●	●	●	●
D505	●	●	●	●	●	●	●	●	●	●	●	●
D506	●	●	●	●	●	●	●	●	●	●	●	●
D507	●	●	●	●	●	●	●	●	●	●	●	●
D508	●	●	●	●	●	●	●	●	●	●	●	●

Dual-Indexed, Eight-Plex (Option 2)

Dual-Indexed, 12-Plex



Dual-Indexed, 16-Plex



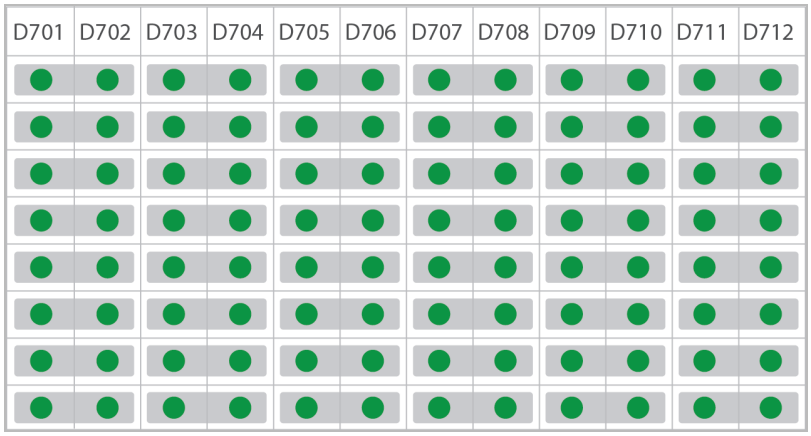
Single-Indexed Sequencing

Use single-indexing when pooling ≤ 12 samples in one pool. The following figures illustrate pooling strategies for 2–12 samples generated with the TruSeq DNA Combinatorial Dual Indexes (96 indexes, 96 samples) or TruSeq RNA Combinatorial Indexes (96 indexes, 96 samples).

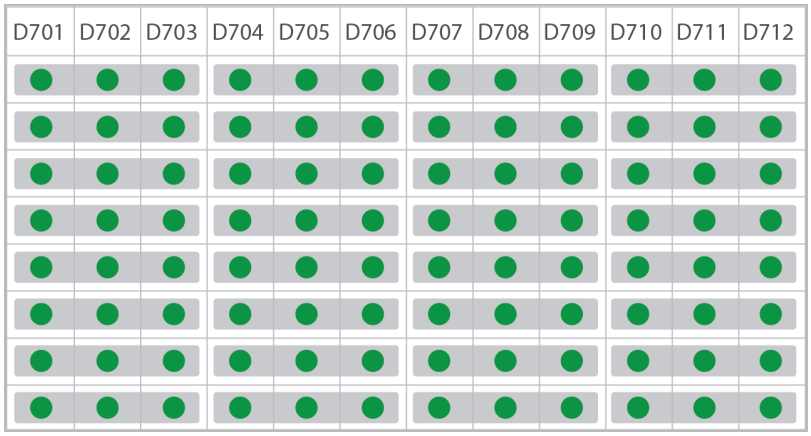
- Color-balanced pools are shaded gray and have green wells.

- Five-plex pools have dark gray wells. The gray wells are not used for sequencing pooled libraries, but can be used for sequencing single libraries.
- For 7–11-plex pools, combine any of the two to six-plex pools.

