10x Genomics Sequencing Handbook

Introduction

10x Genomics Chromium and Visium workflows generate sequencing-ready libraries that include assay-specific dsDNA insert and barcodes. These libraries are standard Illumina paired-end constructs. They begin with P5, end with P7, and have i5 and i7 sample indices. Additionally, they have Read 1 and Read 2 standard Illumina sequencing primer sites (TruSeq or Nextera). An example configuration of a generic dual index sequencing-ready 10x Genomics library is shown below.

This document describes best practices and provides guidance for sequencing 10x Genomics libraries to maximize data output. General guidance for library storage, quality control (QC), pooling, and loading along with sequencing run recommendations are provided in this handbook. For additional product-specific information, consult the relevant User Guides on the 10x Genomics Support website.

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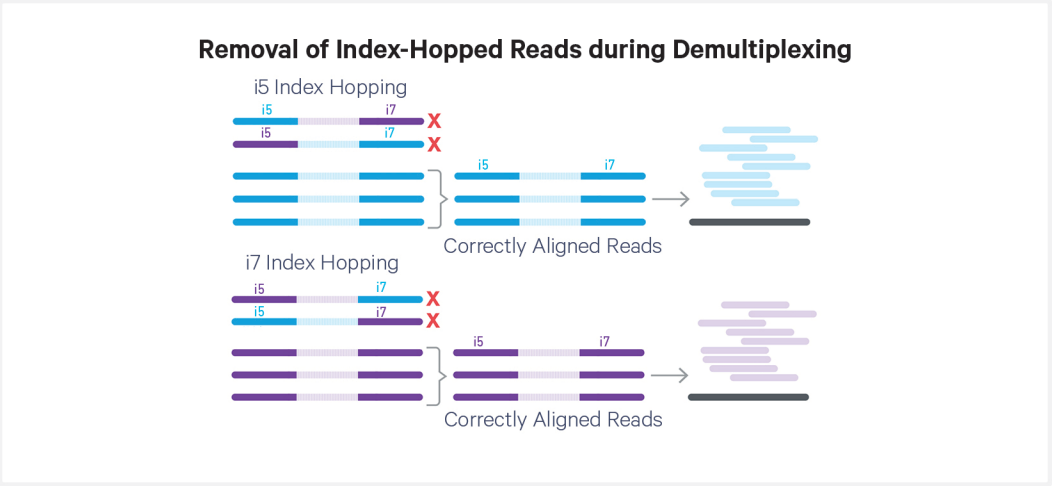
1. Library Storage & QC

1.0 Library Storage

Final 10x Genomics libraries can be stored at **4°C** for up to **72 h** or at **–20°C** for long-term storage. Libraries should not be pooled prior to storage as storing pooled libraries may result in elevated index hopping. Index hopping is a phenomenon that occurs during cluster generation of libraries on the sequencing flow cell and can result in incorrect assignment of insert reads to a sample. Dual indexing mitigates index hopping during demultiplexing by enabling the computational identification of reads that contain an expected pair of unique i5 and i7 index sequences.



If storing libraries for more than a month, requantifying the library before sequencing using the KAPA Library Quantification Kit is recommended.

1.1 Library Size QC

Bioanalyzer, TapeStation, LabChip, or Fragment Analyzer electrophoresis instruments are recommended to check fragment length distribution and to determine the average fragment size that will be used as the library size. Confirm that the fragment length distribution for a generated library is similar to the representative QC trace provided in the User Guide. If additional lower molecular weight or higher molecular weight fragments are observed, contact 10x Genomics Support for assistance for determining if additional cleanup steps are needed prior to sequencing. For example, lower molecular weight fragments in Gene Expression libraries may be from adaptors that were not removed via the cleanup step during library construction. If these are not

removed, adaptor dimers will generate sequencing data that will not map to the transcriptome, reducing overall usable yield.

1.2 Library Quantification

Accurate library quantification is important to ensure uniform sample pooling and optimal sequencing yield. The most common cause of under and overclustering is inaccurate library quantification. Ensure that the quantified library is diluted to the Quantitative Range (ng/µl) to ensure accurate concentration measurements.

The KAPA Library Quantification Kit for Illumina Platforms is recommended for quantifying 10x Genomics libraries. A protocol can be found in this Sequencing Handbook and the Appendix of each User Guide. The KAPA kit is recommended because it only quantifies sequenceable library molecules, such as fragments that contain both the Illumina P5 and P7 sequences. Other quantification methods (such as Bioanalyzer and Qubit) measure all nucleic acids, including incomplete library molecules. Compared to the Bioanalyzer measurements, the KAPA kit detects diverse DNA fragments with similar efficiency, making it ideal for accurate quantification for sequencing.

If using a quantification method other than KAPA, perform a comparison between the KAPA qPCR and the quantification method of choice before performing a sequencing run. When performing these comparisons, keep instruments and reagents consistent as results may vary.

Consider the following scenario:

- A library quantified by KAPA is measured to be 300 nM.
- The same library quantified by Qubit is measured at 200 nM.

Loading this library at the recommended concentration based on the Qubit quantification would result in loading 50% more library vs. using the measured concentration from KAPA. Overloaded sequencing runs may lead to overclustering on the flow cell. Overloading and overclustering can lead to poor run performance, lower Q30 scores, possible introduction of sequencing artifacts, and lower total data output (i.e., fewer usable reads) as compared to optimally loaded runs.

1.3 Post Library Construction Quantification using KAPA qPCR

a. Thaw KAPA Library Quantification Kit for Illumina Platforms.

b. Dilute **2 µl** of sample with deionized water to appropriate dilutions that fall within the linear detection range of the KAPA Library Quantification Kit for Illumina Platforms. (For more accurate quantification, make dilutions in duplicate).

c. Make enough Quantification Master Mix for the DNA dilutions per sample and the DNA Standards (plus 10% excess) using the guidance for 1 reaction volume below.

Quantification Master Mix	1X (µl)
SYBR Fast Master Mix + Primer	12
Water	4
Total	**16**

d. Dispense **16 µl** Quantification Master Mix for sample dilutions and DNA Standards into a 96 well PCR plate.

e. Add **4 µl** sample dilutions and **4 µl** DNA Standards to appropriate wells. Centrifuge briefly.

f. Incubate in a thermal cycler with the following protocol.

Step	Temperature	Run Time
1	95°C	00:03:00
2	95°C	00:00:05
3	67°C Read Signal	00:00:30
4	Go to Step 2, 29X (Total 30 cycles)	

g. Follow the manufacturer's recommendations for qPCR-based quantification, as well as additional best practices from Illumina. For library quantification for sequencer clustering, determine the concentration based on library size derived from the Bioanalyzer, TapeStation, LabChip, or Fragment Analyzer trace.

2. Library Pooling

2.0 Library Pooling Overview

Pooling 10x Genomics libraries for sequencing will depend on factors such as library type, target cell number (Chromium libraries), percentage of capture area covered by tissue (Visium libraries), and desired read depth. Accurate quantification of final libraries is critical for obtaining the desired pool representation. See 1.2 Library Quantification on page 4, the KAPA Kit User Guide, and the 10x Genomics User Guides for additional information. The KAPA kit will yield a concentration in nM for each library.

