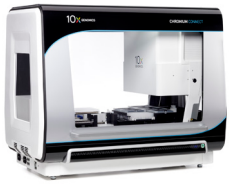

USER GUIDE

Automated Gene Expression Library Construction

FOR USE WITH

Automated Library Construction Kit, 24 rxns PN-1000428
Automated Library Construction Kit, 4 rxns PN-1000429
Dual Index Kit TT Set A, 96 rxns PN-1000215

Notices

Document Number

CG000474 • Rev B

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. In the event of an Instrument failure resulting from an update, such failed Instrument will be replaced or repaired in accordance with the 10x Limited Warranty, Assurance Plan or service agreement, only if such Instrument is covered by any of the foregoing at the time of such failure. Instruments not covered under a current 10x Limited Warranty, Assurance Plan or service agreement will not be replaced or repaired.

Support

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Document Revision Summary

Document Number	CG000474
Title	Automated Gene Expression Library Construction User Guide
Revision	Rev A to B
Revision Date	April 2023
Specific Changes	• Updated to include Automated Library Construction Kit, 4 rxns PN-1000429 (page 8 & other instances) • Revised to include the updated cDNA Generation Kit name (no change in kit configuration, kit components, reagent compositions, and part numbers; page 6)
General Changes	• Updated for general minor consistency of language and terms throughout

Table of Contents

Introduction

Workflow Overview
Automated Library Construction Kit
Additional Kits, Reagents & Equipment
Recommended Thermal Cyclers
Protocol Steps & Timing
Stepwise Objectives

Automated Library Construction Workflow

This User Guide provides an overview of the Automated Library Construction workflow along with a list of 10x Genomics reagents needed to generate Gene Expression Libraries from cDNA prepared by manual or automated workflows. As part of this automated flexible workflow, Gene Expression Libraries can be generated from freshly prepared or stored cDNA using the Automated Library Construction Kit (PN-1000428/1000429).

For information on sample preparation, carrier loading and protocol guidelines, refer to the relevant Chromium Next GEM Single Cell Automated User Guide.

For instrument operation, refer to the Chromium Connect Instrument User Guide (CG000180).

Automated Library Construction Workflow Overview

Workflow Step	Kit Name
Sample Prep	
Manual / OR / Automated	*Manual - Refer to relevant manual User Guide for more information on kits and reagents* *Automated - User Guide Supplements (CG000472) and (CG000473) include a list of 10x Genomics reagents*
cDNA → cDNA	Automated- Use Chromium Next GEM Automated cDNA Generation kits to generate cDNA (3' cDNA Generation Kit v3.1, PN-1000424) (5' cDNA Generation Kit v2, PN-1000425)
STOP — Store cDNA at **4°C** for ≤**72 h** or **-20°C** for ≤**4 wks**	
GEX Library → qPCR and Pooling	Use Automated Library Construction Kit (PN-1000428/1000429) to generate Gene Expression libraries *Automated Library Construction User Guide (CG000474)*
Manual **Automated**	

Automated Library Construction Kit

Automated Library Construction Kit, 24 rxns PN-1000428

Reagent volumes and colors are different in each of the module types, not all module tubes contain reagents.

Automated Library Construction Kit, Module 1, 24 rxns PN-1000399 (store at 4°C)

Automated Library Construction Kit,
Module 1, 24 rxns

	#
Single Cell Library Module 1	24 tube strips

10xGenomics.com

Library Module 1

Automated Library Construction Kit, Module 2, 24 rxns PN-1000400 (store at -20°C)

Automated Library Construction Kit,
Module 2, 24 rxns

	#
Single Cell Library Module 2	24 tube strips

10xGenomics.com

Library Module 2

Automated Library Construction Kit, Module 3, 24 rxns PN-1000401 (store at -20°C)

Automated Library Construction Kit,
Module 3, 24 rxns

	#
Single Cell Library Module 3	24 tube strips

10xGenomics.com

Library Module 3

Automated Library Construction Kit, 4 rxns PN-1000429

Reagent volumes and colors are different in each of the module types, not all module tubes contain reagents.

Automated Library Construction Kit, Module 1, 4 rxns PN-1000412 (store at 4°C)

Automated Library Construction Kit, Module 1, 4 rxns

	#
Single Cell Library Module 1	4 tube strips

10xGenomics.com

Library Module 1

Automated Library Construction Kit, Module 2 & 3, 4 rxns PN-1000413 (store at -20°C)

Automated Library Construction Kit, Module 2 & 3, 4 rxns

	#
Single Cell Library Module 2	4 tube strips
Single Cell Library Module 3	4 tube strips

10xGenomics.com

Library Module 2

Library Module 3

10xGenomics.com

Dual Index Kit TT Set A, 96 rxns PN-1000215 (store at –20°C)

DUAL INDEX

Dual Index Kit TT Set A

	#	PN
Dual Index Plate TT Set A	1	3000431

Additional Kits, Reagents & Equipment

The items in the table below have been validated by 10x Genomics and are required for the Automated Library Construction protocol. DO NOT substitute any of the listed materials.

Supplier	Description	Part Number (US)
Plastics		
Hamilton	CO-RE Tips 50 µl Filtered Tips* CO-RE Tips 300 µl Filtered Tips* 60 ml Reagent Reservoir Self-Standing Hamilton PCR ComfortLid **CO-RE pipette tips will be phased out and replaced by new CO-RE II pipette tips (same part number as CO-RE tips) in 2022/2023. CO-RE II tips include a new sealing surface to interface with the CO-RE II stop disk. Geometry that interfaces with the current CO-RE stop disk is identical between the two tip designs and performance remains unaffected.*	235948 235903 194051 814300
Eppendorf	96-well Full-Skirted Plate 96-well Semi-Skirted Plate *(Blue color listed; other colors are acceptable)*	951020460 951020362
Thermo Fisher Scientific	MicroAmp 8-Tube Strip, 0.2 ml MicroAmp 8-Cap Strip, clear	N8010580 4323032
Kits & Reagents		
Thermo Fisher Scientific	Nuclease-free Water	AM9937
Millipore Sigma	Ethanol, Pure (200 Proof, anhydrous)	E7023-500ML
Qiagen	Qiagen Buffer EB	19086
Additional materials ONLY for optional assays – qPCR and pooling		
Bio-Rad	10% Tween 20 96-well PCR Plates	1662404 HSP9665
Thermo Fisher Scientific	2 ml-Screw-cap Tube 0.5 ml-Screw-cap Tubes	3488NK 3472NK
KAPA Biosystems	KAPA Library Quantification Kit for Illumina Platforms	KK4824
Qiagen	Qiagen Buffer EB	19086
Additional materials for Chromium Connect maintenance Use only indicated cleaning agents. DO NOT use bleach or organic oxidizers		
Thor Labs	Lens tissues	MC-5
VWR	Microcide SQ Broad Spectrum Disinfectant	25099
Contec	70% Isopropanol (alternative to VWR disinfectant)	SB167030IR

