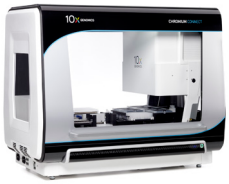
CG000286 Rev D



USER GUIDE

Chromium Next GEM Automated Single Cell 3' Reagent Kits v3.1

FOR USE WITH

Chromium Next GEM Automated Single Cell 3' Library and Gel Bead Kit v3.1, 24 rxns PN-1000141
Chromium Next GEM Automated Single Cell 3' Library and Gel Bead Kit v3.1, 4 rxns PN-1000147
Chromium Next GEM Automated Chip G Single Cell Kit, 48 rxns PN-1000136
Chromium Next GEM Automated Chip G Single Cell Kit, 16 rxns PN-1000146
Single Index Kit T Set A, 96 rxns PN-1000213
Dual Index Kit TT Set A, 96 rxns PN-1000215

Notices

Document Number

CG000286 • Rev D

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

Email: support@10xgenomics.com

10x Genomics

6230 Stoneridge Mall Road

Pleasanton, CA 94588 USA

Document Revision Summary

Document Number	CG000286
Title	Chromium Next GEM Automated Single Cell 3' Reagent Kits v3.1 User Guide
Revision	Rev D
Revision Date	December 2021

Specific Changes:

Updated to include information on Chromium Automated modular workflow compatibility.

General Changes:

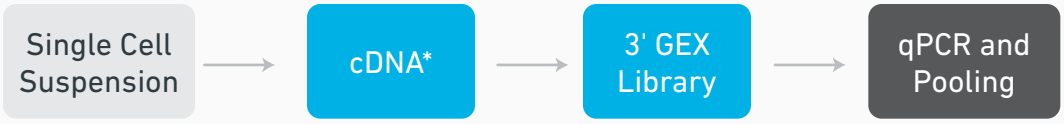
Updated for general minor consistency of language and terms throughout.

Table of Contents

Introduction

Chromium Automated Single Cell 3' Workflow
Chromium Next GEM Automated Single Cell 3' Reagent Kits v3.1
Additional Kits, Reagents & Equipment
Recommended Thermal Cyclers
Protocol Steps & Timing
Stepwise Objectives

Chromium Automated Single Cell 3' Workflow

Assay	3' Gene Expresssion Workflow
3' Gene Expression	

*For Automated Gene Expression flexible workflow with cDNA storage option, refer to Chromium Next GEM Single Cell 3' cDNA Kit v3.1 User Guide Supplement (CG000472) and Automated Library Construction User Guide (CG000474).

Chromium Next GEM Automated Single Cell 3' Reagent Kits v3.1

Chromium Next GEM Automated Single Cell 3' Library and Gel Bead Kit v3.1, 24 rxns PN-1000141

Reagent volumes and colors are different in each of the module types.

Chromium Next GEM Automated Single Cell 3' Library Kit v3.1, Module 1, 24 rxns PN-1000138 (store at 4°C)

Chromium
Next GEM Automated
Single Cell 3' v3.1, Module 1, 24 rxns

	#
Module 1	24 tube strips
Dynabeads™ MyOne™ SILANE	6 tubes (PN-2000048)

10xGenomics.com 10x GENOMICS

Chromium Next GEM Automated Single Cell 3' Library Kit v3.1, Module 2, 24 rxns PN-1000139 (store at -20°C)

Chromium
Next GEM Automated
Single Cell 3' v3.1, Module 2, 24 rxns

	#
Module 2	24 tube strips

10xGenomics.com 10x GENOMICS

Chromium Next GEM Automated Single Cell 3' Library Kit v3.1, Module 3, 24 rxns PN-1000140 (store at -20°C)

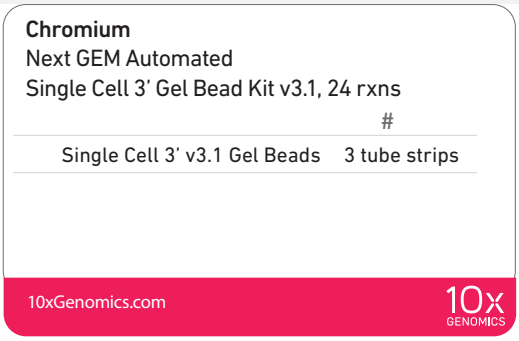
Chromium
Next GEM Automated
Single Cell 3' v3.1, Module 3, 24 rxns

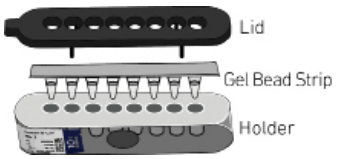
	#
Module 3	24 tube strips
Template Switch Oligo	6 tubes (PN-3000228)

10xGenomics.com 10x GENOMICS

Chromium Next GEM Automated Single Cell 3' Library and Gel Bead Kit v3.1, 24 rxns PN-1000141

Chromium Next GEM Automated Single Cell 3' Gel Bead Kit v3.1, 24 rxns PN-1000137 (store at -80°C)





Chromium Next GEM Automated Single Cell 3' Library and Gel Bead Kit v3.1, 4 rxns PN-1000147

Reagent volumes and colors are different in each of the module types.

Chromium Next GEM Automated Single Cell 3' Library Kit v3.1, Module 1, 4 rxns PN-1000149 (store at 4°C)

Chromium
Next GEM Automated
Single Cell 3' v3.1, Module 1, 4 rxns

	#
Module 1	4 tube strips
Dynabeads™ MyOne™ SILANE	2 tubes (PN-2000048)

10xGenomics.com

Chromium Next GEM Automated Single Cell 3' Library Kit v3.1, Module 2 & 3, 4 rxns PN-1000150 (store at -20°C)

Chromium
Next GEM Automated
Single Cell 3' v3.1, Module 2 & 3, 4 rxns

	#
Module 2	4 tube strips
Module 3	4 tube strips
Template Switch Oligo	2 tubes (PN-3000228)

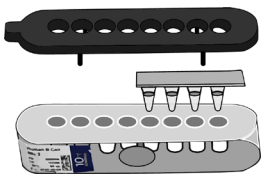
10xGenomics.com

Chromium Next GEM Automated Single Cell 3' Gel Bead Kit v3.1, 4 rxns PN-1000148 (store at -80°C)

Chromium
Next GEM Automated
Single Cell 3' Gel Bead Kit v3.1, 4 rxns

	#
Single Cell 3' v3.1 Gel Beads	4 rxn-tube strip

10xGenomics.com



Chromium Next GEM Automated Chip G Single Cell Kit, 48 rxns PN-1000136 (store at ambient temperature)