2.1 Unique and Diverse Indices

To ensure that each library is correctly assigned to an input sample, select a unique index combination for each library type when pooling them. Samples using the same sample index should not be pooled together, or run on the same flow cell lane, as this would not enable correct sample demultiplexing.

Index plates

10x Genomics index plates contain a defined index setup (single or dual indexes) and specific sequencing primer binding sites in the adapters. Refer to the relevant User Guide to select the appropriate index plate based on the assay, version and library type.

The list of sequences in 10x Genomics index plates can be found here:

- PN-1000215/PN-3000431: Dual Index Kit **TT**, Set A: CSV | JSON
- PN-1000251/PN-3000511: Dual Index Kit **TS**, Set A: CSV | JSON
- PN-1000242/PN-3000483: Dual Index Kit **NT**, Set A: CSV | JSON
- PN-1000250/PN-3000510: Dual Index Kit **TN**, Set A: CSV | JSON
- PN-1000243/PN-3000482: Dual Index Kit **NN**, Set A: CSV | JSON
- PN-1000212/PN-3000427: Single Index Kit **N** Set A: CSV | JSON

Two part numbers are listed for each index plate, one of which is the kit-level part number and one of which is the plate-level part number.

The list of sequences contain both the forward and reverse complement sequence for the i5 index. Illumina sequencers read the i7 index in the forward orientation, whereas the i5 index is read in either the forward or reverse

complement orientation depending on the instrument and chemistry. Whether you use the forward or reverse complement sequence also depends on the software that is being used. Refer to Illumina's documentation: Guidelines for reverse complementing i5 sequences for demultiplexing.

The types of oligos present in each well of the plate differ between single index and dual index plates. For dual index plates, each well is a mix of 2 oligonucleotides, one of which contains a unique i7 sample index and one of which contains a unique i5 sample index. For single index plates, each well is a mix of 4 oligonucleotides, each of which contains a unique i7 sample index. Using 4 oligos per sample index ensures that the i7 index read is balanced across all 4 bases during sequencing.

Each sample index in each of the 96-well sample index plates is unique. For 10x Genomics dual index plates, each index sequence differs by at least 3 bp from every other index sequence from every dual index plate (TT, TS, NT, TN, and NN plates).

Index combinations for low plex pools

Color balance in the index read is important for optimal run quality and demultiplexing results. For more information, consult the following Illumina resources:

- What is nucleotide diversity and why is it important?
- Index color balancing for XLEAP SBS reagents on the NextSeq 1000/2000 and NovaSeq X/X Plus
- Index color balancing for the NovaSeq 6000 system
- Index color balancing for the NextSeq 1000/2000 system using standard SBS reagents

The tables below contain suggested index combinations when sequencing low-plex pools on Illumina 2-channel systems.

Criteria used

For 2-channel systems with **XLEAP-SBS** chemistry: *NovaSeq X Series, NextSeq 1000/2000 XLEAP-SBS*

- Illumina recommends selecting index combinations that allow for signals in both channels for every cycle. If this is not possible, Illumina recommends selecting index combinations that have signals in at least the green channel for every cycle.

- The suggested 3-plex and 4-plex pools in the tables below have signals in both the blue channel (**C or A**) and the green channel (**C or T**) for every cycle.
- The suggested 2-plex pools in the tables below have signals in at least the green channel (**C or T**) for every cycle.

For 2-channel systems with **pre-XLEAP SBS** chemistry: *NovaSeq 6000, NextSeq 2000 standard SBS*

- Illumina recommends selecting index combinations that allow for signal in both channels for every cycle. If this is not possible, Illumina recommends selecting index combinations that have signal in at least one channel for every cycle.
- The suggested 3-plex and 4-plex pools in the tables below have signal in both the green channel (**A or T**) and the red/blue channel (**A or C**) for every cycle. (Blue channel is for NextSeq 2000 standard SBS and red channel is for NovaSeq 6000).
- The suggested 2-plex pools in the tables below have signal in at least one channel for every cycle (i.e., at least one A, T or C).

Consideration

The index combinations in the tables below are suggested theoretical combinations to optimize color balance. The lists are non-overlapping within each index plate and plex type. The lists are not exhaustive and may not cover all possible index combinations. Not all index combinations have been tested. 10x Genomics has not performed extensive testing of low plex pools.

The tables below are for 2-channel systems. 10x Genomics has not generated a list of suggested index combinations for other Illumina instruments.

The tables below are for 10x Genomics libraries prepared using Dual Index Plates TT, TS, or TN. 10x Genomics has not generated a list of suggested low plex index combinations for other 10x Genomics Dual Index Plates. Lists are not generated for 10x Genomics Single Index plates (e.g. Single Index Plate N used for Epi ATAC libraries) as individual wells contain a mixture of 4 oligonucleotides and thus have sufficient diversity.

For 5-plex or higher, the provided 4-plex lists can be used as a starting point and additional indexes can be added.

For optimal demultiplexing results, accurate library quantification and appropriate loading concentrations are also critical, as discussed in this article.

Recommended pooling for 2-channel systems with XLEAP-SBS chemistry *(e.g. NovaSeq X Series, NextSeq 2000 XLEAP-SBS)*			
	SI-TT Plate	**SI-TS Plate**	**SI-TN Plate**
2-plex	A5, B9	A11, C8	B4, E9
	B2, B10	A8, C5	C7, H3
	C1, F8	B2, B7	D1, F4
	D5, H6	D1, H2	E1, G1
	F6, H3	F7, E4	E6, G7
		G4, H4	F1, H1
		H9, G3	
	SI-TT Plate	**SI-TS Plate**	**SI-TN Plate**
3-plex	A3, C9, H11	A2, F10, G11	A1, B3, C5
	A5, B9, F5	A3, D5, E9	A4, E8, E10
	A6, E9, G3	A4, D1, E1	A8, F12, H3
	A10, C3, E4	A6, H5, H3	A9, B8, G10
	A11, G10, H5	A7, D8, F5	A10, F2, H9
	A12, C2, D7	A9, G10, G5	B4, E6, F3
	B1, G11, H12	A11, B1, F4	B5, G7, H7
	B2, B10, G9	A12, B8, G3	B10, D4, D7
	B3, B7, E12	B2, F7, G4	B12, B1, D3
	B4, D5, H6	B3, C11, H6	C2, C12, F11
	B5, D8, C10	B5, E4, E10	C4, E5, H1
	B8, F11, F1	B6, E2, G1	C7, E7, F8
	B11, E3, F12	B7, E7, F2	C10, F10, H2
	B12, C11, H3	B10, D9, F11	D1, D8, F4
	C4, F6, F7	B11, H1, H11	D2, F7, H5
	D1, G12, H2	C3, F9, E12	D12, F6, H6
	D11, F4, H7	C6, B12, D2	E1, F9, G2
	D6, D9, H8	D4, E8, H4	E11, G1, G12
	E7, F9, G2	D6, E5, H8	
	F3, F8, F10	C12, H9, H2	