Additional Kits, Reagents & Equipment

Supplier	Description	Part Number (US)
Quantification & Quality Control		
Agilent	2100 Bioanalyzer Laptop Bundle High Sensitivity DNA Kit 4200 TapeStation High Sensitivity D1000 ScreenTape/Reagents High Sensitivity D5000 ScreenTape/Reagents	G2943CA 5067-4626 G2991AA 5067-5592/ 5067-5593 5067-5584/ 5067-5585
Thermo Fisher Scientific	Qubit 4.0 Fluorometer Qubit dsDNA HS Assay Kit	Q33226 Q32854
Advanced Analytical	Fragment Analyzer Automated CE System - 12 cap Fragment Analyzer Automated CE System - 48/96 cap High Sensitivity NGS Fragment Analysis Kit	FSv2-CE2F FSv2-CE10F DNF-474

Choose Bioanalyzer, TapeStation, or Qubit based on availability & preference.

Recommended Thermal Cyclers

Thermal cyclers for off-deck use.

Supplier	Description	Part Number
Bio-Rad	C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module	1851197
Eppendorf	MasterCycler Pro	North America 950030010 International 6321 000.019
Thermo Fisher Scientific	Veriti 96-Well Thermal Cycler	4375786

Recommended Real Time qPCR System

Supplier	Description	Part Number
Bio-Rad	CFX96 Real-time System	1855096

The qPCR system should be compatible with the KAPA Library Quantification Kit dye. Refer to manufacturer`s recommendation.

Protocol Steps & Timing

The table below provides an overview of the complete automated workflow steps and timing

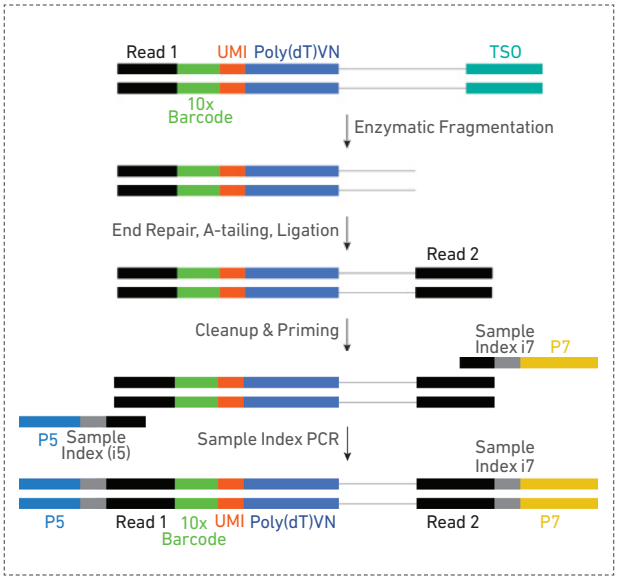
Steps	Timing
Manual	
Cell Preparation (Dependent on Cell Type)	~1-1.5 h
Gather & Load Reagents and Consumables	~60 min
Automated Gene Expression cDNA	
Master Mix Preparation	~4.5 h Walk-away time
Chromium Automated Controller Loading	
GEM Generation	
OPTIONAL Confirm GEM Generation (Manual, 5 min)	
Post GEM RT-Cleanup – Dynabead	
cDNA Amplification	
cDNA Cleanup – SPRIselect	
STOP Stop after SPRI clean up, store cDNA at 4°C for ≤72 hrs or -20°C for ≤4 wks cDNA QC & Quantification (Manual, 50 min; best practice)	
Automated Gene Expression Library	
Fragmentation, End Repair & A-tailing	~4 h Walk-away time
Post Fragmentation, End Repair & A-tailing Double Sided Size Selection – SPRIselect	
Adaptor Ligation	
Post Ligation Cleanup- SPRIselect	
Sample Index PCR	
Post Sample Index PCR Double Sided Size Selection- SPRIselect	
Manual	
Post Library Construction QC	50 min
OPTIONAL Library Quantification qPCR & Library Pooling	

Stepwise Objective – Automated Gene Expression Library Construction

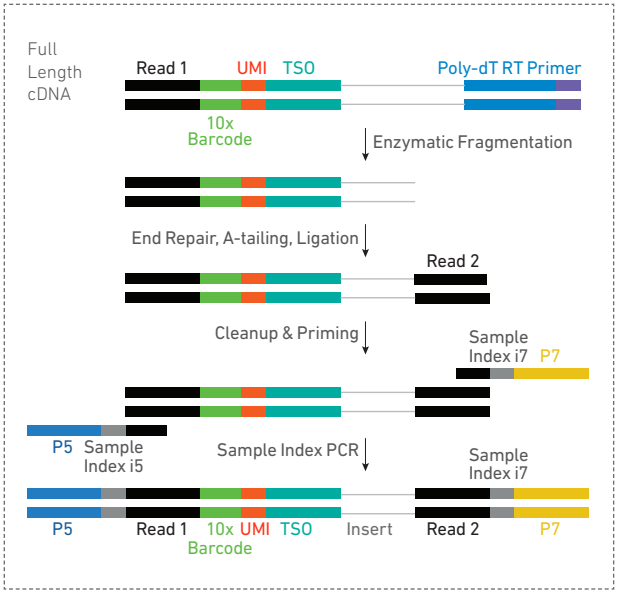
DUAL INDEX

Automated Gene Expression Library construction includes enzymatic fragmentation and size selection for optimizing the input cDNA amplicon size. P5, P7, i7 and i5 sample indexes, and TruSeq Read 2 (read 2 primer sequence) are added via End Repair, A-tailing, Adaptor Ligation, and PCR. The final libraries contain the P5 and P7 primers used in Illumina bridge amplification.