Chromium
Partitioning Oil

	#	PN
Partitioning Oil	6	2000190

Chromium
50% Glycerol

	#	PN
50% Glycerol	6	2000109

Chromium
Next GEM Chip G
Automated Single Cell

	#	PN
Next GEM Chip G Automated Single Cell (gasket attached)	6	2000181

10xGenomics.com

10x GENOMICS

Chip (gasket attached)

Chromium Next GEM Automated Chip G Single Cell Kit, 16 rxns PN-1000146 (store at ambient temperature)

Chromium
Partitioning Oil

	#	PN
Partitioning Oil	2	2000190

Chromium
50% Glycerol

	#	PN
50% Glycerol	2	2000109

Chromium
Next GEM Chip G
Automated Single Cell

	#	PN
Next GEM Chip G Automated Single Cell (gasket attached)	2	2000181

10xGenomics.com

10x GENOMICS

Chip (gasket attached)

Choose either Single Index or Dual Index kit based on the type of library (single index or dual index) being generated.

Single Index Kit T Set A, 96 rxns PN-1000213 (store at –20°C)

Single Index Kit T

	#	PN
Single Index Plate T Set A	1	2000240

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 Chromium Connect Automated Single Cell 3' 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	235948 235903 194051 814300
Eppendorf	96-well Full-Skirted Plate* 96-well Semi-Skirted Plate **Alternatively, use Amplifyt 96-Well PCR Plates, Full-Skirted, Clear from Thomas Scientific (485096/1149K05) or Fisher Scientific (NC1959287 for 25/case, NC1959288 for 100/case)*	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 Low TE Buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA)	AM9937 12090-015
Millipore Sigma	Ethanol, Pure (200 Proof, anhydrous)	E7023-500ML
Qiagen	Qiagen Buffer EB	19086
Equipment		
10x Genomics - - - -	10x Vortex Adapter Benchtop Vortex Benchtop Centrifuge Plate Centrifuge Benchtop Thermal Cycler	330002 - - - -
Additional materials ONLY for optional assays – qPCR and pooling		
Bio-Rad	10% Tween 20 96-well PCR Plates	1662404 HSP9665
Qiagen	Qiagen Buffer EB	19086
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
Additional materials for Chromium Connect maintenance		
Thor Labs	Lens tissues	MC-5
VWR	Microcide SQ Broad Spectrum Disinfectant (For cleaning metal surfaces use 70% sterile Isopropanol (Contec, SB167030IR) or other polycarbonate compatible disinfectant)	25099

Use only indicated cleaning agents. DO NOT use bleach or organic oxidizers.

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

		Steps	Timing
3 h	MANUAL	Cell Preparation (Dependent on Cell Type)	~1-1.5 h
		Gather & Load Reagents and Consumables	~60 min
	AUTOMATED	Master Mix Preparation	~8.5 h Walk-away time
		Chromium Automated Controller Loading	
		GEM Generation	
		OPTIONAL Confirm GEM Generation (Manual, 5 min)	
6 h		Post GEM RT-Cleanup – Dynabead	
		cDNA Amplification	
		cDNA Cleanup – SPRIselect	
		OPTIONAL cDNA QC & Quantification (Manual, 50 min; best practice)	
9 h		Fragmentation, End Repair & A-tailing	
		Post Fragmentation, End Repair & A-tailing Double Sided Size Selection – SPRIselect	
		Adaptor Ligation	
12 h		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 Objectives

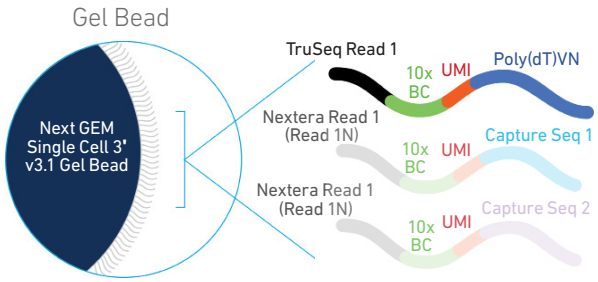
Chromium Connect automates the preparation of sequencing-ready, single cell libraries from input samples with walk-away convenience. Generation of Chromium Single Cell 3' Gene Expression libraries on the Chromium Connect instrument includes automated Gel Beads-in-emulsion (GEM) generation, barcoding, and library preparation from single cell suspensions, along with additional functionalities for library quantification and pooling.

The Chromium Connect platform for 3' digital gene expression profiles 500-10,000 individual cells per sample. A pool of ~3,500,000 10x Barcodes is sampled separately to index each cell's transcriptome. It is done by partitioning thousands of cells into nanoliter-scale GEMs, where all generated cDNA share a common 10x Barcode. Libraries are generated and sequenced from the cDNA and 10x Barcodes are used to associate individual reads back to the individual partitions.

This document outlines the key automated protocol steps for generating Single Cell 3' Gene Expression libraries.

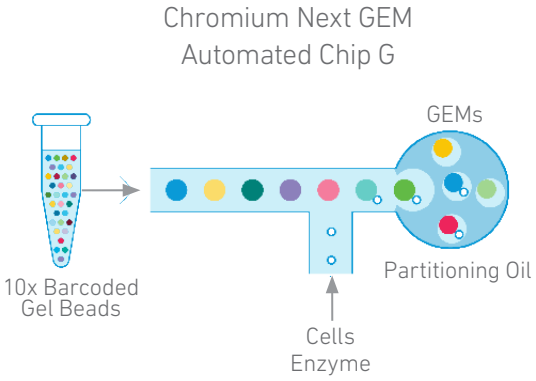
Single Cell 3' v3.1 Gel Beads

The Single Cell 3' v3.1 Gel Bead poly(dT) primer enables the production of barcoded, full-length cDNA from poly-adenylated mRNA, for generating Single Cell 3' Gene Expression libraries.



Automated GEM Generation & Barcoding

Automated GEM generation is done by combining barcoded Single Cell 3' v3.1 Gel Beads, a Master Mix containing cells and enzymes, and Partitioning Oil onto Chromium Next GEM Automated Chip G. To achieve single cell resolution, cells are delivered at a limiting dilution, such that the majority (~90-99%) of generated GEMs contain no cell, while the remainder largely contain a single cell.