Recommended pooling for 2-channel systems with XLEAP-SBS chemistry *(e.g. NovaSeq X Series, NextSeq 2000 XLEAP-SBS)*			
	SI-TT Plate	**SI-TS Plate**	**SI-TN Plate**
4-plex	A1, C9, E5, H7	A1, B2, C1, E9	A1, F3, G12, H4
	A2, B7, E9, G3	A2, B5, C11, G12	A2, D2, E4, F7
	A3, D1, G11, H1	A3, C7, G4, G6	A3, A9, G10, H8
	A4, B4, C10, F2	A4, F9, F2, H8	A4, A8, B5, F1
	A5, A12, D12, E2	A5, A6, D1, D5	A5, C8, B7, C4
	A6, B3, B6, C3	A7, E7, F4, H7	A6, C2, C12, G6
	A7, C7, A11, E3	A8, F7, F3, H1	A7, B4, B3, C6
	A8, D7, G1, H10	A9, C8, E8, G10	A10, C1, D8, E3
	A9, B10, F1, F10	A10, A12, F8, G11	A11, D11, E9, H10
	A10, G5, G12, H2	A11, G5, G8, H6	A12, C5, D9, G3
	B2, B1, G8, H12	B1, D4, E10, F6	B1, C11, C3, H9
	B5, B11, F4, H11	B3, B10, D12, G1	B2, D4, D7, D10
	B8, C11, E8, H6	B6, C3, F1, H3	B6, F12, H11, H12
	B9, E4, F8, H8	B7, D3, D6, E1	B8, F10, E6, F6
	B12, D4, E1, H3	B8, C12, G2, H12	B9, D1, D12, F8
	C1, D2, G4, H5	B11, D2, E3, G7	B11, E7, H1, H5
	C2, D10, F5, H4	C2, D11, H9, G3	B12, F2, G2, H7
	C4, E10, F3, G10	C4, C5, H10, F12	C7, E1, D6, H6
	C5, E6, F7, G7	C6, B4, C10, E12	C10, G7, F9, G11
	C6, F11, E7, G9	D7, D10, E11, H2	D3, E10, E11, F11
	D5, E11, F12, H9	D9, D8, E6, H5	D5, C9, G9, H3
	D8, C8, D6, G2	E5, F5, F11, H11	E2, E5, G5, H2
	C12, D11, F9, F6	F10, E4, G9, H4	E8, G1, F5, G8

Recommended pooling for 2-channel systems with non-XLEAP SBS chemistry *(e.g. NovaSeq 6000, NextSeq 2000 standard SBS)*			
	SI-TT Plate	**SI-TS Plate**	**SI-TN Plate**
2-plex	A1,A12	A10, E4	A1, C1
	A10, F12	A11, A12	A10, E2
	A11, D4	A2, C1	A11, F2
	A2, H8	A3, B11	A12, C8
	A3, F2	A4, D7	A2, B4
	A4, E10	A5, B8	A3, G9
	A5, D11	A6, H9	A4, E12
	A6, B8	A7, F5	A5, D9
	A7, H12	A8, H5	A6, E10
	A8, C8	A9, B5	A7, B8
	B1, H10	B1, E7	A8, H9
	B10, F5	B12, D6	A9, B12
	B11, C11	B2, D8	B1, F6
	B12, H3	B4, H11	B10, H7
	B2, G6	B7, C10	B11, C5
	B3, G11	B9, F1	B2, H1
	B4, F4	C11, H12	B3, C10
	B5, D6	C12, E6	B6, G3
	B6, D3	C3, G12	B7, C7
	B7, H4	C5, G8	B9, F7
	B9, F7	C6, E8	C11, G7
	C1, G10	C7, H7	C12, H5
	C10, E7	C8, E2	C2, E9
	C12, H5	C9, D9	C3, G6
	C2, C9	D1, F3	C4, E4
	C3, C4	D10, D11	C6, G10
	C5, F3	D12, H2	C9, D10
	C6, H11	D2, C4	D1, F8
	C7, F11	D3, G7	D12, E11
	D1, H2	D4, G6	D3, F5
	D10, G2	D5, E5	D4, D2
	D5, G12	E1, E10	D5, G8
	D7, E3	E11, G9	D6, H10
	D8, D12	E12, H3	D7, E3
	D9, G7	E3, H1	D8, H2
	E1, H7	E9, F12	E1, G12
	E11, H9	F10, B10	E5, F4

Recommended pooling for 2-channel systems with non-XLEAP SBS chemistry *(e.g. NovaSeq 6000, NextSeq 2000 standard SBS)*			
	SI-TT Plate	**SI-TS Plate**	**SI-TN Plate**
2-plex	E12, E8	F11, G2	E6, F11
	E2, F8	F2, F8	E7, G2
	E4, F10	F4, G1	E8, H11
	E5, H6	F6, H8	F1, H6
	E6, E9	F7, H6	F10, D11
	F6, G5	F9, G5	F3, F12
	F9, F1	G3, H4	F9, G11
	G1, G9	G4, G11	G1, H12
	G3, G8	H10, G10	G4, H8
	G4, H1		G5, H3
	SI-TT Plate	**SI-TS Plate**	**SI-TN Plate**
3-plex	A1, G6, H1	A4, B12, D3	A2, D2, F7
	A3, C12, E9	A5, B7, F11	A3, H4, H6
	A4, B10, E1	A7, B10, G7	A4, D1, G1
	A5, F9, F1	A9, D12, E6	A7, D7, H3
	A9, D1, G12	A10, G6, H6	A10, B12, B1
	A10, C3, F4	A11, F7, H1	A12, B6, D10
	A11, D10, G8	A12, B4, G4	B3, B7, C9
	A12, B8, H9	B1, E1, H9	B8, D5, E6
	B1, G9, G11	B2, D7, G12	B9, G7, F8
	B2, E2, F8	B3, D5, E12	B10, C4, H1
	B4, G3, G4	B5, D8, H3	C1, D4, H2
	B5, B6, H11	B8, C3, H8	C2, C12, E3
	B7, D11, F11	C1, G3, G11	C5, E9, F5
	B9, A8, H5	C2, C4, D6	C6, D9, G12
	B11, E3, G7	C6, B11, E9	C11, G4, H9
	B12, D12, E11	C8, F9, E5	D3, G10, G11
	C2, F5, H12	C10, D1, G1	D8, E11, G3
	C4, D2, F10	C11, E2, F12	D11, F3, H12
	C6, C10, E8	C12, D10, H11	E1, F2, F12
	C7, F3, F7	D2, D11, G2	E5, F6, F11
	C9, E5, E6	E8, F3, G10	E7, G8, H10
	C11, H6, H7	F10, E4, G8	F9, G6, H7
	E4, E7, H8	H2, H4, H7	F10, E8, G5
	D8, E10, G5		