Amplified Single Cell 3' cDNA processing (dual index)



Amplified Single Cell 5' cDNA processing (dual index)



Sequencing

Gene Expression library comprises standard Illumina paired-end constructs which begin and end with P5 and P7. The 16 bp 10x Barcode and 12 bp UMI are encoded in Read 1, while Read 2 is used to sequence the cDNA fragment. Sample index sequences are incorporated as the i7 and i5 reads for Dual Index Libraries. TruSeq Read 1 and TruSeq Read 2 are standard Illumina sequencing primer sites used in paired-end sequencing.

Illumina sequencer compatibility, sample indices, library loading and pooling for sequencing are summarized in the Sequencing chapter.

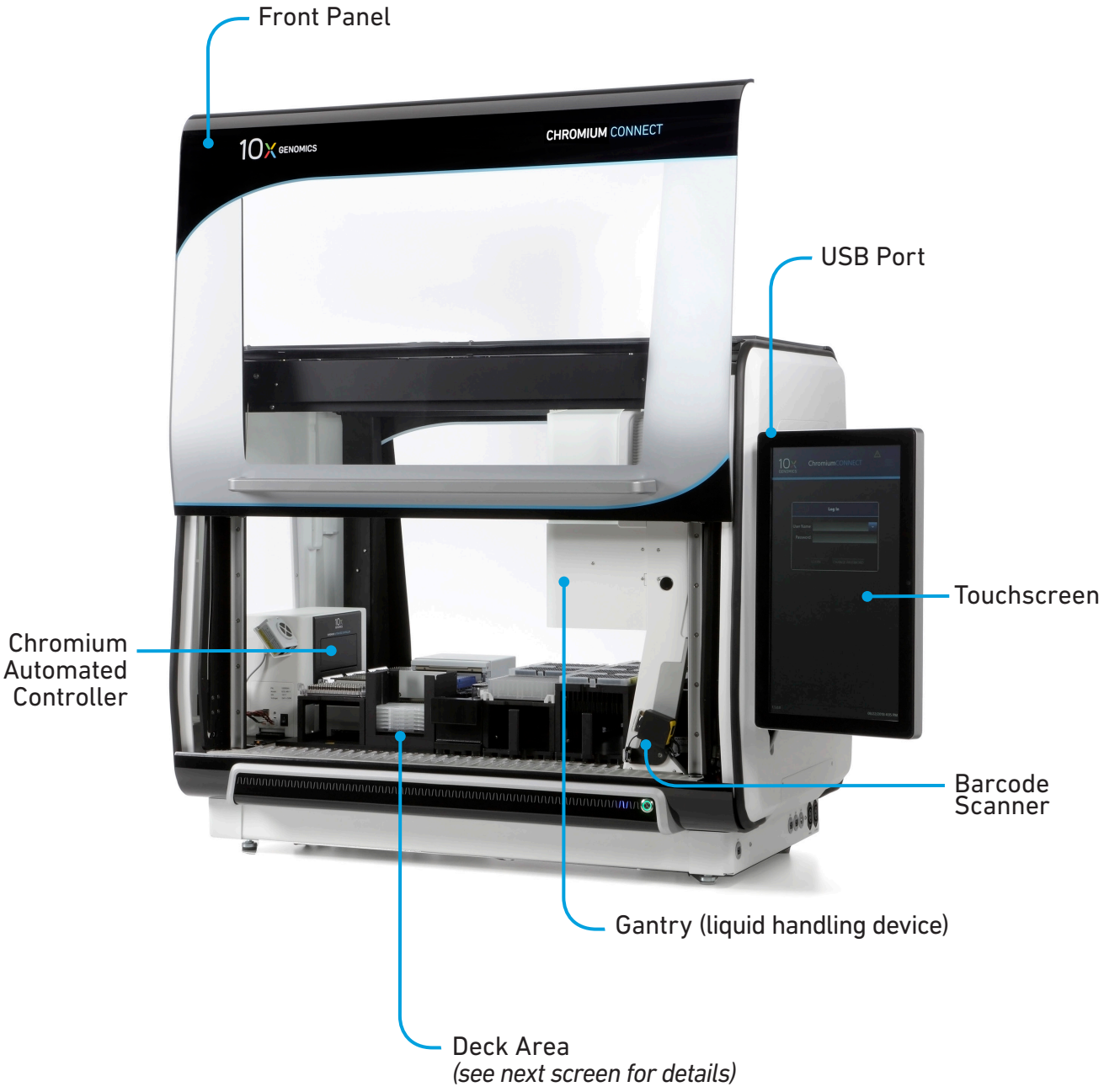
Chromium Connect

Instrument Orientation

Deck Orientation

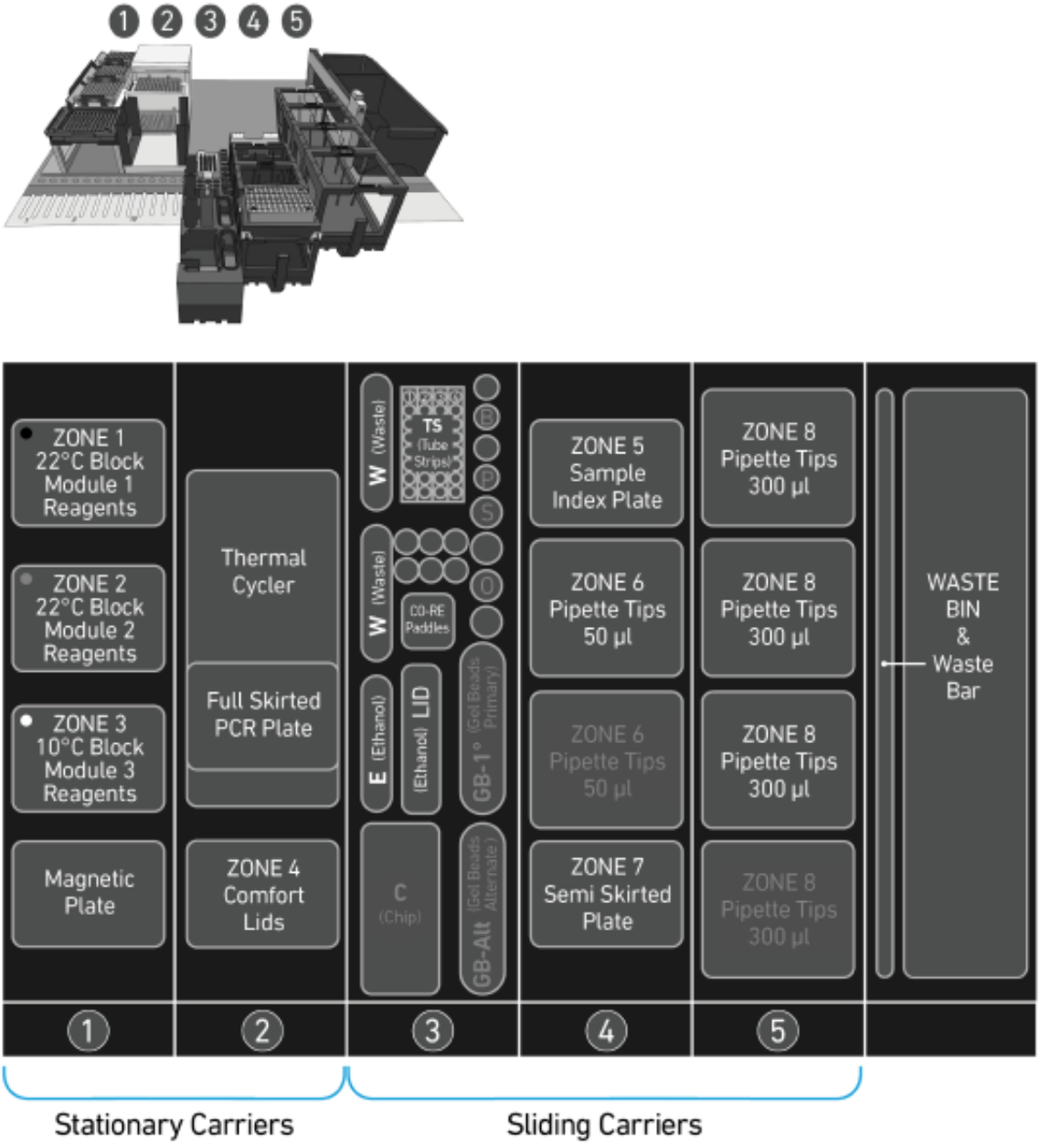
CSV Setup

Instrument Orientation



Refer to the Chromium Connect Instrument User Guide (CG000180) and Quick Reference Cards (CG000256) for more information.

Deck Orientation

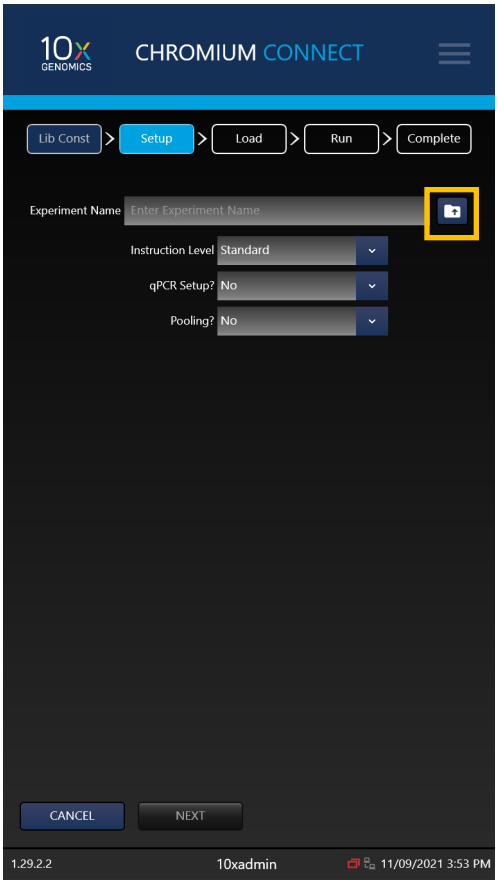


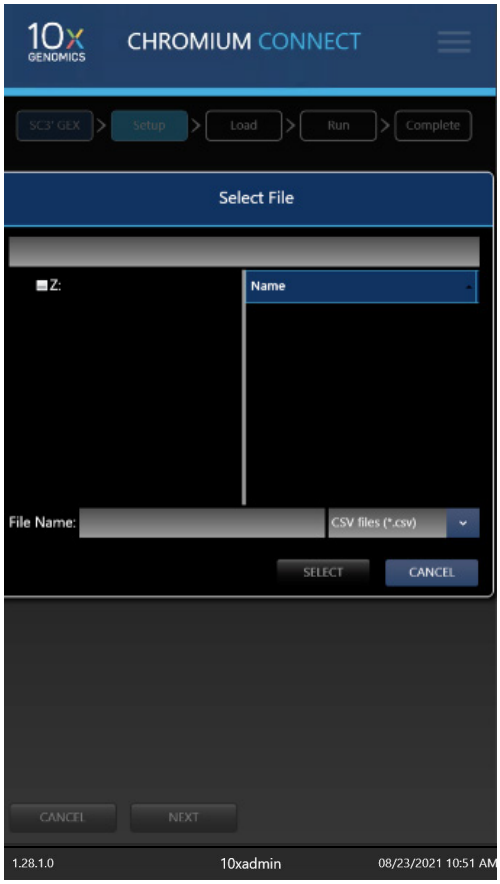
Refer to the Chromium Connect Instrument User Guide (CG000180) and Quick Reference Cards (CG000256) for more information.