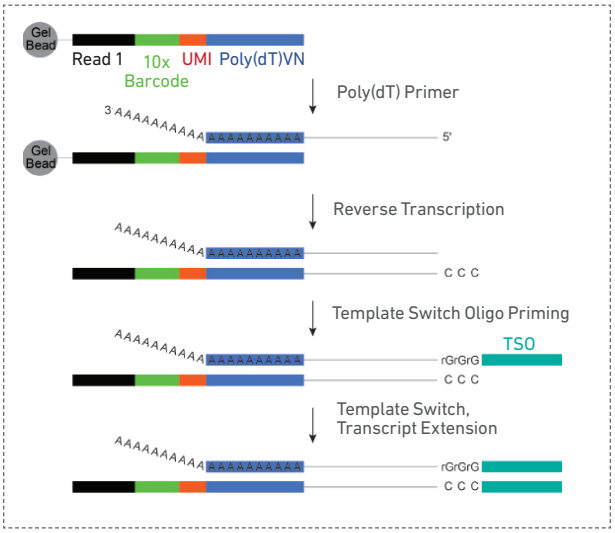
Automated GEM Generation & Barcoding

Immediately following GEM generation, the Gel Beads are dissolved, primers released, and any co-partitioned cell is lysed. Primers containing:

- an Illumina TruSeq Read 1 (read 1 sequencing primer)
- 16 nt 10x Barcode
- 12 nt unique molecular identifier (UMI)
- 30 nt poly(dT) sequence

are mixed with the cell lysate and a Master Mix containing reverse transcription (RT) reagents. Incubation of the GEMs produces barcoded, full-length cDNA from poly-adenylated mRNA.

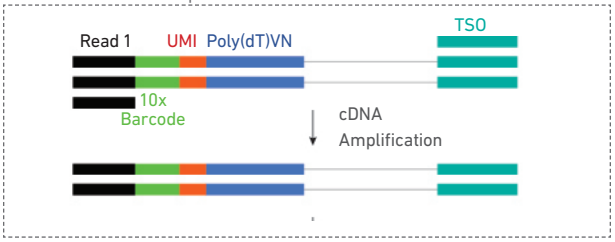
Inside individual GEMs



Automated Post GEM-RT Cleanup & cDNA Amplification

After incubation, GEMs are broken and pooled fractions are recovered. Silane magnetic beads are used to purify the first-strand cDNA from the post GEM-RT reaction mixture, which includes leftover biochemical reagents and primers. Barcoded, full-length cDNA is amplified via PCR to generate sufficient mass for library construction.

Pooled cDNA amplification

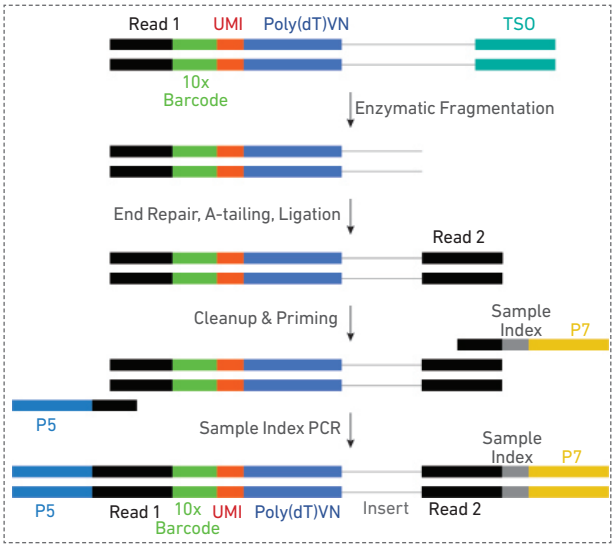


Automated 3' Gene Expression Library Construction

Single Index

Enzymatic fragmentation and size selection are used to optimize the cDNA amplicon size. P5, P7, a sample index, 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 cDNA processing (single index)

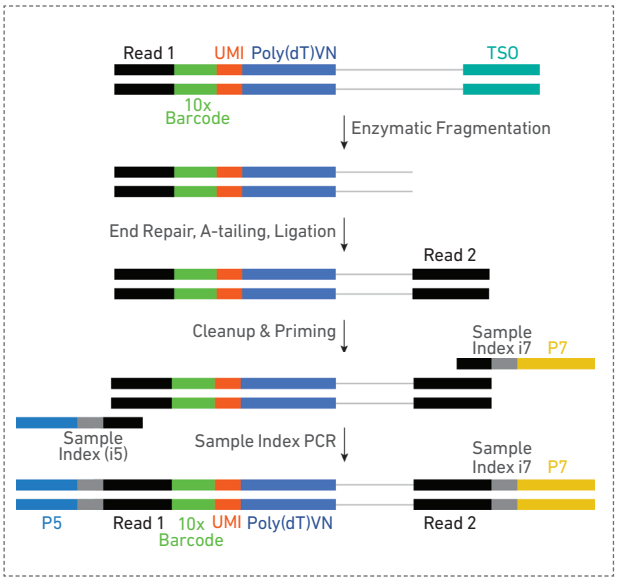


DUAL INDEX

Dual Index

Enzymatic fragmentation and size selection are used to optimize the 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 cDNA processing (dual index)

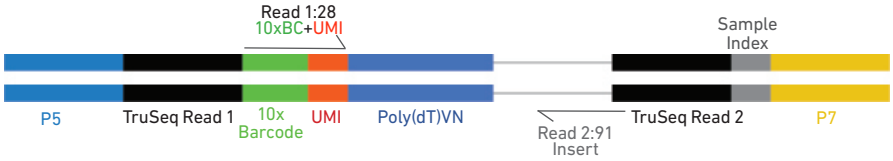


Sequencing

A Chromium Single Cell 3' 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 index read for Single Index Libraries. 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.

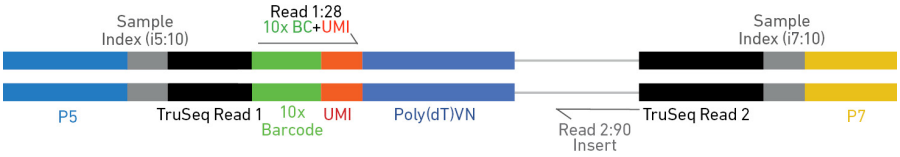
Illumina sequencer compatibility, sample indices, library loading and pooling for

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



DUAL INDEX

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



See Appendix for Oligonucleotide Sequences

Tips & Best Practices

Consult the Chromium Connect User Guide (CG000180) and follow the Chromium Connect Touchscreen prompts for specifics of assay execution.