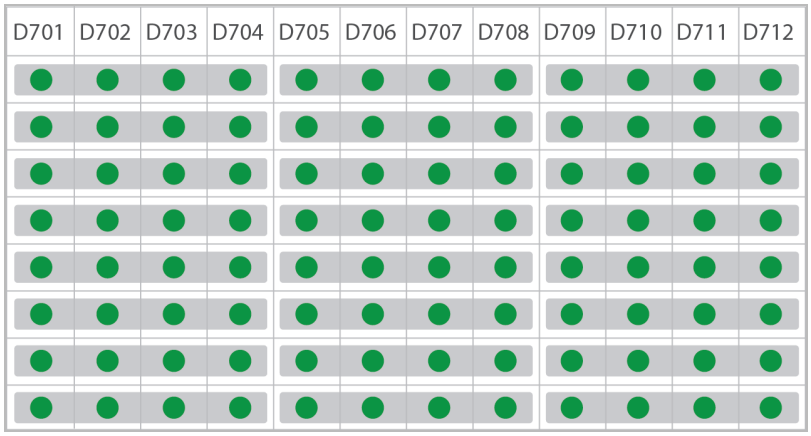
Single-Indexed, Two-Plex



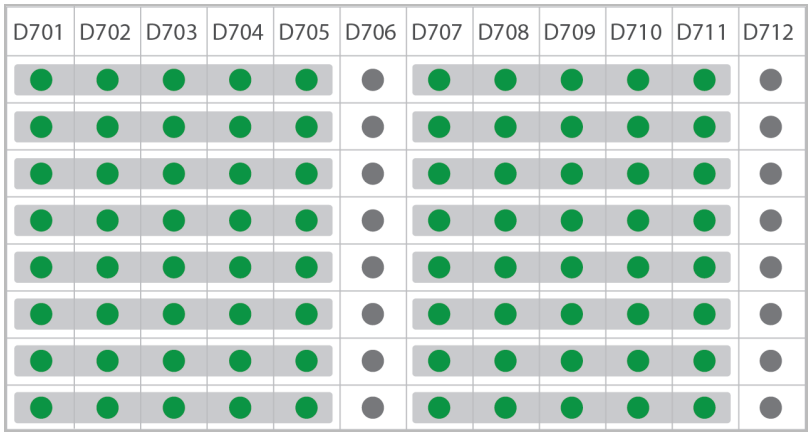
Single-Indexed, Three-Plex



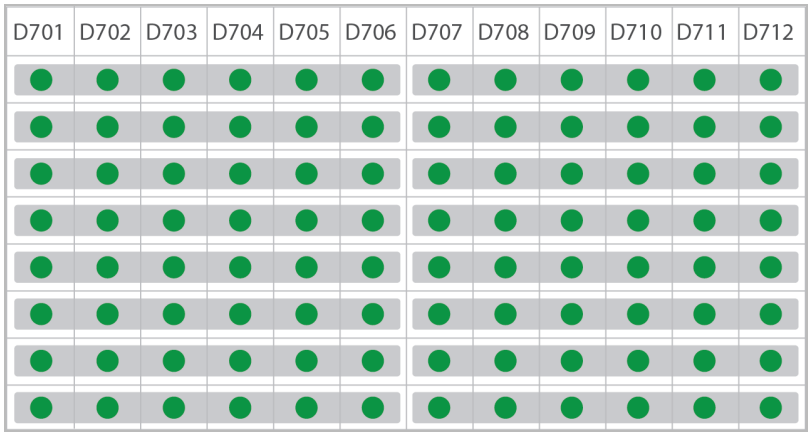
Single-Indexed, Four-Plex



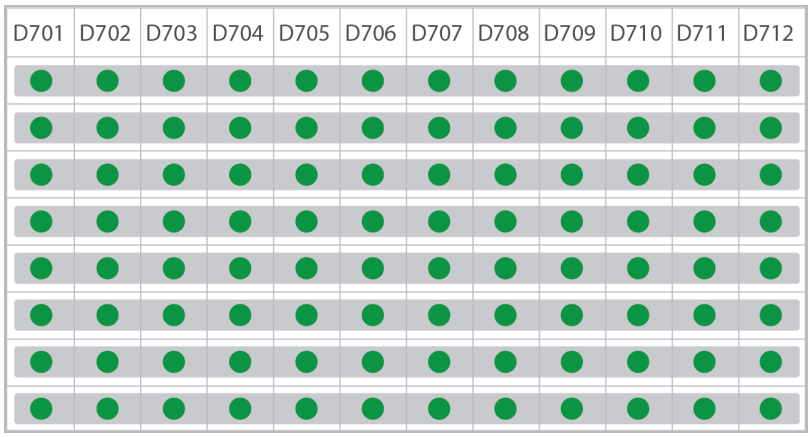
Single-Indexed, Five-Plex



Single-Indexed, Six-Plex



Single-Indexed, 12-Plex



Nextera Pooling Guidelines

This section details pooling strategies for index adapter tubes and index adapter plates for Nextera based index adapters (indexed by ligation).

Nextera DNA 96 CD Indexes (96 indexes, plated)

Kit Contents

Table 3 Index i7 Adapters

Index Name	Bases in Adapter	Bases for Sample Sheet	Type
H701	TCGCCTTA	TAAGGCGA	i7
H702	CTAGTACG	CGTACTAG	i7
H703	TTCTGCCT	AGGCAGAA	i7
H705	AGGAGTCC	GGACTCCT	i7
H706	CATGCCTA	TAGGCATG	i7
H707	GTAGAGAG	CTCTCTAC	i7
H710	CAGCCTCG	CGAGGCTG	i7
H711	TGCCTCTT	AAGAGGCA	i7
H712	TCCTCTAC	GTAGAGGA	i7
H714	TCATGAGC	GCTCATGA	i7
H720	AGGCTCCG	CGGAGCCT	i7
H723	GAGCGCTA	TAGCGCTC	i7

Plate Layout

Table 4 96 Plex, Dual Indexed Plate Layout

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

Low Plexity Guidelines

NovaSeq

The NovaSeq sequencer does not require color balanced indexes; any quantity (including 1-plex), or combination of indexes, is supported.