CSV Setup

Sample information can also be uploaded using a CSV file at the run set-up screen. Use the folder icon to search a network file system or USB drive. Navigate to the appropriate CSV file and click "SELECT".
The Chromium Connect Input Sample Template (CG000309) is available on the 10x Genomics support website.

Run Set-up Screen





Sample CSV File

A sample CSV file is shown below. The columns highlighted in blue are mandatory to start a run. Any missing fields/corrections can be added during sample information setup. Final selections will be recorded in the final run report CSV file.

SAMPLEPARAMETERS	SAMPLENAME	SIINDEX	VOLUME	CellCount	ExpressionI	Cycles	USERDEFINED1	USERDEFINED2	USERDEFINED3	USERDEFINED4
ID1	aaa	A1	5	2001-6000	High					
ID2	bbb	B1	5	2001-6000	High					
ID3	ccc	C1	5	2001-6000	High					
ID4	ddd	D1	5	2001-6000	High					
ID5	eee	E1	5	2001-6000	High					
ID6	fff	F1	5	2001-6000	High					
ID7	ggg	G1	5	2001-6000	High					
ID8	hhh	H1	5	2001-6000	High					
RUNPARAMETERS	SELECTION									
runName	Sample Run									
Instruction Level	Standard									
qPCR Setup?	No									
Pooling?	No									
SI Cycles	14									
Notes										

Uploading Sample Information Using a CSV File

The following tables provide specific guidelines on sample entry in the CSV file.

Sample Parameters	Information
Sample Name	Alphanumeric and up to 32 characters
Sample Index	Location on dual index plate to be used for each sample during SI PCR
Cell Expression	User defined field for tracking Example: High cell expression: Cell lines Low cell expression: PBMCs
Volume	5-22 µL
Cell Count	User defined field for tracking (enter applicable option EXACTLY as shown below) 500-2000 2001-6000 6001-10000 DO NOT use commas. Space between symbol & number required.
Sample Index Cycles	User defined field. Refer to approriate Assay User Guide for guidance on optimal cycles.

Up to four user-defined fields (LIMS data) can be passed through the instrument for additional sample tracking.

Run Parameters	Information
Run Name	Alphanumeric and up to 32 characters
Instruction Level	Standard, Advanced, Expert Refer to the Chromium Connect Instrument User Guide (CG000180) for details
qPCR Setup	Opt-in for optional assay step: Yes/No
Pooling	Opt-in for optional assay step: Yes/No

Items & Reagents

Gather Items & Reagents

Follow prompts on the Chromium Connect touchscreen to gather the listed items and reagents for loading the Deck Carriers.
Gather the quantities specified for each of the items and reagents.

Item	Qty
Nuclease-free Water	10 ml
Ethanol, Pure (200 Proof, anhydrous)	40 ml
Hamilton	
ComfortLids	4
50 µl CO-RE/CO-RE II Pipette Tips, with filter (Black, Conductive)	1-2 rack
300 µl CO-RE/CO-RE II Pipette Tips, with filter (Black, Conductive)	3 racks
Reagent Reservoir, 60 ml	3
Eppendorf	
96-well Semi Skirted Plate	1
96-well Full Skirted Plate	1
Thermo Fisher Scientific	
MicroAmp 8-Tube Strip, 0.2 ml	1
10x Genomics	
Automated Library Construction Kit	
Library Module 1(stored at 4°C) *Black tube strip*	1 tube strip/sample
Library Module 2 (stored at -20°C) *Gray tube strip*	1 tube strip/sample
Library Module 3 (stored at -20°C) *White tube strip*	1 tube strip/sample
Dual Index Plate TT Set A (stored at -20°C)	1 plate

See Additional Kits, Reagents & Equipment list for performing optional QC.

Thaw & Prep Reagents

Follow prompts on the touchscreen to thaw and prepare reagents.
Some important guidelines are highlighted below.

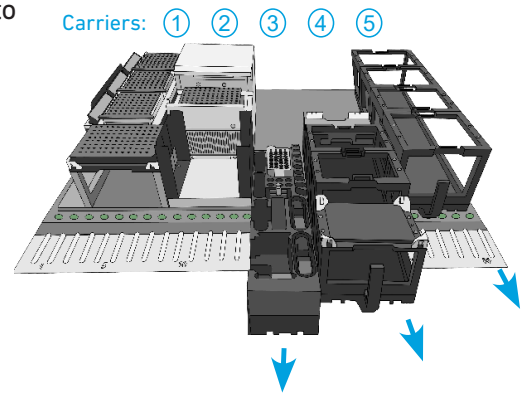
ACTION	GUIDELINES *Follow touchscreen prompts for specifics and timing*
Thaw Reagents	• Thaw reagents as indicated on the touchscreen. Verify no precipitate is present. • Ensure that the correct thawing locations and temperatures are used. • During reagent thaw load the consumables following touchscreen prompts.
Prepare Ethanol	• Prepare **50 ml** 80% Ethanol in Nuclease-free water and dispense in Ethanol Reservoir when prompted.
Library Modules	• Thaw Library Modules as prompted on the touchscreen. • After reagent thaw, invert rack holding Module tube strips and vortex Library Modules 1 and 2 for **30 sec**; verify no precipitate. • Centrifuge Library Modules 1 and 2 at **300 rcf** for **1 min** at **22°C.** • Retrieve Library Module 3 from **4°C** storage. Invert-mix; DO NOT vortex. Centrifuge Module 3 (separately from Module 1, to avoid reagent precipitation). • Confirm there are no bubbles at the bottoms of any module tubes

Confirm that there are no bubbles at the bottoms of any module tubes, Dual Index Plate wells, or Primer tubes.

Carrier Loading Guidelines

Carrier Loading Guidelines

Follow the instructions on the touchscreen to load the carriers.