Recommended pooling for 2-channel systems with non-XLEAP SBS chemistry *(e.g. NovaSeq 6000, NextSeq 2000 standard SBS)*			
	SI-TT Plate	**SI-TS Plate**	**SI-TN Plate**
4-plex	A1, A2, G6, H1	A1, B10, G3, G6	A1, F5, G6, H9
	A3, B5, C10, F9	A2, B5, B7, C3	A2, E3, F11, G12
	A5, A9, A11, D1	A3, F1, D5, H6	A3, D8, G7, H6
	A6, D9, D11, H4	A4, D4, H2, H5	A4, F10, D11, H2
	A8, E1, E8, H7	A5, C1, D6, F9	A5, B3, E1, D1
	A10, D8, C6, E7	A6, D12, G11, H3	A6, C12, D3, F2
	A12, C2, E3, F5	A7, A9, E11, F8	A7, B9, D6, E8
	B1, C11, F8, H9	A10, D9, E8, F2	A8, C4, E4, E11
	B2, A4, C12, E11	A11, B12, C12, E5	A10, C1, E6, F7
	B3, B6, D7, F12	A12, B6, B9, G4	A11, C8, D12, H5
	B4, F6, F7, G7	B1, F6, F11, F12	A12, C10, G10, G11
	B7, E12, G5, H12	B2, H10, F4, H4	B1, B7, B11, D7
	B8, G3, H3, G11	B4, F10, E9, H7	B2, B6, C2, D4
	B9, A7, E9, H11	B11, C5, D10, E12	B4, C3, E2, F8
	B10, D3, G4, G8	C2, C11, G2, H12	B5, E5, F6, F4
	B11, D2, E10, G2	C4, F3, G8, G7	B8, B10, H4, H11
	B12, D4, E2, G1	C6, B3, G9, H1	C5, E7, F1, G8
	C1, C9, D10, H8	C7, C9, F5, G1	C6, C7, D2, E9
	C3, F11, E6, G10	C10, D1, G5, H11	C9, F9, G2, G9
	C5, D6, H10, H5	D2, E4, G10, H8	C11, G1, F12, G5
	C7, C4, G12, H2	D3, D11, E2, H9	D10, H1, H3, H12
	C8, E4, F3, F10	E7, D7, E1, G12	D9, E12, G4, H8
	D5, D12, F1, F4	F7, D8, E6, E10	F3, G3, H7, H10
	E5, F2, G9, H6		

2.2 Library Pooling Calculations

To calculate the necessary volumes for library pooling, gather the following info:

- Average Library Size (bp): Determined by Bioanalyzer (or TapeStation / LabChip / Fragment Analyzer)
- Library Concentration (nM): Determined by qPCR
- Target Volume: can be adjusted up or down to make sure the 'final transfer volume' is not too high (e.g. use up too much of the final library); or too low (pipette volume too low / inaccurate).

- Target Pool Concentration: This is the expected concentration of the final pool. 10x Genomics highly recommends re-quantifying the pool prior to sequencing. If the expected pool concentration is significantly different from the observed concentration (by qPCR) this could indicate a problem with pooling, individual library quantification, or final library re-quantification.

Information necessary for Chromium libraries:

- Cell Number Expected: How many cells expected for each library (e.g. targeted cell recovery)
- Reads per Cell: Outlined in each User Guide and in 3.4 Sequencing Parameters on page 24. Different sequencing depths may be necessary depending on the experimental design and cell types of interest

Information necessary for Visium libraries:

- Percentage of Capture Area covered by tissue (Visium HD, Visium HD 3') or number of tissue-covered spots (Visium v1, Visium v2)
- Read Depth Requirements: Outlined in each User Guide and in 3.4 Sequencing Parameters on page 24.

Input the information above into these example 10x Genomics worksheets for 8 reactions or 32 reactions to determine necessary input volumes.

Alternatively, each library can be diluted to the same nM (by qPCR) and pooled in equal molar ratios.

2.3 Pooling Verification

To verify correct library pooling, sequencing QC may be performed on a small output sequencer or as a spike-in. Refer to Illumina's recommendations for assessing library representation in a pool using iSeq for pooling QC and for final pool quantification. Select a sequencer with comparable clustering chemistry when possible. Sequencing QC will help verify the library quality, the sample representation in the pool, and confirm that the sample indices are represented in the expected ratios before proceeding with the final sequencing run.

2.4 Pooling Compatibility

10x Genomics recommends pooling Feature Barcode libraries with their respective Gene Expression libraries to increase nucleotide diversity. For example, pool Single Cell 5' v3 CRISPR Screening libraries with Single Cell 5' v3 Gene Expression libraries. Refer to the relevant User Guide for recommended pooling ratios based on the sequencing read depth requirements for each library type.

It may also be possible to pool libraries together from different 10x Genomics assays. This is low risk if the libraries share the same sequencing configurations and have similar recommended loading concentrations. 10x Genomics has not extensively tested all possible pooling configurations across all sequencing platforms. When pooling different library types, consider that short library molecules cluster more efficiently. This different clustering efficiency is also dependent on instrument type; therefore, the optimal pooling ratio will have to be determined empirically. Optimization of loading concentrations and % PhiX input may also be required depending on the pooled library types and sequencing platform.

The following table provides guidance for pooling libraries from different 10x Genomics assays.

Product	
Universal 3' Gene Expression	It is low risk to pool these library types together for sequencing as they share the same sequencing configuration (R1: 28, i7: 10, i5:10, R2:90) * 5' v2/HT libraries require 26 cycles for Read 1 if sequenced alone. If pooled with other 10x library types, Read 1 should be increased to 28 cycles. ** The pooling guidelines in this table apply to Flex libraries when sequencing using Read 2 Sequencing Configuration (R1: 28, i7: 10, i5:10, R2:90). If sequencing Flex libraries using Read 1 Sequencing Configuration (e.g. R1: 54, i7:10, i5:10, R2:50 for Flex v2 Multiplex libraries), pooling with other 10x library types is not recommended as it is untested. *** Flex Multiplex libraries require higher PhiX spike-in (5% or 10% PhiX depending on sequencing platform). However, Flex Multiplex libraries can be mixed with 1% PhiX if the pool comprises at least 20% Single Cell 3' or Single Cell 5' Gene Expression libraries. A higher PhiX is not required in this situation because the Single Cell 3' or Single Cell 5' Gene Expression libraries contribute adequate diversity to the Probe Barcode of Flex Multiplex libraries. See CG000677 for further information.
Single Cell 3' v4 Gene Expression	
Single Cell 3' v4 Cell Surface Protein	
Single Cell 3' v3.1/HT Gene Expression	
Single Cell 3' v3.1/HT Cell Surface Protein	
Single Cell 3' v3.1/HT CRISPR	
Single Cell 3' v3.1/HT CellPlex	
Universal 5' Gene Expression	
Single Cell 5' v3 Gene Expression	
Single Cell 5' v3 V(D)J	
Single Cell 5' v3 Cell Surface Protein	
Single Cell 5' v3 CRISPR	
Single Cell 5' v2/HT Gene Expression*	
Single Cell 5' v2/HT V(D)J*	
Single Cell 5' v2/HT Cell Surface Protein*	
Single Cell 5' v2/HT CRISPR*	
Epi Multiome	
Single Cell Multiome - Gene Expression	
Flex**	
Flex v2 Singleplex Gene Expression	
Flex v2 Singleplex Protein Expression	
Flex v2 Multiplex Gene Expression***	
Flex v2 Multiplex Protein Expression***	
Flex v1 Singleplex Gene Expression	
Flex v1 Singleplex Protein Expression	
Flex v1 Multiplex Gene Expression***	
Flex v1 Multiplex Protein Expression***	
Visium	
Fresh Frozen Direct Placement (v1) Gene Expression	