NOTE

As an exception, H705 cannot be used as the sole i7 index on a NovaSeq run.

Other Sequencers

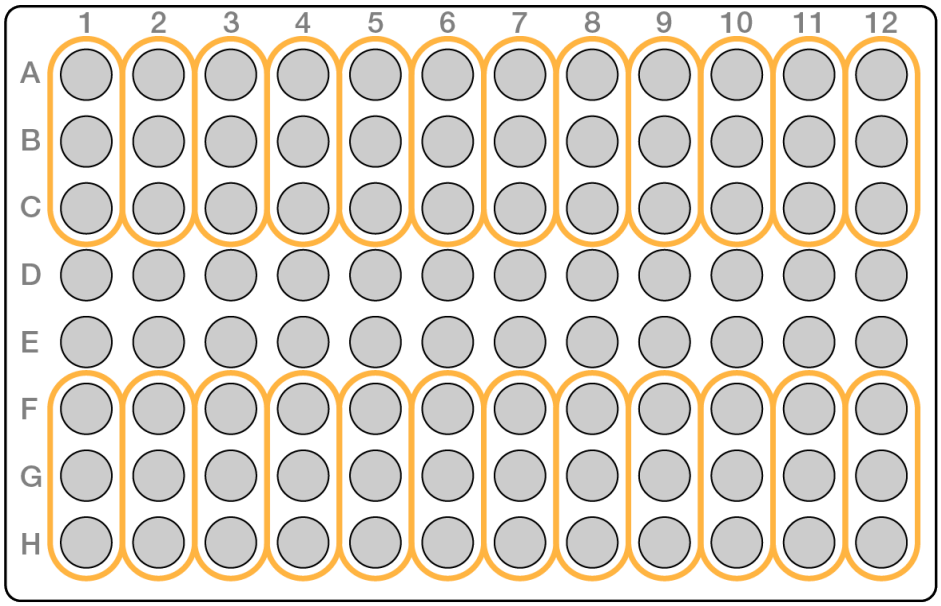
All pools listed are color-balanced (2-dye and 4-dye) on any Illumina sequencer.

NOTE

For more information on setting up a run in the MiniSeq Local Run Manager (LRM) software, see *Trim Adapters for Nextera DNA Flex Kits in MiniSeq LRM Quick Reference Card (document # 1000000040431)* .

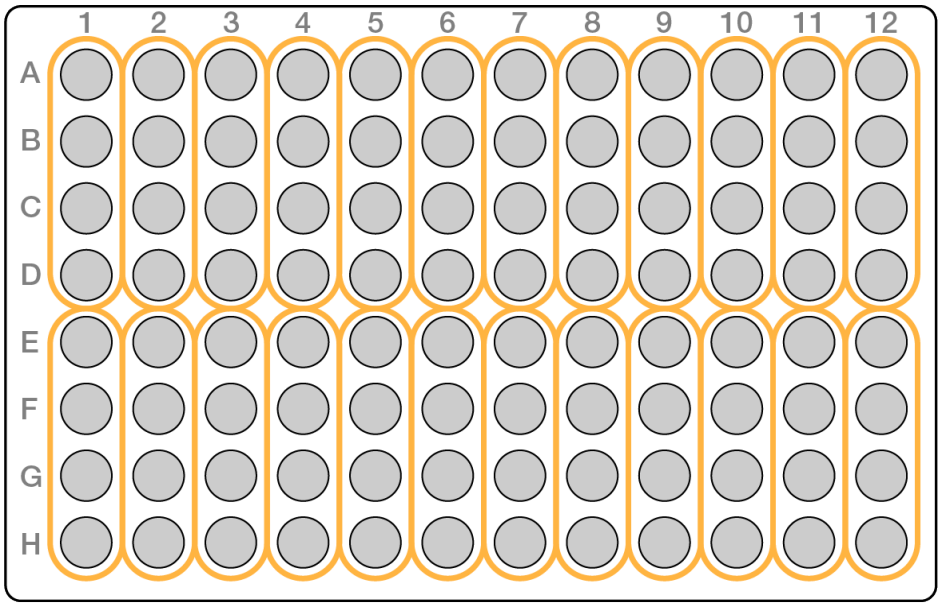
- 3-plex—use the first 3, or last 3, wells in a column
  - In 3-plex usage, rows D and E are not used.