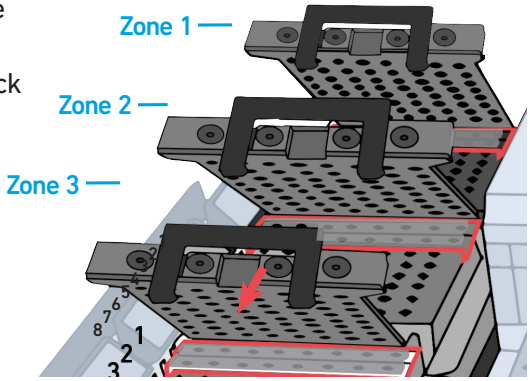


Carriers

- Handle the carriers as prompted.
- Ensure that Carriers 3, 4, and 5 are completely slid out and placed on an off-deck workspace before loading.
- Align the carriers to the corresponding Deck Rails when sliding them in or out of the deck.
- Ensure correct orientation of tube labels with barcodes to enable Barcode Scanning.

Modules

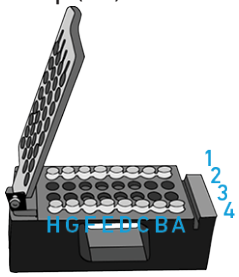
- Load one tube strip/sample of each of the indicated modules in the corresponding positions on the Carrier, starting from back to front row.
- DO NOT skip any rows when loading.
- Use pinhole alignment to place module tube strips in the correct orientation (as shown on the touchscreen).



Label Tube Strip Orientation

- The tube strip to receive the final libraries should be placed in Position 4 of the Tube Strip Holder.
- Label tube strip orientation for collecting final libraries.

Tube Strip (TS) Holder



Consult the Chromium Connect User Guide (CG000180) for more information.

Gene Expression Library Construction Guidelines

cDNA QC and Quantification Recommendations

Sample Index PCR

Post Library Construction QC

cDNA QC & Quantification

Before proceeding with automated library construction, QC and quantify the input cDNA as per guidelines provide in the relevant user guide and then proceed to automated Sample Index PCR.

Sample Index PCR

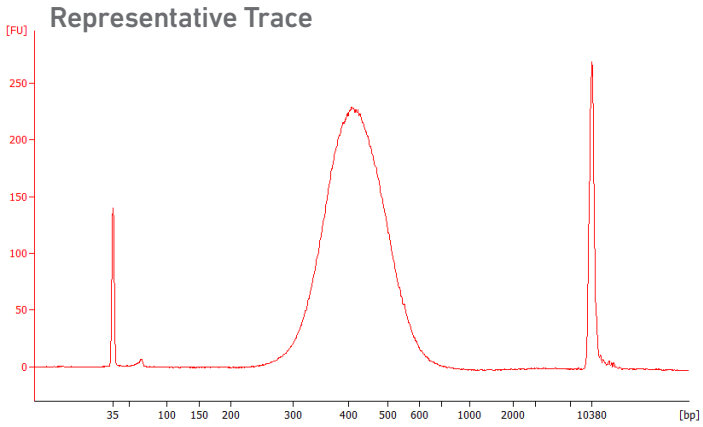
- The SI-PCR cycle number should be selected based on cDNA input. A range of cycle number options is available, to be determined based on specific experimental considerations.
- Follow the guidelines in the **relevant automation workflow user guides (listed below)** for quantification of the cDNA to be used in library construction. For Single Cell 3' Gene Expression library construction, use fixed volume of 15 µl cDNA. For Single Cell 5' Gene Expression library construction, use up to 60 ng cDNA in 5-22 µl. Note that the intended cDNA input amounts differ slightly from those used in manual protocols in order to account for pipetting differences in automation.

  For Single Cell 3' Gene Expression libraries, refer to Chromium Next GEM Automated Single Cell 3' Reagent Kits v3.1 (CG000286)

  For Single Cell 5' Gene Expression libraries, refer to Chromium Next GEM Automated Single Cell 5' Reagent Kits v2 (CG000384)
- Cycle number selected will apply to all the samples in the run.

Post Library Construction QC

Run sample on an Agilent Bioanalyzer High Sensitivity chip.



Determine the average fragment size from the Bioanalyzer trace. This will be used as the insert size for library quantification.

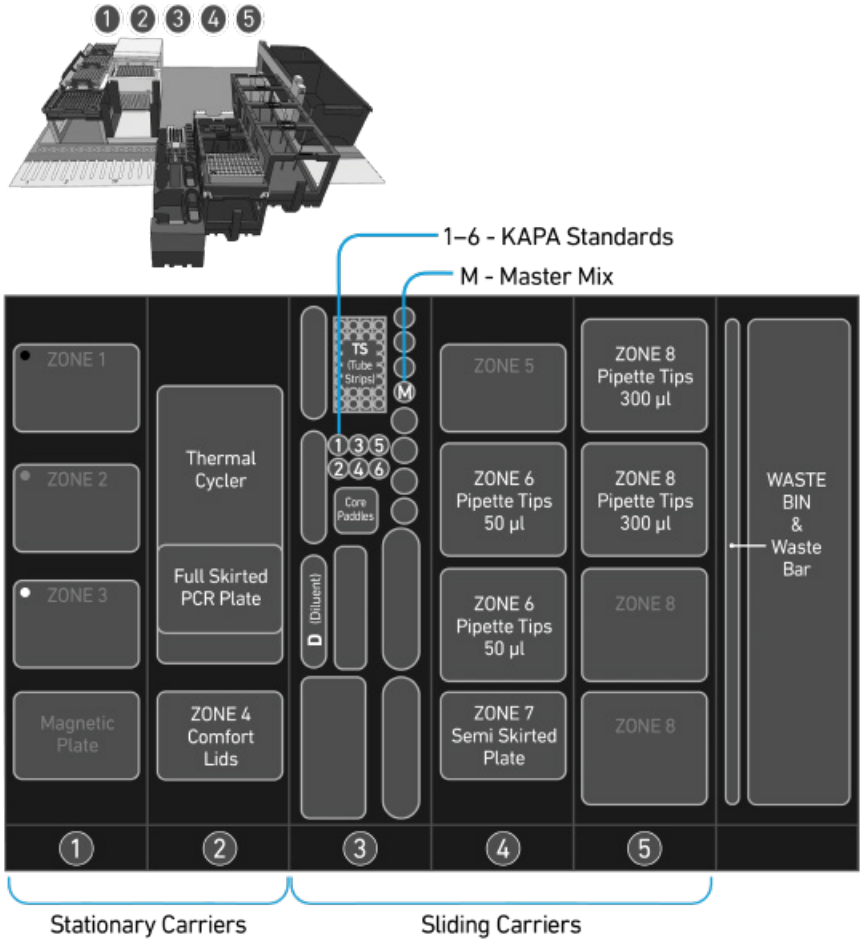
Post Library Construction Quantification & Pooling