Product	
Visium	
HD 3' Spatial Gene Expression	10x Genomics has not tested pooling Visium HD 3' libraries with other 10x Genomics libraries; therefore, pooling is not recommended due to possible impact on assay performance.
HD Spatial Gene Expression	Pooling Visium HD libraries with other 10x Genomics libraries is not recommended due to their unique sequencing configuration: R1: 43, i7:10, i5:10, R2: 50. Additionally, on NovaSeq X Plus, Visium libraries cluster optimally at a higher loading concentration (300-400 pM) than most Single Cell Gene Expression libraries (150-200 pM). Limited testing of Visium HD and Flex libraries pooled at a 1:1 molar ratio at range of concentrations on NovaSeq X Plus did not yield any equal sequencing representation of Visium HD and Flex libraries, likely due to the differences in optimal loading concentrations for each library type.
CytAssist Spatial Gene Expression (v2)	CytAssist v2 libraries have different sequencing configurations than the library types above (R1: 28, i7:10, i5:10, R2: 50). It may be possible to pool these libraries with the library types at the beginning of this table as long as (1) Read 2 is increased to 90 cycles, (2) 10% PhiX is used if pooling with Multiplex Flex libraries, and (3) the CytAssist v2 libraries do not comprise >40% of the pool. When pooling, optimization may be required depending on the sequencing platform. For example, for NovaSeq X Series instruments, the optimal loading concentrations differ between CytAssist v2 libraries and other 10x Genomics library types; thus optimization may be required to determine the optimal pooling ratios and the optimal loading concentration for the library pool.
CytAssist Spatial Gene & Protein Expression	
Epi ATAC	
Single Cell ATAC v2	ATAC v2 libraries have a different sequencing configuration (R1: 50, i7: 8, i5: 16, R2: 50). 10x Genomics has not tested and does not recommend pooling these libraries with other 10x Genomics library types (exception: see notes below for Multiome ATAC).

Product	
Epi Multiome ATAC	
Single Cell Multiome - ATAC	Multiome ATAC libraries have a different sequencing configuration (R1: 50, i7: 8, i5: 24, R2: 49). 10x Genomics has not tested and does not recommend pooling these libraries with other 10x Genomics library types, other than standalone ATAC libraries (see below). Pooling Multiome ATAC libraries with standalone ATAC libraries may be possible when using Illumina sequencers that run the forward strand workflow (eg. MiSeq), with the following adjustment: R1: 50, i7 :8 , i5: 16, R2: 49. 10x Genomics does not recommend pooling these libraries on reverse complement sequencing workflows.

3. Library Sequencing

3.0 Loading Concentration

The following table provides recommended final library loading concentrations (pM) based on internal testing. Recommended loading concentrations are starting points and may need adjustments.

Product	Kit	MiSeq (pM)	NextSeq 500/550 (pM)	NextSeq 1000/2000 (pM)	NovaSeq 6000 (pM)	NovaSeq X Series (pM)
Universal 3' Gene Expression	**Single Cell 3' v4**					
	Gene Expression	12	1.6	650	150*/150	150–200
	Cell Surface Protein	Recommended Pooling - Pool with 3' Gene Expression libraries				
	Single Cell 3' v3.1/HT					
	Gene Expression	11	1.8	650	150*/150	150–200
	CRISPR	Required Pooling - Pool with 3' Gene Expression libraries				
	CellPlex	Required Pooling - Pool with 3' Gene Expression libraries				
	Cell Surface Protein	Recommended Pooling - Pool with 3' Gene Expression libraries				
Universal 5' Gene Expression	**Single Cell 5' v3**					
	Gene Expression V(D)J	10	1.6	650	150*/150	150–200
	CRISPR	Required Pooling - Pool with 5' Gene Expression libraries				
	Cell Surface Protein	Recommended Pooling - Pool with 5' Gene Expression libraries				
	Single Cell 5' v2, 5' HT					
	Gene Expression V(D)J	10	1.5	650	150*/150	150–200
	CRISPR	Required Pooling - Pool with 5' Gene Expression libraries				
	Cell Surface Protein	Recommended Pooling - Pool with 5' Gene Expression libraries				
Epi ATAC	**Single Cell ATAC v2**					
	ATAC v2	11	1.7	650	150*/150	100–150
Epi Multiome	**Single Cell ATAC Multiome ATAC + GEX**					
	Multiome - ATAC	10	1.5	650	150*/150	100–150
	Multiome - Gene Expression	11	1.8	650	150*/150	150–200

Product	Kit	MiSeq (pM)	NextSeq 500/550 (pM)	NextSeq 1000/2000 (pM)	NovaSeq 6000 (pM)	NovaSeq X Series (pM)
Flex	**Flex v1, v2**					
	Singleplex Gene Expression	12	2.5	650	150–200*/ 100–150	150–200
	Multiplex Gene Expression					
	Singleplex Protein Expression	Recommended - Pool with Gene Expression libraries				
	Multiplex Protein Expression	Recommended - Pool with Gene Expression libraries				
Visium	**Visium**					
	HD 3' Spatial Gene Expression	-	-	650	100–150*/ 100–150	150–200
	HD Spatial Gene Expression	-	-	650	150–200*/ 100–150	300–400
	CytAssist Spatial Gene Expression (v2)	11	1.8	650	150–200*/ 100–150	300–400
	CytAssist Spatial Gene & Protein Expression	11	1.8	650	150–200*/ 100–150	300–400
	Fresh Frozen Direct Placement (v1)	11	1.8	650	150*/300	150–200

*Refers to guidance for the XP (lane splitting) workflow

10x Genomics recommends a PhiX spike-in for all library types. The exact recommendation can be found in the respective User Guides. Generally, a 1% PhiX spike-in is recommended for QC purposes, unless sequencing a low diversity library or a low diversity stretch (e.g. Flex libraries when reading the Probe Barcode information). In these cases, a 5% PhiX spike-in is recommended (10% PhiX for Flex Multiplex libraries on the NovaSeq 6000 and NovaSeq X Series).

3.1 Denaturing and Diluting

Once quantified and normalized, libraries should be denatured and diluted as recommended for Illumina sequencing platforms. Consult Illumina documentation for denaturing and diluting libraries.

3.2 Sequencer Compatibility

Choosing a sequencing platform will depend on many factors including, but not limited to:

- Number of reads desired
- Price
- Access

Compatibility of the sequencers listed below has been verified by 10x Genomics for many, but not all, 10x Genomics libraries. Consult the sequencing page for the relevant product on the 10x Support website to confirm sequencer compatibility for a specific library type. Some variation in assay performance is expected based on sequencer choice.