Figure 2 3-plex plate layout example



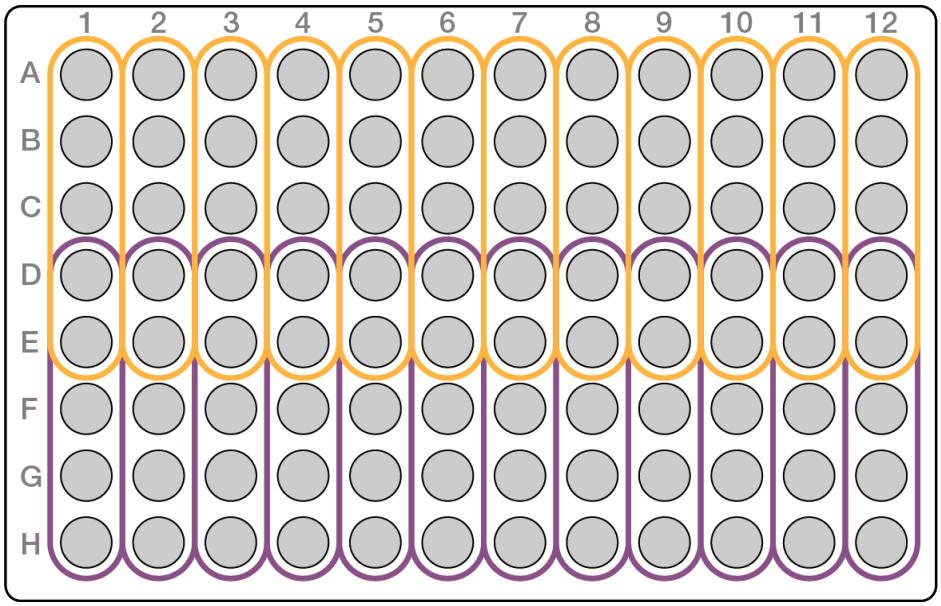
- 4-plex—use the first 4, or last 4, wells in a column

Figure 3 4-plex plate layout example



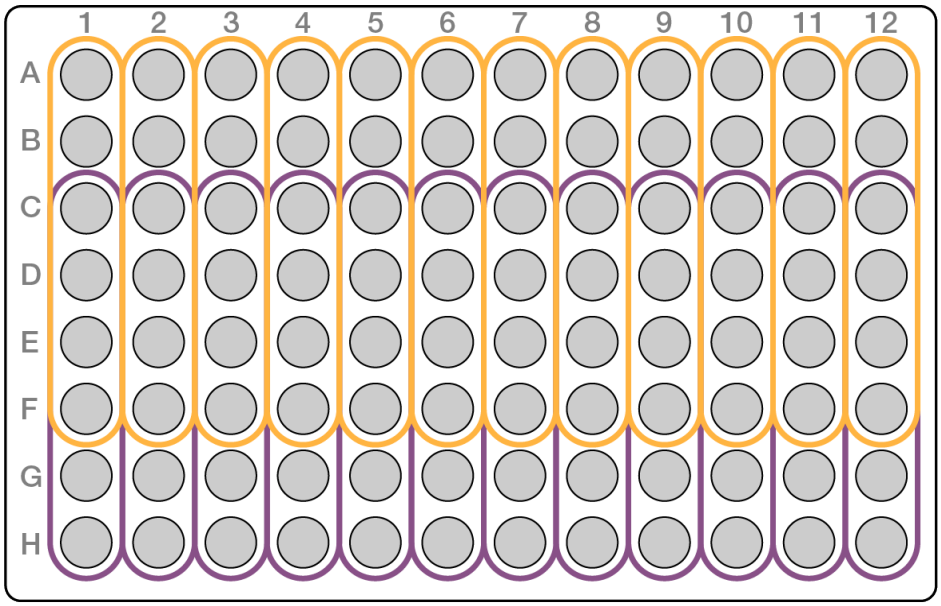
- **5-plex**—use the first 5 or last 5 wells in a column