Deck Orientation – Library Quantification
Post Library Construction Quantification
Deck Orientation – Library Pooling
Library Pooling

Deck Orientation – Library Quantification

Library quantification using qPCR is recommended for accurate pooling and loading on sequencers. If the option is selected during gene expression run-setup, automated qPCR plate set-up can be run directly on Chromium Connect after library generation and final library QC. Alternatively, the option can be selected from the instrument home screen, at the user's convenience. Up to 8 samples can be quantified on a 96 well reaction plate, including duplicates for each sample. The minimum sample volume required is **25 µl.** Only **6 µl** of the sample will be used for qPCR plate setup.

The Chromium Connect deck layout for Library Quantification setup is shown below.



Gather Items & Reagents

Follow prompts on the Chromium Connect touchscreen to gather the listed items and reagents for loading the Deck Carriers for Library Quantification.

Gather the quantities specified for each of the items and reagents.

Item	Qty
Hamilton	
Comfort Lid	1
50 µl CO-RE/CO-RE II Pipette Tips, with filter (Black, Conductive)	1-2 racks
300 µl CO-RE/CO-RE II Pipette Tips, with filter (Black, Conductive)	1-2 racks
60-ml Reagent Reservoir	1
Eppendorf	
96-well Semi Skirted Plate	1
Thermo Fisher Scientific	
2-ml Tube with Screw Cap	6
Bio-Rad	
96-well Hard-Shell Full Skirted Plate	1
Reagent	**Qty**
Qiagen Buffer EB	50 ml
Nuclease-free Water	1 ml
10% Tween-20	250 µl
Libraries (in up to four 8-tube strips)	up to 8 libraries
KAPA Library Quantification Kit, thawed	
SYBR FAST Master Mix Primer Mix Standards	5 ml 1 ml 6

Post Library Construction Quantification

- Prepare reagents as prompted on the touchscreen.
- Vortex and centrifuge KAPA standards and libraries before use.
- Retrieve previously prepared Master Mix + Primer Mix
  OR
  Add **1 ml** Primer Mix to **5 ml** SYBR FAST Master Mix.
- Prepare specified Quantification Master Mix in the 2-ml tube using the guidance below.

# Sample	Master Mix + Primer Mix (µl)	Water (µl)	Total Vol (µl)
8	1305	435	1740
7	1200	400	1600
6	1095	365	1460
5	990	330	1320
4	885	295	1180
3	780	260	1040
2	675	225	900
1	570	190	760

Volumes listed take into account volume for 6 standards

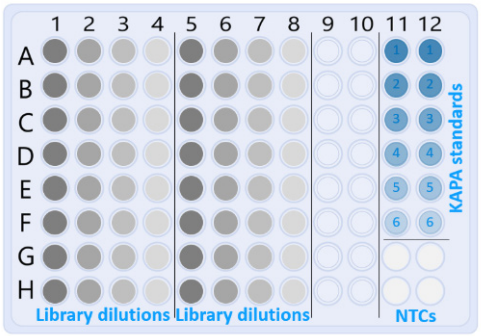
Post Library Construction Quantification

- Follow the touchscreen prompts for loading, scanning, and executing the run.
- During the run, the following steps will be executed by the instrument:
  -KAPA Master Mix transfer to the 96-well Hard Shell Full Skirted Plate (layout below)
  -Diluent transfer to dilution plate
  -Serial dilutions of libraries
  -Addition of library dilutions, KAPA Standards, and negative controls to the plate

Total reaction volume (20 µl)=
16 µl Master Mix
+
4 µl Library Dilution/
KAPA Standard/
Negative Control (NTC)

Dilutions:

1:12,500
1:62,500
1:312,500
1:1,562,500



- After the run is completed, follow the unloading instructions on the touchscreen.
- Cap and store libraries at **4˚C ≤72 h** or **-20˚C ≤4 weeks**.
- Remove Full Skirted Plate. Seal plate and centrifuge at **300 rcf** for **1 min** at **22˚C**.
- Follow the manufacturer's recommendations for qPCR-based quantification. For library quantification for sequencer clustering, determine the concentration based on average size (bp) derived from the Bioanalyzer/TapeStation trace.

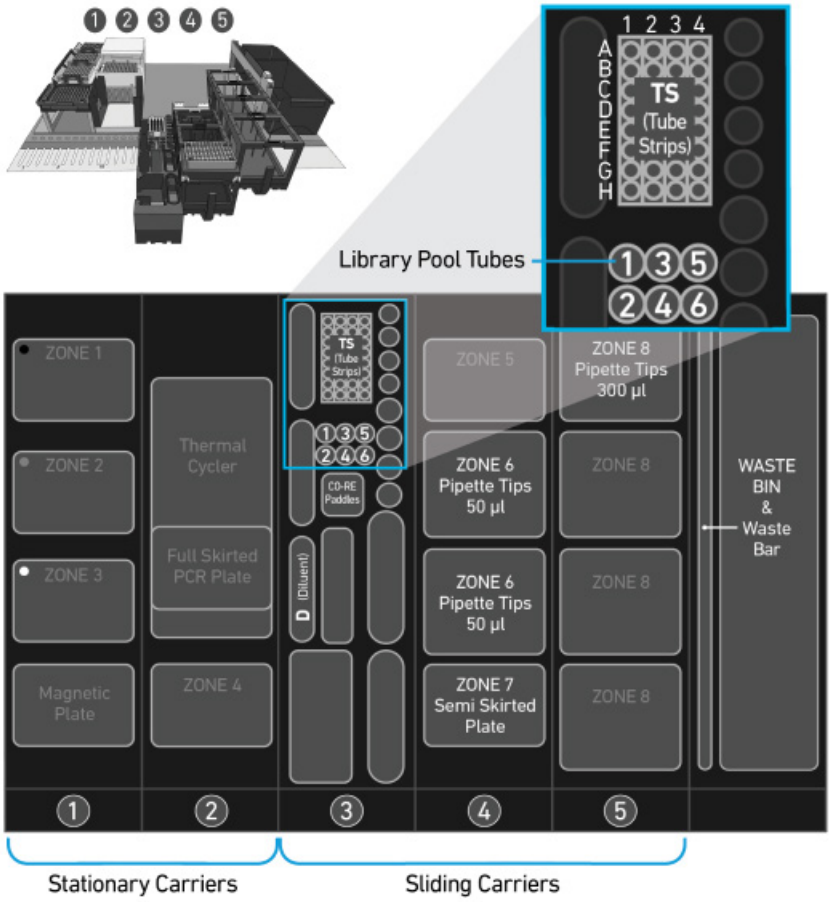
Step	Temperature	Run Time
1	95°C	00:05:00
2	95°C	00:00:30
3	60°C	00:00:45
4	Go to Step 2, 29X (Total 30 cycles)	