Consumables

- Use validated and recommended emulsion-safe plastic consumables as some plastics can destabilize GEMs.

Cell Concentration

- Resuspend samples in PBS+ 0.04% BSA. Total volume loaded onto the sample plate is **10 µl**.
- Based on stock cell concentration, do sequential stock dilutions, if needed.
- Use 3 independent cell counts to determine cell concentration.
- The presence of dead cells in the suspension may also reduce the recovery rate. Consult the 10x Genomics Single Cell Protocols Cell Preparation Guide and the Guidelines for Optimal Sample Preparation flowchart (Documents CG00053 and CG000126 respectively) for more information on preparing cells.

Multiplet Rate (%)	# of Cells Loaded	# of Cells Recovered
~0.4%	~800	~500
~0.8%	~1,600	~1,000
~1.6%	~3,200	~2,000
~2.3%	~4,800	~3,000
~3.1%	~6,400	~4,000
~3.9%	~8,000	~5,000
~4.6%	~9,600	~6,000
~5.4%	~11,200	~7,000
~6.1%	~12,800	~8,000
~6.9%	~14,400	~9,000
~7.6%	~16,000	~10,000

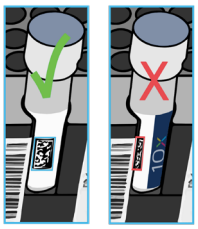
Cell Preparation

- Ensure cell counts are accurate.
- Based on stock cell concentration, do sequential stock dilutions, if needed. Refer to the Cell Suspension Volume Calculator Table for optimal pipetting volumes and concentrations.
- Load cell samples when prompted on the touchscreen.
- The cDNA amplification cycle number will be based on the cell count. The cycle number chosen for one sample will apply to all the samples in a run. Refer to cDNA Amplification Cycle Number for more information.

Reagent Handling

- Fully thaw and thoroughly mix reagents before use.
- Ensure there are no air bubbles at the bottom of reagent tubes.
- Follow the prompts on the touchscreen for handling Library Modules 1, 2, and 3 during setup and use.
- Ensure correct reagent tube barcode orientation (on tubes and racks) as prompted by the touchscreen.
- Prepare and dispense 80% ethanol off-deck to avoid spilling on consumables.
- When specified, promptly move reagents back to the recommended storage.

Barcode Orientation



Chromium Automated Chip Handling

- The automated chip includes a pre-installed gasket.
- Minimize exposure of reagents and chips to sources of particles and fibers, laboratory wipes, frequently opened flip-cap tubes, clothing that sheds fibers, and dusty surfaces.
- Keep chip and gasket in sealed package until prompted to load.
- After removing the chip from the sealed bag, use in ≤ **24 h**.
- Avoid contacting the bottom surface of the chip with gloved hands and other surfaces.
- DO NOT use chips or gaskets specific to other 10x Genomics protocols.

Chip (gasket attached)