Figure 4 5-plex plate layout example



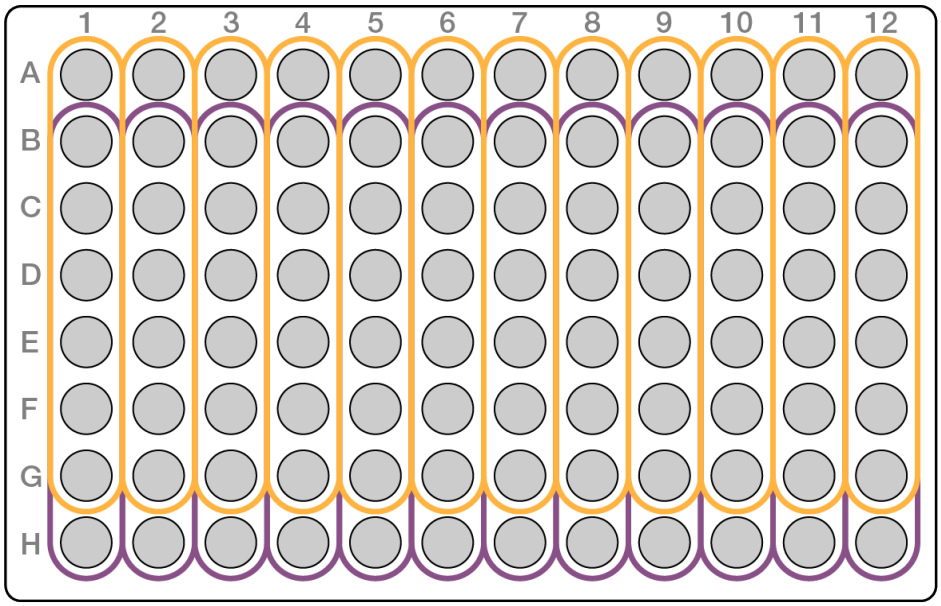
- **6-plex**—use the first 6 or last 6 wells in a column, or 2 from one column, and 4 from an adjacent column

Figure 5 6-plex plate layout example



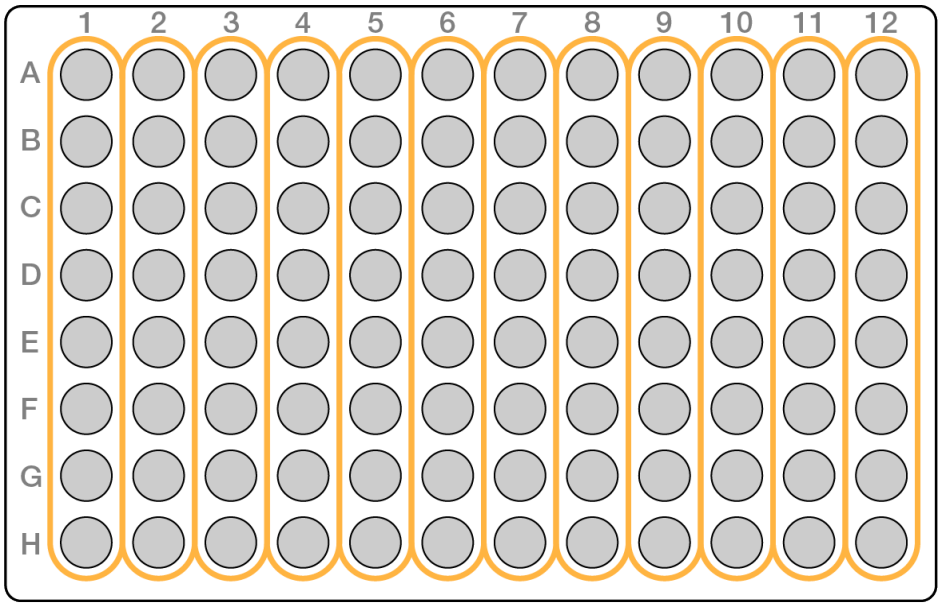
- **7-plex**—use the first 7, or last 7, wells in a column