- Resource:
  Use the Chromium Connect Library Quantification Worksheet (CG000157) provided on the 10x Genomics Support website for calculating library concentrations.

Deck Orientation – Library Pooling

The libraries may be pooled on the Chromium Connect instrument and used for sequencing, taking into account the preferred cell numbers and per-cell read depth requirements for each library. Samples utilizing the same sample index should not be pooled together, or run on the same flow cell lane, as this would prevent correct sample demultiplexing.

The Chromium Connect deck layout for Library Pooling is shown below.



Gather Items & Reagents

Follow prompts on the Chromium Connect touchscreen to gather the listed items and reagents for loading the Deck Carriers for Library Pooling.

Gather the quantities specified for each of the items and reagents.

Item	Qty
Hamilton	
50 µl CO-RE Pipette Tips, with filter (Black, Conductive)	1-2 racks
300 µl CO-RE Pipette Tips, with filter (Black, Conductive)	1-2 racks
Reagent Reservoir, 60 ml	1
Eppendorf	
96-well Semi Skirted Plate	1
Thermo Fisher Scientific	
0.5-ml Tube with Screw Cap	6

Reagent	Qty
Qiagen Buffer EB	50 ml
Libraries (in up to four 8-tube strips)	up to 32 libraries

Library Pooling

- Follow the touchscreen prompts for loading, scanning, and executing the run.
- Briefly vortex and centrifuge libraries in the 8-tube strips.
- Ensure a minimum **25 µl** library volume is available in the tubes.
- After run is complete, follow touchscreen prompts to unload and store the libraries.
- Unload remaining items and clean as prompted on the touchscreen.
- Resource:
  Use the Chromium Connect Library Pooling Worksheet (CG000466) provided on the 10x Genomics Support website to calculate volumes to be pooled. The calculated volumes can be input into the instrument either manually, or via the CSV file generated from this workbook.

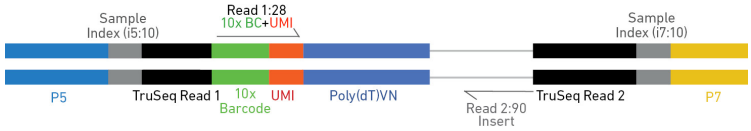
Sequencing

Sequencing Libraries

DUAL INDEX

Gene Expression libraries comprise standard Illumina paired-end constructs which begin with P5 and end with P7. 16 bp 10x Barcodes are encoded at the start of TruSeq Read 1, while 10 bp i5 and i7 sample index sequences are incorporated as sample index reads. TruSeq Read 1 and Read 2 are standard Illumina sequencing primer sites used in paired-end sequencing. TruSeq Read 1 is used to sequence 16 bp 10x Barcodes and 12 bp UMI. Sequencing these libraries produce a standard Illumina BCL data output folder.

Chromium Single Cell 3' Gene Expression Library (Dual Index)



Chromium Single Cell 5' Gene Expression Library (Dual Index)

Illumina Sequencer Compatibility

The compatibility of the listed sequencers has been verified by 10x Genomics. Some variation in assay performance is expected based on sequencer choice. For more information about performance variation, visit the 10x Genomics Support website.

- MiSeq
- NextSeq 500/550
- NextSeq 1000/2000
- HiSeq 3000
- NovaSeq

Sample Indices

Each sample index in the Dual Index Kit TT (PN-1000215) is a mix of 1 unique i7 and one unique i5 sample index. If multiple samples are pooled in a sequence lane, the sample index name (i.e. the Dual Index TT Set A plate well ID, SI-TT-) is needed in the sample sheet used for generating FASTQs with "cellranger mkfastq". Samples utilizing 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.

Gene Expression Library Sequencing Depth & Run Parameters

Sequencing Depth	Minimum 20,000 read pairs per cell
Sequencing Type	Paired-end, dual indexing
Sequencing Read	Recommended Number of Cycles
For Dual Index	
Read 1	28 cycles
i7 index	10 cycles
i5 index	10 cycles
Read 2	90 cycles

Library Loading

Once quantified and normalized, the Gene Expression libraries should be denatured and diluted as recommended for Illumina sequencing platforms. Refer to Illumina documentation for denaturing and diluting libraries. Refer to the 10x Genomics Support website for more information.

For Chromium Singel Cell 3' Gene Expression libraries

Instrument	Loading Concentration (pM)	PhiX (%)
MiSeq	11	1
NextSeq 500/550	1.8	1
NextSeq 1000/2000	650	1
NovaSeq	150*/300	1

For Chromium Singel Cell 5' Gene Expression libraries

Instrument	Loading Concentration (pM)	PhiX (%)
MiSeq	10	1
NextSeq 500	1.5	1
NovaSeq	150*/300	1
NextSeq 2000	650	1

* Use 150 pM loading concentration for Illumina XP workflow.

Library Pooling

The Gene Expression libraries may be pooled for sequencing, taking into account the differences in cell number and per-cell read depth requirements between each library. Samples utilizing the same sample index should not be pooled together, or run on the same flow cell lane, as this would prevent correct sample demultiplexing.

Refer to Post Library Construction Quantification & Pooling chapter for library pooling on the Chromium Connect instrument.