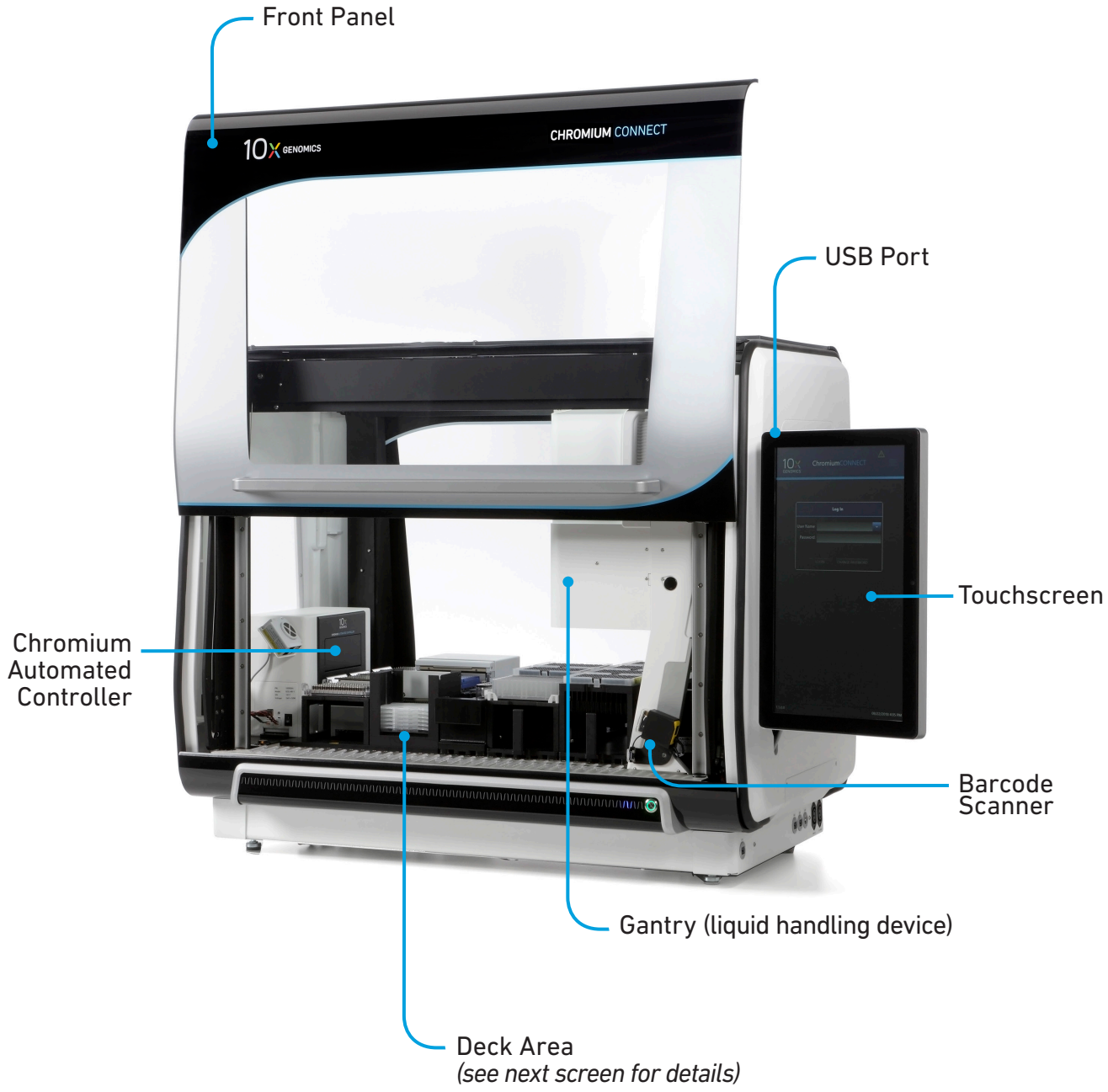
Chromium Connect

Instrument Orientation

Deck Orientation

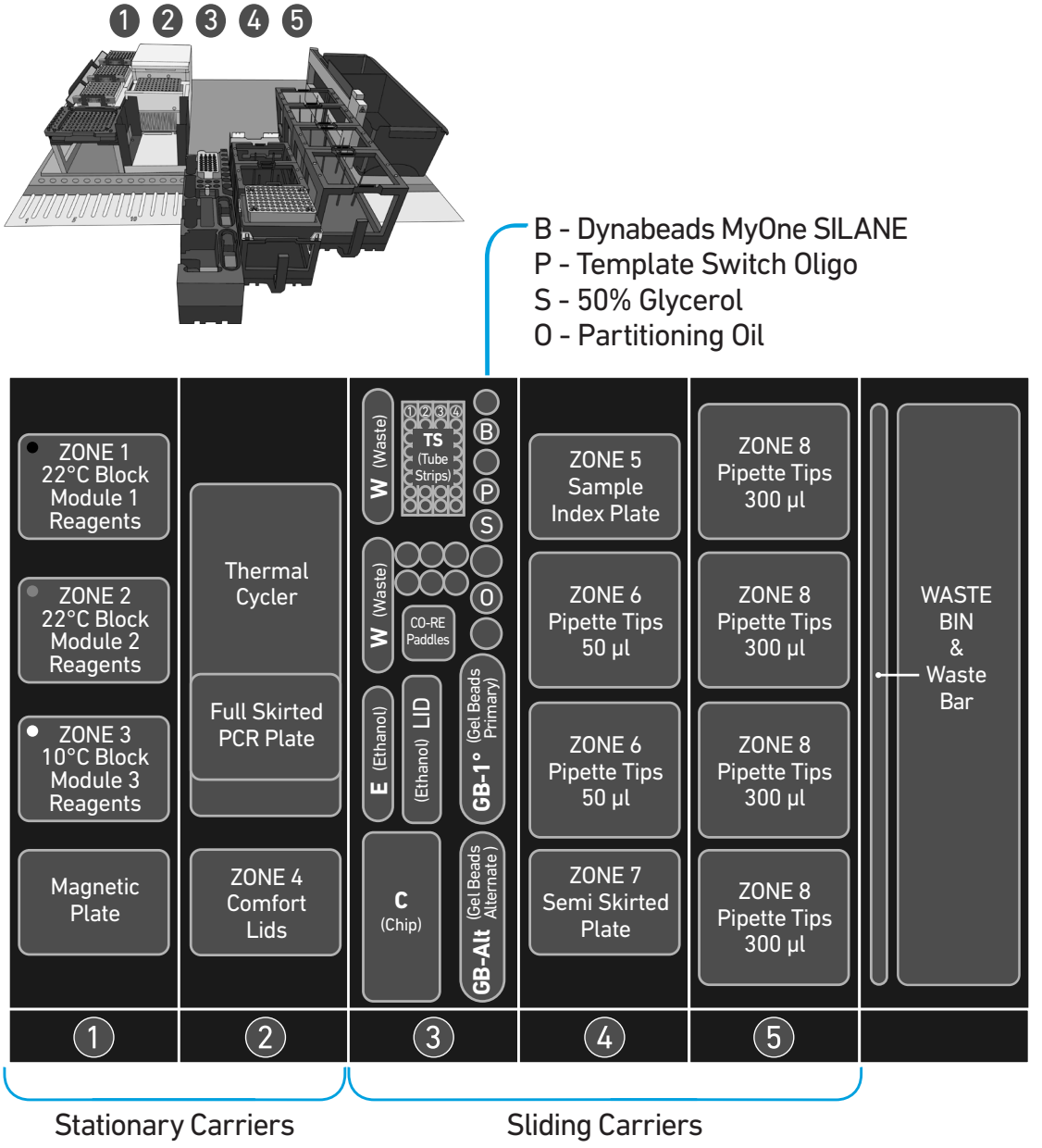
CSV Setup

Instrument Orientation



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

Deck Orientation



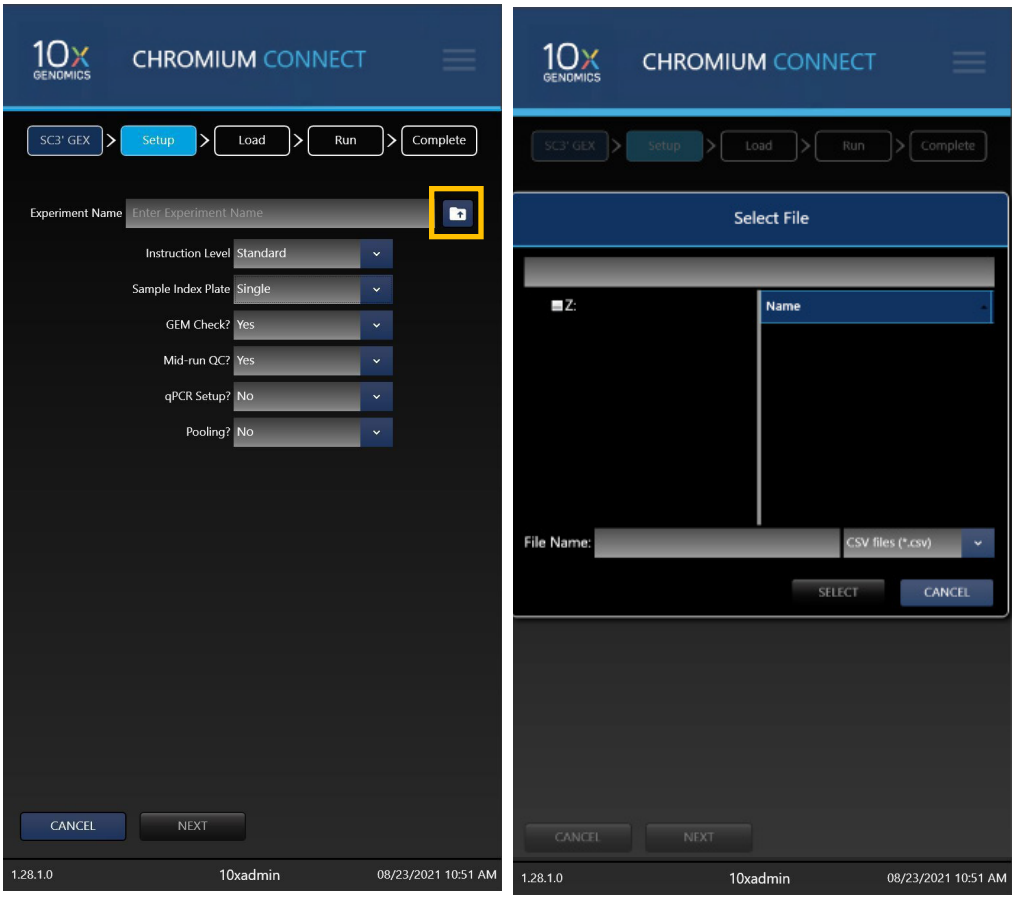
Refer to the Chromium Connect Instrument User Guide (CG000180) and Quick Reference Cards (CG000256) for more information.