- MiSeq
- NextSeq 500/550
- NextSeq 1000/2000
- NovaSeq 6000
- NovaSeq X Series

The 10x Genomics Flowcell Capacity Calculator (CG000604) may be used to calculate the total reads required to sequence a pool of 10x Genomics Single Cell libraries, and to select an Illumina platform and flow cell with sufficient read output. Not all library types can be pooled and sequenced together. This calculator will NOT call out these incompatibilities.

10x Genomics libraries can also be modified to enable sequencing on various long and short-read sequencing platforms, with some platforms requiring third-party analysis tools. Consult the sequencing page for the relevant product on the 10x Support website for guidance on compatible sequencing platforms.

3.3 Sequencing by Synthesis Kit Selection

In addition to selecting a sequencer and flow cell, an appropriate SBS cycle kit with sufficient reagents to sequence a specific library type is required. Illumina provides SBS reagents for all sequencing platforms. Having sufficient volume of sequencing reagents is critical for proper run performance. Each cycle of sequencing generates one base of sequencing information for each cluster on the flow cell. Consult this article for more information on how many cycles of sequencing reagents are contained in various Illumina kits.

For example, the NextSeq 1000/2000 P3 flow cell v3 100 cycle kit can generate ~1.2 billion clusters in the flow cell and contains enough sequencing reagents for 138 cycles of sequencing. This means a maximum of 138 bp could be sequenced with this SBS kit, including the index cycles.

At 10x Genomics, libraries are sequenced as specified in the Sequencing section of the appropriate User Guides. It may be possible to modify the sequencing configuration to utilize a SBS kit with fewer cycles; however, this may compromise assay performance and is not recommended.

To determine the appropriate flow cell and SBS kit:

- Based on the total number of read pairs required for a library or library pool, determine the appropriate flow cell. The desired read pairs per cell correspond to Illumina's single-end reads (i.e., the number of clusters) when assessing yield specifications.
- Calculate the total number of required sequencing cycles to identify the appropriate SBS kit by summing all the read lengths from R1, R2, and the index reads (see examples below). Illumina sequencers with forward strand workflows will require an additional 7 cycles of sequencing reagents when performing a dual indexing run. This will also need to be accounted for in the total number of cycles. Consult this article to ensure that the bp required is less than the cycles included for each kit.

Example 1

10x Genomics 3' v3.1 Dual Index libraries have the following recommended sequencing configuration, which can be found in the relevant User Guides.

	Read 1	i7 Index	i5 Index	Read 2
Purpose	Cell Barcode & UMI	Index	Index	Insert
Length	28	10	10	90

Given this sequencing configuration, 138 bp (28 + 10 + 10 + 90) are needed. This would be appropriate for any Illumina 100 cycle kit, as the cycle number

contained in these kits (138) contains the required number of base pairs (138 bp).

Example 2

10x Genomics 5' v2 Dual Index libraries have the following recommended sequencing configuration, which can be found in the relevant User Guides.

	Read 1	i7 Index	i5 Index	Read 2
Purpose	Cell Barcode & UMI	Index	Index	Insert
Length	26	10	10	90

Given this sequencing configuration, 136 bp (26 + 10 + 10 + 90) are needed. This would be appropriate for any Illumina 100 cycle kit, as the cycle number contained in these kits (138) exceeds the required number of base pairs (136 bp).

Some sequencers may not have an available 100 cycle kit. Exceptions include:

- NovaSeq S4: 200 cycle kit available
- iSeq: 300 cycle kit available
- MiSeq, MiniSeq, and NextSeq: 150 cycle kit is available

3.4 Sequencing Parameters

Number of Cycles Required for Each Read

The following table summarizes the number of cycles required for each read for 10x Genomics libraries. The recommended configuration can also be found in the relevant User Guide. Read lengths described below have been optimized by 10x Genomics. In general, modification of these lengths is not recommended due to negative impacts on assay performance.

Product	Read 1	i7	i5	Read 2	Total
Universal 3' Gene Expression					
Single Cell 3' v4 Gene Expression	28	10	10	90	138
Single Cell 3' v4 Cell Surface Protein					
Single Cell 3' v3.1/HT Gene Expression					
Single Cell 3' v3.1/HT Cell Surface Protein					
Single Cell 3' v3.1/HT CRISPR					
Single Cell 3' v3.1/HT CellPlex					
Universal 5' Gene Expression					
Single Cell 5' v3 Gene Expression	28	10	10	90	138
Single Cell 5' v3 V(D)J					
Single Cell 5' v3 Cell Surface Protein					
Single Cell 5' v3 CRISPR					
Single Cell 5' v2, 5' HT Gene Expression	26	10	10	90	136
Single Cell 5' v2, 5' HT V(D)J					
Single Cell 5' v2, 5' HT Cell Surface Protein					
Single Cell 5' v2, 5' HT CRISPR					
Epi ATAC					
Single Cell ATAC v2	50	8	16	50	124
Epi Multiome					
Single Cell Multiome - ATAC	50	8	24	49	131
Single Cell Multiome - Gene Expression	28	10	10	90	138
Flex					
Flex v2 Multiplex Gene Expression	28	10	10	90	138
Flex v2 Multiplex Protein Expression	or 54*	10	10	50	124

Product	Read 1	i7	i5	Read 2	Total
Flex					
Flex v2 Singleplex Gene Expression	28 or 48*	10	10	90	138
Flex v2 Singleplex Protein Expression					
Flex v1 Singleplex Gene Expression					
Flex v1 Multiplex Gene Expression					
Flex v1 Singleplex Protein Expression		10	10	50	118
Flex v1 Multiplex Protein Expression					
Visium					
HD 3' Spatial Gene Expression	43	10	10	75	138
HD Spatial Gene Expression	43	10	10	50	113
CytAssist Spatial Gene Expression (v2)	28	10	10	50	98
CytAssist Spatial Gene & Protein Expression					
Fresh Frozen Direct Placement (v1)	28	10	10	90	138

* Flex libraries can be sequenced using two different configurations. These are referred to as Read 1 or Read 2 Sequencing Configuration, depending on which read is used to read the sequences required for sample demultiplexing. Refer to the Flex sequencing page on the 10x Support website for further information.

Sequencing Depth

The following table summarizes the sequencing depth for 10x Genomics libraries. In the sequencing section of the User Guide, the sequencing depth required for data analysis is listed as read pairs per cell. Read pair per cell (reads per cell) correspond to the number of clusters passing filter needed for single cell analysis. One single end read = 1 cluster = 1 read pair = 2 paired end reads. For information on library pooling, see 2.4 Pooling Compatibility on page 15.