Figure 6 7-plex plate layout example



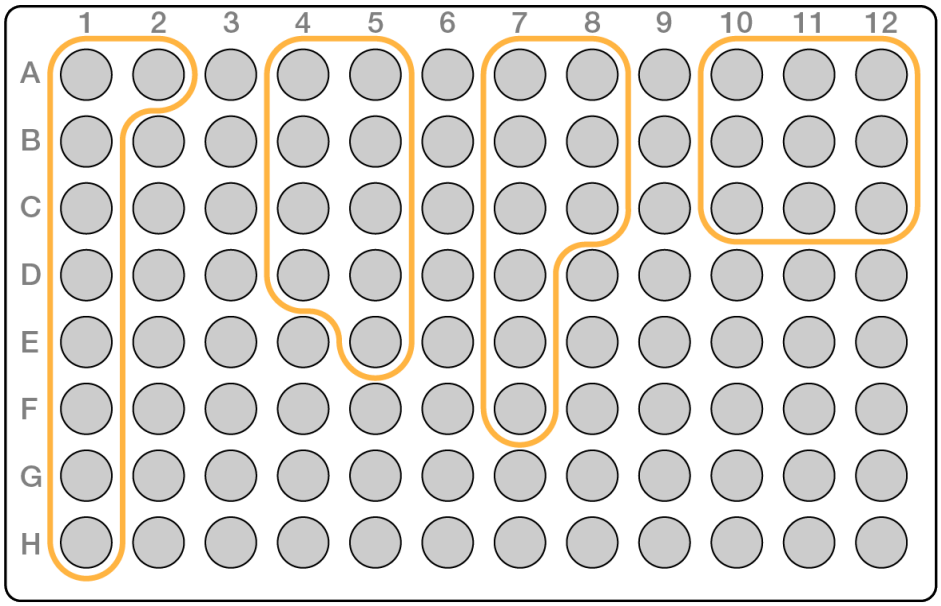
- **8-plex**—use a full column

Figure 7 8-plex plate layout example



- 9-plex+—use any collection of indexes containing color balanced pools (for example, A1-H1 + A2, or A4-D4 + A5-E5)

Figure 8 Various valid 9-plex plate layout examples



Nextera DNA 24 CD Indexes (24 indexes, tubed)

Kit Contents

Table 5 Index i7 Adapters

Index Name	Bases in Adapter	Bases for Sample Sheet	Type
H705	CGTCTAAT	ATTAGACG	i7
H706	TCGACTAG	CTAGTCGA	i7
H707	CCTAGAGT	ACTCTAGG	i7
H710	GCGTAAGA	TCTTACGC	i7
H711	TTATGCGA	TCGCATAA	i7
H714	TCGCCTTA	TAAGGCGA	i7

Table 6 Index i5 Adapters

Index Name	Bases in Adapter	Bases for Sample Sheet NovaSeq, MiSeq, HiSeq 2000/2500	Bases for Sample Sheet MiniSeq, NextSeq, HiSeq 3000/4000/X	Type
H503	TATCCTCT	TATCCTCT	AGAGGATA	i5
H505	GTAAGGAG	GTAAGGAG	CTCCTTAC	i5
H506	ACTGCATA	ACTGCATA	TATGCAGT	i5
H517	GCGTAAGA	GCGTAAGA	TCTTACGC	i5

Low Throughput Guidelines

NovaSeq

The NovaSeq sequencer does not require color balanced indexes; any quantity (including 1-plex), or combination of indexes, is supported.

NOTE

As an exception, H705 cannot be used as the sole i7 index on a NovaSeq run.

The pooling guidelines for other Illumina instruments are applicable.

Other Sequencers

All pools listed below are color-balanced (2-dye and 4-dye) on any Illumina sequencer.

- 2-plex is not supported
- 3-plex
  - i5—use H505, H506, and H517
  - i7—use either (H705, H706, H707) or (H710, H711, H714)
- 4-plex
  - i5—use all i5s

- i7—use either 3-plex pool + any i7
- Example: H503-H705, H505-H706, H506-H707, H517-H710

- 5-plex
  - i5—use the 4-plex pool + any i5
  - i7—use either 3-plex pool + any two i7s
  - Example: H503-H705, H505-H706, H506-H707, H517-H710, H503-H711
- 6-plex
  - i5—use the 4-plex pool + any two i5s OR two 3-plex pools
  - i7—use all six of the i7s ***or*** two 3-plex pools
  - Example: H503-H705, H505-H706, H506-H707, H517-H710, H503-H711, H505-H714
- 7-plex
  - i5—use the 4-plex pool + any three i5s OR two 3-plex pools + any i5
  - i7—use all six of the i7s + an additional i7 ***or*** two 3-plex pools + any i7
- 8-plex
  - i5—use all of the i5s (twice each)
  - i7—use all six of the i7s + any two additional i7s
- 9-plex+
  - Use as many valid pools in your set as possible; at a minimum, use at least one valid pool, plus remaining indexes.

NOTE

For more information on setting up a run in the MiniSeq Local Run Manager (LRM) software, see *Trim Adapters for Nextera DNA Flex Kits in MiniSeq LRM Quick Reference Card (document # 1000000040431)* .

AmpliSeq for Illumina Pooling Guidelines

This section details pooling strategies for index adapter plates for AmpliSeq for Illumina based index adapters.