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 Info Template (CG000309) is available on the 10x Genomics support website.

Select either single index or dual index in the sample index plate drop down menu

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	CELLEXPRESSION	CELLCOUNT	CYCLES	Valid	cDNA(pg/ul)	Qc3	Qc4	USERDEFINED1	USERDEFINED2	USERDEFINED3	USERDEFINED4
ID1	Sample1	A4		High	< 500	DFLT								
ID2	Sample2	B4		High	< 500	DFLT								
ID3	Sample3	C4		High	< 500	DFLT								
ID4	Sample4	D4		High	< 500	DFLT								
ID5	Sample5	E4		High	< 500	DFLT								
ID6	Sample6	F4		High	< 500	DFLT								
ID7	Sample7	G4		High	< 500	DFLT								
ID8	Sample8	H4		High	< 500	DFLT								
RUNPARAMETERS	SELECTION													
runName	Experiment1													
Instruction Level	Standard													
GEM Check?	Yes													
Mid-run QC?	Yes													
qPCR Setup?	No													
Pooling?	No													
Notes														

Uploading Sample Information Using a CSV File

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 single or 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
Cell Count	User defined field for tracking (enter applicable option EXACTLY as shown below) < 500 500-6000 > 6000 DO NOT use commas. Space between symbol & number required.
Cycles	User defined field. Refer to cDNA Amplification Cycle Number 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
GEM Check	Opt-in for optional QC step: Yes/No
Mid-run QC	Opt-in for optional QC step: Yes/No
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	
Comfort Lids	6
50 µl CO-RE Pipette Tips, with filter (Black, Conductive)	2 racks
300 µl CO-RE Pipette Tips, with filter (Black, Conductive)	4 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	2
Low TE Buffer	2 ml
10x Genomics	
Chromium Next GEM Chip G Automated Single Cell Kit (stored at room temperature) *Partitioning Oil* *50% Glycerol* *Chip G (keep chip sealed)*	1
Chromium Next GEM Automated Single Cell 3' Gel Bead Kit v3.1(stored at -80°C) *Single Cell 3' Gel Bead Kit v3.1*	1 tube/sample
Chromium Next GEM Automated Single Cell 3' Library Kit v3.1	
Library Module 1(stored at 4°C) *Black tube strip* *Dynabeads*	1 tube strip/sample 1 tube/run
Library Module 2 (stored at -20°C) *Gray tube strip*	1 tube strip/sample
Library Module 3 (stored at -20°C) *White tube strip* *Template Switch Oligo*	1 tube strip/sample 1 tube/run
Single Index Plate T Set A (stored at -20°C)	1 plate
Dual Index Plate TT Set A (stored at -20°C)	1 plate

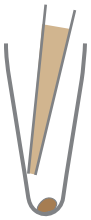
Choose either Single Index or Dual Index kit based on the type of library (single index or dual index) being generated.

See Additional Kits, Reagents & Equipment list for performing optional assays and/or 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.
Resuspend TSO	• Resuspend only when prompted on the touchscreen after samples are loaded. • Centrifuge TSO briefly. Add **1,120 µl** Low TE Buffer to resuspend. Vortex **15 sec** at maximum speed, centrifuge briefly.
Dynabeads	• Vortex Dynabeads for **≥30 sec**. • Pipette mix to resuspend completely by using a 200 µl pipettor set to 150 µl; pipette mix at least 20 times until fully resuspended. DO NOT centrifuge. • Confirm there are no bubbles at the bottom of the tube.
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. • Confirm there are no bubbles at the bottoms of any module tubes • 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).

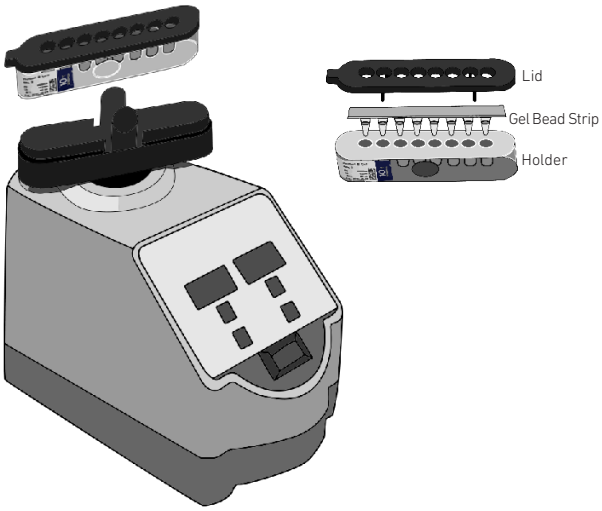


Resuspend Clump

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

Thaw & Prep Reagents

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

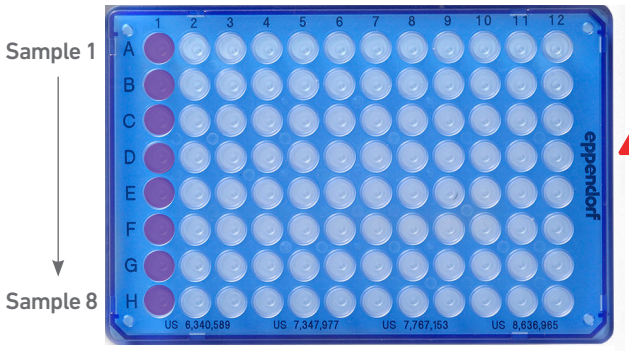
ACTION	GUIDELINES *Follow touchscreen prompts for specifics and timing*
Prepare Gel Beads	• Equilibrate the Gel Beads for **30 min** at **room temperature** before use. • Snap the tube strip holder with the Gel Bead strip into a 10x Vortex Adapter. Vortex **30 sec**. • Centrifuge the Gel Bead strip for **~5 sec** after removing from the holder. Confirm there are no bubbles at the bottom of the tubes and the liquid levels look even. • Place the Gel Bead strip back in the holder and secure the holder lid. • Store unused Gel Beads at **–80°C** and avoid more than 12 freeze-thaw cycles. DO NOT leave Gel Beads at room temperature for **>24 h**. • Remove Gel Beads from the Deck during any of the optional QCs and store the holder with the unused Gel Beads at **–80°C**. 