Product	Minimum Sequencing Depth	Additional Notes
Universal 3' Gene Expression	**Read Pairs per Cell**	
Single Cell 3' v4 Gene Expression	20,000	
Single Cell 3' v3.1 Gene Expression	20,000	
Single Cell 3' v4 Cell Surface Protein	5,000	Pool libraries with Single Cell 3' v4 Gene Expression libraries to maintain nucleotide diversity
Single Cell 3' v3.1/HT Cell Surface Protein/CRISPR/CellPlex	5,000	Pool libraries with Single Cell 3' v3.1 Gene Expression libraries to maintain nucleotide diversity
Universal 5' Gene Expression	**Read Pairs per Cell**	
Single Cell 5' v3 Gene Expression	20,000	
Single Cell 5' v2, 5' HT Gene Expression	20,000	
Single Cell 5' v3 V(D)J	5,000	Pool libraries with Single Cell 5' Gene Expression libraries to maintain nucleotide diversity
Single Cell 5' v3 Cell Surface Protein		
Single Cell 5' v3 CRISPR		
Single Cell 5' v2, 5' HT Gene Expression	20,000	
Single Cell 5' v2, 5' HT V(D)J	5,000	
Single Cell 5' v2, 5' HT Cell Surface Protein		Pool libraries with Single Cell 5' HT Gene Expression libraries to maintain nucleotide diversity
Single Cell 5' v2, 5' HT CRISPR		Pool libraries with Single Cell 5' HT Gene Expression libraries to maintain nucleotide diversity
Epi ATAC	**Read Pairs per Nucleus**	
Single Cell ATAC v2	25,000	

Product	Minimum Sequencing Depth	Additional Notes
Epi Multiome	**Read Pairs per Nucleus**	
Single Cell Multiome - ATAC	25,000	
Single Cell Multiome - Gene Expression	20,000	
Flex	**Read Pairs per Cell**	
Flex v2 Singleplex Gene Expression	10,000	
Flex v2 Multiplex Gene Expression		
Flex v1 Singleplex Gene Expression		
Flex v1 Multiplex Gene Expression		
Flex v2 Singleplex Protein Expression	5,000	Pool Protein Expression libraries with Gene Expression libraries to maintain nucleotide diversity
Flex v2 Multiplex Protein Expression		
Flex v1 Singleplex Protein Expression		
Flex v1 Multiplex Protein Expression		
Visium	**Read Pairs per tissue-covered spot (v1/v2) or fully-covered Capture Area (HD/HD 3')**	
HD 3' Spatial Gene Expression	550,000,000	Calculating sequencing depth requires estimating the approximate Capture Area (%) covered by tissue. This may be performed visually or by using the Visium Manual Alignment Wizard in Loupe Browser for a more accurate measurement. For more information, consult the Manual Alignment for Visium page and this article on the 10x Genomics Support website. If estimating the approximate Capture Area (%) covered by tissue visually, multiply the Capture Area (%) covered by tissue by the recommended number of read pairs per fully-covered Capture Area. For example, for a Capture Area that is 60% covered, the formula is (0.60 x 555,000,000) for 275,000,000 total read pairs. The 550M minimum recommendation achieved >75% sequencing saturation for >50% of fresh frozen tissues tested at 10x Genomics.

Product	Minimum Sequencing Depth	Additional Notes
Visium	**Read Pairs per tissue-covered spot (v1/v2) or fully-covered Capture Area (HD/ HD 3')**	
HD Spatial Gene Expression	275,000,000 (6.5 mm) 825,000,000 (11 mm)	Calculating sequencing depth requires estimating the approximate Capture Area (%) covered by tissue. This may be performed visually or by using the Visium Manual Alignment Wizard in Loupe Browser for a more accurate measurement. For more information, consult the Manual Alignment for Visium page and this article on the 10x Genomics Support website. If estimating the approximate Capture Area (%) covered by tissue visually, multiply the Capture Area (%) covered by tissue by the recommended number of read pairs per fully-covered Capture Area (275 million read pairs for 6.5 mm slides and 825 million for 11 mm Visium HD slides). For example, for a 6.5-mm Capture Area that is 60% covered, the formula is (0.60 x 275,000,000) for 165,000,000 total read pairs. The 275M or 825M minimum recommendation achieved >50% sequencing saturation for >50% of formalin fixed, paraffin embedded (FFPE) tissues tested at 10x Genomics. To achieve similar saturation with fixed and fresh frozen tissues deeper sequencing will be required due to higher sensitivity expected with these tissue preservation methods. For more information, consult the Visium HD Spatial Gene Expression User Guide 2.0 (CG001679).
CytAssist Spatial Gene Expression (v2)	Gene Expression: 25,000 Protein Expression: 5,000	Calculating sequencing depth requires estimating the approximate Capture Area (%) covered by tissue. This may be performed visually or by using the Visium Manual Alignment Wizard in Loupe Browser for a more accurate measurement. For more information, consult the Manual Alignment for Visium page on the 10x Genomics Support website. If estimating the approximate Capture Area (%) covered by tissue visually, multiply the Capture Area (%) covered by tissue by the number of spots and by the recommended read depth to determine sequencing depth. For example, for a Capture Area that is 60% covered, the formula is (0.60 x 5,000 total spots) x recommended read pairs per tissue-covered spot. For a v1 sample, this would be (0.60 x 5,000 total spots) x (50,000 read pairs/spot) for 150 million total read pairs.
CytAssist Spatial Gene & Protein Expression		
Fresh Frozen Direct Placement (v1)	50,000	

4. Library Demultiplexing

4.0 Library Demultiplexing Overview

Multiplexing is the technique of combining multiple samples in a single sequencing run. Each sample is tagged with a unique identifier called an index barcode, allowing them to be processed together. During the sequencing process, the machine reads all the combined samples as a single dataset, producing a mixture of sequences from all the indexed samples. Therefore, demultiplexing is a crucial step in bioinformatics analysis, involving the identification of indices, the sorting of sequences into separate files (one for each original sample), and concatenating the bases from the individual sequencing cycles to a continuous sequence. If demultiplexing is done incorrectly, sequences could be assigned to the wrong samples or no samples, leading to potential errors in downstream analysis. Once the sequences have been demultiplexed from the binary base calling files (BCL files), they are typically stored in the FASTQ format—a text-based file format that contains both nucleotide sequence information and quality scores for each nucleotide. These FASTQ files, usually provided by a sequencing core, are essential inputs for most Cell Ranger and Space Ranger pipelines. However, if the starting with BCL files, they must be converted to FASTQ format through the demultiplexing process. 10x Genomics recommends using Illumina's BCL Convert software to generate compatible FASTQ files.

4.1 Sample Sheets

A sample sheet instructs the sequencer how many cycles each read should be and how to assign generated FASTQ files to the appropriate sample. Though a sample sheet is not required for sequencing 10x Genomics libraries when running the sequencer in 'standalone' mode, a sample sheet is required for demultiplexing after sequencing. If a sample sheet will not be used, the run setup must specify the appropriate number of cycles for Read 1, the i7 and i5 index reads, and Read 2. Information on product-specific sequencing configurations can be found in 3.4 Sequencing Parameters on page 24.