AmpliSeq for Illumina Combinatorial Dual (CD) Indexes

AmpliSeq CD Indexes for Illumina are compatible with all Illumina sequencing systems. Indexes are intended for dual index sequencing.

NOTE

For pools that contain 8–96 samples, any index combination can be used.

The following table shows the plate layout of AmpliSeq CD indexes for Illumina.

Figure 9 Plate Layout for AmpliSeq CD indexes for Illumina

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

Index 1 (i7) Adapters

CAAGCAGAAGACGGCATACGAGA[i7]GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG

Table 7 Index i7 Adapters

Index Name	Bases for Sample Sheet
Q7005	GTGAATAT
Q7006	ACAGGCGC
Q7007	CATAGAGT
Q7008	TGCGAGAC
Q7015	TCTCTACT
Q7016	CTCTCGTC
Q7017	CCAAGTCT
Q7018	TTGGACTC
Q7023	GCAGAATT
Q7024	ATGAGGCC
Q7025	ACTAAGAT
Q7026	GTCGGAGC

Index 2 (i5) Adapters

AATGATACGGCGACCACCGAGATCTACAC[i5]TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG

Table 8 Index i5 Adapters

Index Name	i5 Bases for Sample Sheet MiSeq	i5 Bases for Sample Sheet MiniSeq, NextSeq
Q5001	AGCGCTAG	CTAGCGCT
Q5002	GATATCGA	TCGATATC
Q5007	ACATAGCG	CGCTATGT
Q5008	GTGCGATA	TATCGCAC
Q5009	CCAACAGA	TCTGTTGG
Q5010	TTGGTGAG	CTCACCAA
Q5013	AACCGCGG	CCGCGGTT
Q5014	GGTTATAA	TTATAACC

Adapter Trimming

The following sequence is needed for adapter trimming.

CTGTCTCTTATACACATCT

Low Plexity Guidelines

To achieve unique dual indexing, pool between two and eight samples according to the following guidelines.

NOTE

These guidelines are for 1–8 samples, there are no specific pooling requirements for pooling more than eight samples (up to 96).

For columns, the fundamental unit is two, however other combinations are supported. All combinations apply to any column on the plate.

The example shown in Figure 10 provides dual indexing strategies for columns when using AmpliSeq for Illumina CD indexes.

- Two in a column as shown in orange in the following combinations:
  - A-B
  - C-D
  - E-F
  - G-H
- Three in a column, as shown in gray in the following combinations:
  - A-C
  - D-F

  or
  - C-E
  - F-H
- Four in a column, as shown in purple in the following combinations:
  - A-D

- E-H

- Five in a column, as shown in blue in the following combinations:
  - A-E

  or
  - D-H
- Six in a column, as shown in green in the following combinations:
  - A-F

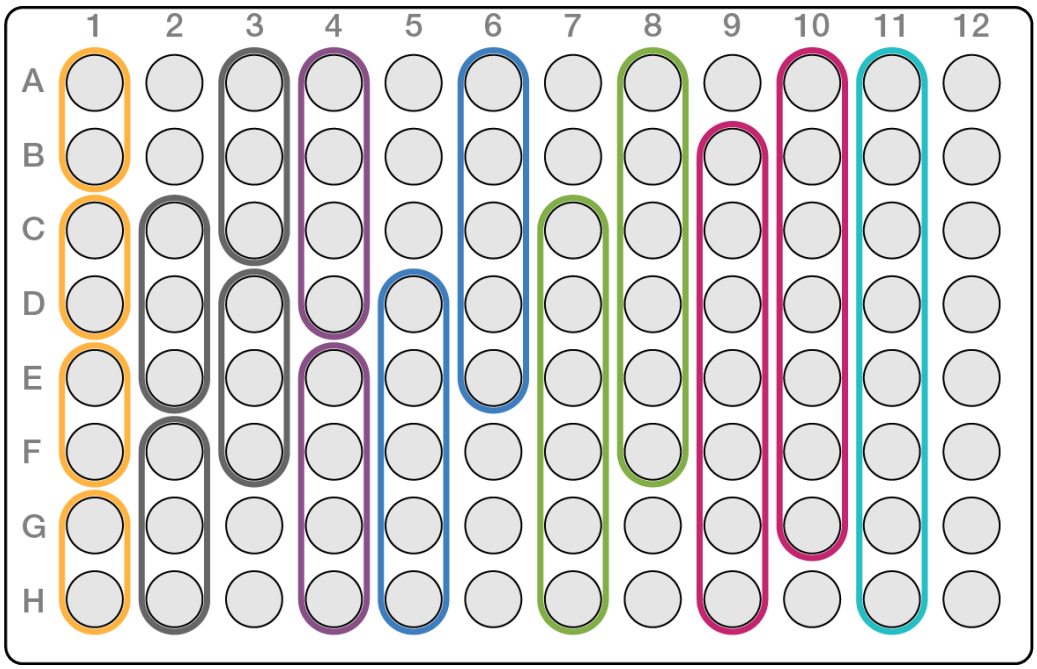
  or
  - C-H
- Seven in a column, as shown in pink in the following combinations:
  - A-G

  or
  - B-H
- Eight in a column, as shown in teal.