Sample Preparation Guidelines

Sample Preparation Guidelines

- Resuspend samples in PBS+ 0.04% BSA. Refer to Cell Suspension Volume Calculator Table for the cell suspension and buffer volumes. Total volume loaded per sample onto the sample plate is **10 µl**.
- Based on stock cell concentration, do sequential stock dilutions, if needed.
- It is recommended to use 3 independent cell counts to determine cell concentration.
- The presence of dead cells in the suspension may also reduce the recovery rate. Consult the 10x Genomics Single Cell Protocols Cell Preparation Guide and the Guidelines for Optimal Sample Preparation flowchart (Documents CG00053 and CG000126 respectively) for more information on preparing cells.
- The cell load impacts PCR cycle numbers for cDNA amplification and other downstream steps in the assay. Refer to Additional Protocol Guidelines chapter for more information.
- Differences in manual and automated sample preparation are outlined below:

	Manual	Automated
Sample Prep	Using 10x Genomics Demonstrated Protocols for cell prep and QC	
Sample Input Volume	Up to 43 µl	10 µl
Sample Loading	PCR strip tubes	96-well skirted plate
Samples per Chip	1-8	1-8
Samples Tested	Various	HEK293T, NIH3T3, PBMCs, Dissociated Embryonic Mouse Brain



Samples are loaded in column 1, starting at A1. DO NOT add glycerol in unused sample wells when running <8 samples.

Cell Suspension Volume Calculator Table

(Chromium Connect Automated Single Cell 3' protocol)

Volume of Cell Suspension Stock per reaction (µl) | Volume of PBS + 0.04% BSA (µl)

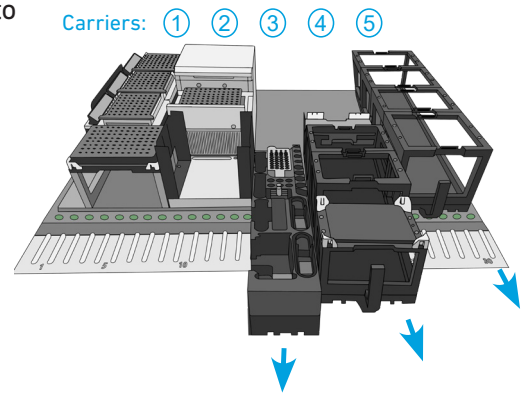
Cell Stock Concentration (cells/ µl)	Targeted Cell Recovery										
	500	1000	2000	3000	4000	5000	6000	7000	8000	9000	10000
100	8.25 1.75	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
200	4.13 5.87	8.25 1.75	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
300	2.75 7.25	5.50 4.50	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
400	2.07 7.93	4.13 5.87	8.25 1.75	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
500	1.65 8.35	3.30 6.70	6.60 3.40	9.90 0.10	n/a	n/a	n/a	n/a	n/a	n/a	n/a
600	1.38 8.62	2.75 7.25	5.50 4.50	8.25 1.75	n/a	n/a	n/a	n/a	n/a	n/a	n/a
700	1.18 8.82	2.36 7.64	4.72 5.28	7.08 2.92	9.43 0.57	n/a	n/a	n/a	n/a	n/a	n/a
800	1.04 8.96	2.07 7.93	4.13 5.87	6.19 3.81	8.25 1.75	n/a	n/a	n/a	n/a	n/a	n/a
900	0.92 9.08	1.84 8.16	3.67 6.33	5.50 4.50	7.34 2.66	9.17 0.83	n/a	n/a	n/a	n/a	n/a
1000	0.83 9.17	1.65 8.35	3.30 6.70	4.95 5.05	6.60 3.40	8.25 1.75	9.90 0.10	n/a	n/a	n/a	n/a
1100	0.75 9.25	1.50 8.50	3.00 7.00	4.50 5.50	6.00 4.00	7.50 2.50	9.00 1.00	n/a	n/a	n/a	n/a
1200	0.69 9.31	1.38 8.62	2.75 7.25	4.13 5.87	5.50 4.50	6.88 3.12	8.25 1.75	9.63 0.37	n/a	n/a	n/a
1300	0.64 9.36	1.27 8.73	2.54 7.46	3.81 6.19	5.08 4.92	6.35 3.65	7.62 2.38	8.89 1.11	n/a	n/a	n/a
1400	0.59 9.41	1.18 8.82	2.36 7.64	3.54 6.46	4.72 5.28	5.90 4.10	7.08 2.92	8.25 1.75	9.43 0.57	n/a	n/a
1500	0.55 9.45	1.10 8.90	2.20 7.80	3.30 6.70	4.40 5.60	5.50 4.50	6.60 3.40	7.70 2.30	8.80 1.20	9.90 0.10	n/a
1600	0.52 9.48	1.04 8.96	2.07 7.93	3.10 6.90	4.13 5.87	5.16 4.84	6.19 3.81	7.22 2.78	8.25 1.75	9.29 0.71	n/a
1700	0.49 9.51	0.98 9.02	1.95 8.05	2.92 7.08	3.89 6.11	4.86 5.14	5.83 4.17	6.80 3.20	7.77 2.23	8.74 1.26	9.71 0.29
1800	0.46 9.54	0.92 9.08	1.84 8.16	2.75 7.25	3.67 6.33	4.59 5.41	5.50 4.50	6.42 3.58	7.34 2.66	8.25 1.75	9.17 0.83
1900	0.44 9.56	0.87 9.13	1.74 8.26	2.61 7.39	3.48 6.52	4.35 5.65	5.22 4.78	6.08 3.92	6.95 3.05	7.82 2.18	8.69 1.31

Grey boxes: Volumes that would exceed the allowable buffer volume in each reaction
Yellow boxes: Indicate a low transfer volume that may result in higher cell load variability
Blue boxes: Optimal range of cell stock concentration to maximize the likelihood of achieving the desired cell recovery target

Carrier Loading Guidelines

Carrier Loading Guidelines

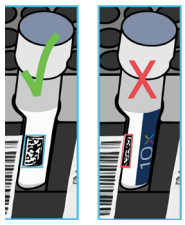
Follow the instructions on the touchscreen to load the carriers.