MiSeq instruments running newer versions of the MiSeq Control Software (MCS) v3.1 and above can be run without a sample sheet. MCS v3.1 added manual mode (similar to standalone mode), which allows users to manually enter run parameters without a sample sheet.

A basic sample sheet for BCL Convert has three sections. Each section is described here and example sample sheets are provided for both single and dual indexed samples.

`[Header]`: Can be used to specify the BCL sample sheet version.

`[BCLConvert_Settings]`: In a V2 sample sheet, this section is used to specify several FASTQ conversion settings including whether or not to create FASTQ files for indices. Use `[Settings]` in a V1 sample sheet.

`[BCLConvert_Data]`: In a V2 sample sheet, this section is used to sort samples and index adaptors based on the following column headers. The `[BCLConvert_Data]` section must be renamed `[Data]` or `[data]` for a V1 sample sheet:

Column Name	Description
`Lane`	Optional. Generates FASTQ files only for the samples with the specified lane number. Allows only one valid integer. If the same sample has been run on multiple lanes of the flow cell, add a new row for each lane. If the lane is not specified, indices are searched in all lanes.
`Sample_ID`	The sample ID.
`index`	i7 index sequence
`index2`	i5 index sequence
`Sample_ Project`	Optional Used when --bcl-sampleproject-subdirectories is specified in BCL Convert run. Only alphanumeric characters, dashes, and underscores are allowed. Logs or Reports should not be used as directory names for this flag, as they are already default output directories. Additional information may be found here.

For additional information on using BCL Convert to generate FASTQ files, please refer to the Direct Demultiplexing pages on the 10x Genomics Support site for each product. These links are collected in this article.

If using the Illumina Experiment Manager sample sheet, the sheet may ask for adaptor sequences for trimming. Leave this line blank, as 10x Genomics does not recommend adaptor trimming or preprocessing FASTQ reads before input into 10x Genomics software pipelines. Remove the following lines from the Settings section of the Illumina Experiment Manager sample sheet: Adaptor or AdaptorRead1 or AdaptorRead2.

BCL Convert can also run in Illumina's BaseSpace Sequencing Hub or on-board the instrument. Consult this article for more information on setting up a sample sheet in BaseSpace. When configuring a sample sheet in BaseSpace, the *OverrideCycles* setting may be changed to adjust the read length configuration of the FASTQ if it is different from the RunInfo.xml file. For more information on using *OverrideCycles*, consult this article.

The following is an example sample sheet for libraries created with the Dual Index Plate TT, Set A or Dual Index Plate TN, Set A. The parameter `CreateFastqForIndexReads,0` under `[BCLConvert_Settings]` tells BCL Convert not to generate FASTQ files for indices. Cell Ranger does not require FASTQ files for indices.

```
[Header]
FileFormatVersion,2
[BCLConvert_Settings]
CreateFastqForIndexReads,0
[BCLConvert_Data]
Lane,Sample_ID,index,index2
1,test_sample,GTAACATGCG,AGGTAACACT
```

For general support on using BCL Convert, contact Illumina technical support.

4.2 Undetermined Reads

A high percentage of reads in the ‘Undetermined’ category is most likely an issue with the sample sheet or a problem during demultiplexing. For example, incorrect indices may be used, causing the reads to not be recognized. This can be checked in the ‘Top Unknown Barcodes’ section of the `Top_Unknown_Barcodes.csv` file from `bcl-convert`. Additionally, poor quality of the index reads can also lead to a large number of 'Undetermined' reads.

4.3 Specifying Input FASTQ Files for 10x Genomics Pipelines

To serve as inputs for Cell or Space Ranger, FASTQ files should conform to the naming conventions of `bcl-convert`:

[Sample Name]_S1_L00[Lane Number]_[Read Type]_001.fastq.gz

Where Read Type is one of:

- I1: Sample index read (optional)
- I2: Sample index read (optional)
- R1: Read 1
- R2: Read 2

The FASTQ files are specified by providing the path to the folder containing them (via the `--fastqs` argument) and then optionally restricting the selection by specifying the samples and or lanes of interest.

Additionally, Space Ranger accepts file names without the lane number [Lane Number], e.g., sample1_S1_R1_001.fastq.gz.

For more information on specifying FASTQ files for 10x Genomics Pipelines, consult the following:

- Specifying input FASTQs for Cell Ranger
  - Universal 3' Gene Expression
  - Universal 5' Gene Expression
  - Flex
- Specifying input FASTQs for Space Ranger
  - Visium
- Specifying input FASTQs for Cell Ranger ARC
  - Epi Multiome
- Specifying input FASTQs for Cell Ranger ATAC
  - Epi ATAC

4.4 Running BCL Convert

The command to run BCL Convert:

```
/path/to/binary/bcl-convert --bcl-input-directory <folder-with-bcls> \
--output-directory <name-of-output-dir-for-FASTQs> \
--sample-sheet <samplesheet-filename.csv>
```

Required arguments:

- `--bcl-input-directory`: path to the input directory containing BCL files
- `--output-directory`: path to an output directory for newly created FASTQ files. This directory must not exist before command execution.
- `--sample-sheet`: path to a CSV file containing sample information as described in 4.1 Sample Sheets on page 30. Providing a path to the directory instead of the specific CSV file can cause the software to hang.

4.5 FastQC

FastQC is a quality control tool designed to assess the quality of high-throughput sequencing data. Run FastQC if poor sequencing quality is expected or a large number of N's are present in the 10x Barcode read (Read 1). A low number of valid 10x Barcodes can lead to a loss of certain reads during data processing and result in a low median gene count per cell. In such cases, generating a quality control report can be helpful for assessing data quality. For instance, generating a FastQC report on the raw sequencing data (i.e. FASTQ files) is a useful routine check after receiving data from a high-throughput sequencing run. For instructions on downloading FastQC, see this page. For installation instructions, see this page.

Example syntax to run FastQC:

```
fastqc -t 8 -f fastq -o ./output_directory_name/ /path_to_fastq_files_
dir/*.gz
```

The example above uses the downloaded FastQC executable file (fastqc), the number of threads for the program to use (`-t`), the input file type (`-f`), an output directory name to save the FastQC reports to a location other than the current directory (`-o`), and the full path to the input FASTQ files.

Notices

Document Number

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Legal Notices

Instrument & Licensed Software Updates Warranties

Updates to existing Instruments and Licensed Software may be required to enable customers to use new or existing products.

Support

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Document Revision Summary

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Description of Changes

- Added index combinations for low plex pools to 2.1 Unique and Diverse Indices on page 6.
- Added information on Flex v2 to 2.4 Pooling Compatibility on page 15, 3.0 Loading Concentration on page 19, and 3.4 Sequencing Parameters on page 24.
- Added information on sequencing depth for Visium HD 11-mm libraries to 3.4 Sequencing Parameters on page 24.
- Changed the order of the assay versions for Visium products to show the most recent one at the top in 2.4 Pooling Compatibility on page 15, 3.0 Loading Concentration on page 19, and 3.4 Sequencing Parameters on page 24
- Fixed various broken and incorrect hyperlinks

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Updated for general minor consistency of language and terms throughout.