NOTE

All eight combinations in any column are unique index combinations.

Figure 10 Example of Column Pooling Guidelines for Low Plexity



For rows, the fundamental unit is three, however any set of pools can be in a row as long as they are contained between columns 1-6 or columns 7-12. All combinations apply to any row on the plate.

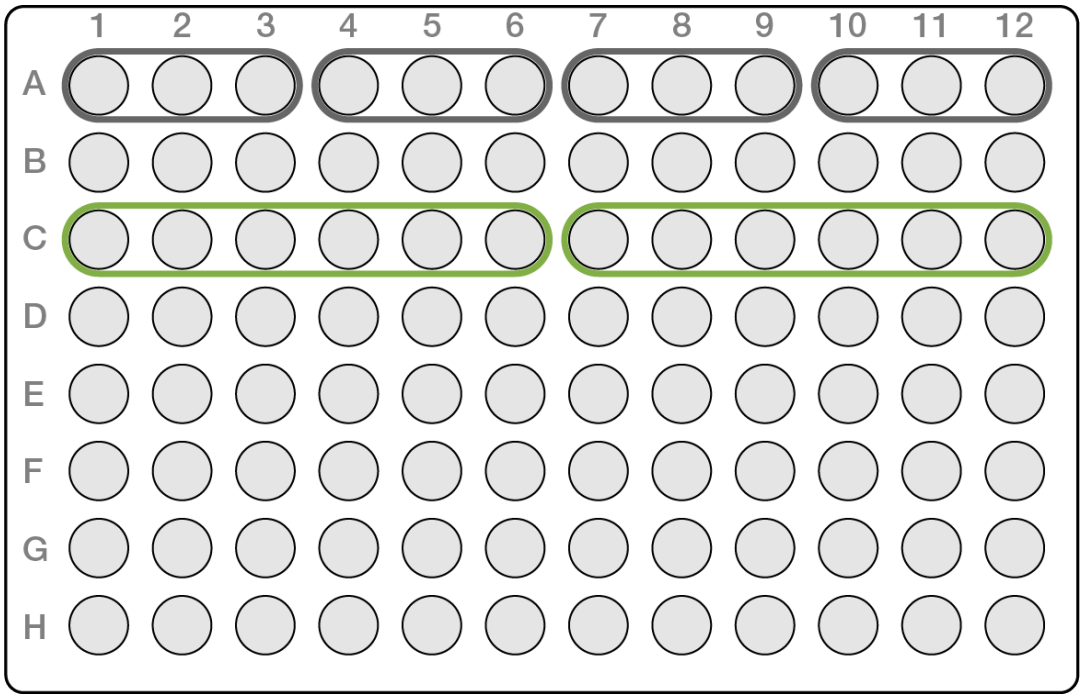
The example shown in Figure 11 provides dual indexing strategies for rows when using AmpliSeq for Illumina CD indexes.

- Three in a row, as shown in gray in the following combinations:
  - 1-3
  - 4-6
  - 7-9

- 10-12

- Six in a row, as shown in green in the following combinations:
  - 1-6
  - 7-12

Figure 11 Example of Row Pooling Guidelines for Low Plexity



Next Steps

When library prep is complete, you are ready to begin sequencing.

For information on setting up an indexed sequencing run, see the system guide for your sequencing system.

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Website: www.illumina.com
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Safety data sheets (SDSs)—Available on the Illumina website at support.illumina.com/sds.html.

Product documentation—Available for download in PDF from the Illumina website. Go to support.illumina.com, select a product, then select **Documentation & Literature**.

Revision History

Document	Date	Description of Change
Document #1000000041074 v02	May 2018	Added One-channel Sequencing section for iSeq.
Document #1000000041074 v01	January 2018	Added section for AmpliSeq for Illumina pooling guidelines. Incorporated Nextera DNA Flex pooling guidelines.
Document #1000000041074 v00	October 2017	Initial release.

Document # 1000000041074 v02

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