Carriers

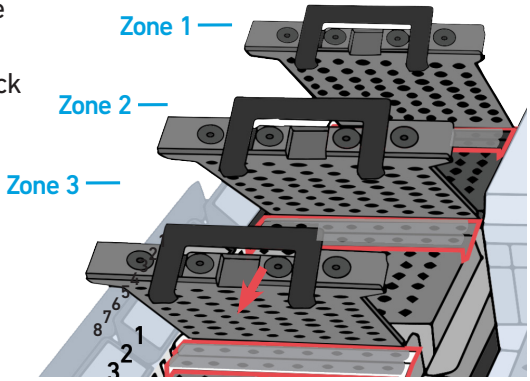
- Handle the carriers as prompted.
- Ensure that Carriers 3, 4, and 5 are completely slid out and placed on an off-deck workspace before loading.
- Align the carriers to the corresponding Deck Rails when sliding them in or out of the deck.
- Ensure correct orientation of tube labels with barcodes to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

Barcode Orientation



Modules

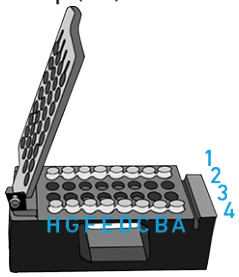
- Load one tube strip/sample of each of the indicated modules in the corresponding positions on the Carrier, starting from back to front row.
- DO NOT skip any rows when loading.
- Use pinhole alignment to place module tube strips in the correct orientation (as shown on the touchscreen).



Label Tube Strip Orientation

- The cDNA tube strip will be at Position 1 and the final library tube strip will be at Position 4 in the Tube Strip Holder.
- Label tube strip orientation for collecting cDNA and final libraries.

Tube Strip (TS) Holder

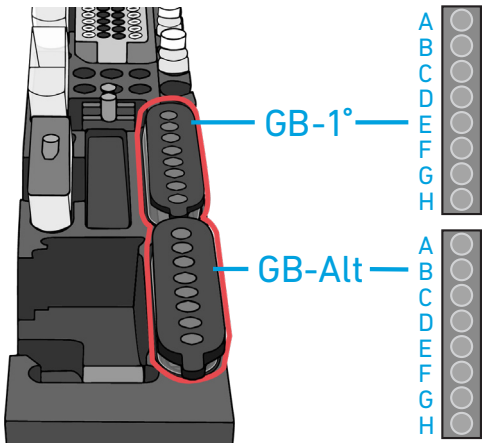


Consult the Chromium Connect User Guide (CG000180) for more information.

Carrier Loading Guidelines

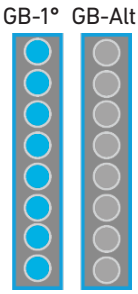
Load Gel Beads

- Up to 2 Gel Bead tube strips may be loaded in the primary (GB-1°)and alternate (GB-Alt) positions. One Gel Bead tube is required/sample.
- If only loading one tube strip, load in the primary position.
- Select the location of the loaded Gel Bead tube on the touchscreen.
- Examples of various Gel Bead loading combinations are illustrated below.



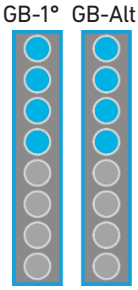
Example 1

8 samples run with 1 Gel Bead tube strip loaded in GB-1° location.



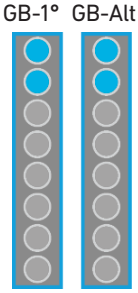
Example 2

8 samples run with 2 Gel Bead tube strips loaded in GB-1° and GB-Alt locations.



Example 3

4 samples run with 2 Gel Bead tube strips loaded in GB-1° and GB-Alt locations.

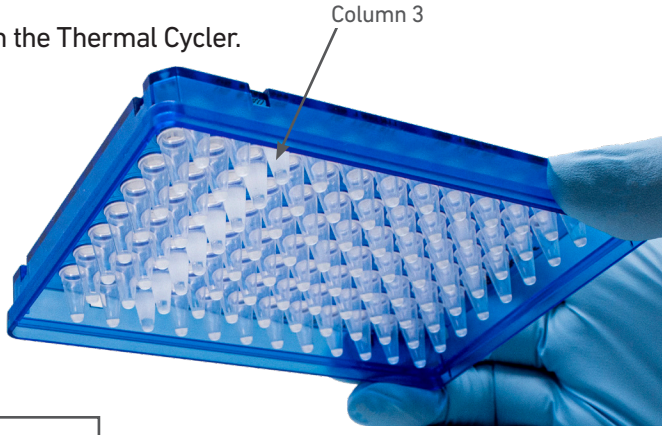


Additional Protocol Guidelines

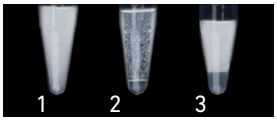
Confirm GEM Generation
cDNA Amplification Cycles
cDNA QC & Quantification
Sample Index PCR

Confirm GEM Generation

- Instrument will pause for **5 min** during GEM QC.
- Carefully remove Full Skirted Plate from the Thermal Cycler.
- Hold up the Full Skirted Plate and view the bottom of the wells in Column 3 to confirm GEM generation (shown below).
- Reload Full Skirted Plate in the Thermal Cycler.



GEM Generation Example
Tube 1 shows normal GEM generation
Tube 2 shows a wetting failure
Tube 3 indicates a clog



cDNA Amplification Cycle Number

- cDNA amplification cycles are determined by target cell number.
- Recommended guidelines for selecting optimal amplification cycle numbers when skipping cDNA QC.

Targeted Cell Recovery	RNA Content	Total cDNA Cycles	Recommended SI PCR Cycles
<500	Low	13	14
	High	13	10
500–6,000	Low	12	14
	High	12	10
>6,000	Low	11	14
	High	11	10

- The optimal number of cycles is a trade-off between generating sufficient final mass for library construction and minimizing PCR amplification artifacts. The number of cDNA cycles should also be reduced if large numbers of cells are sampled.

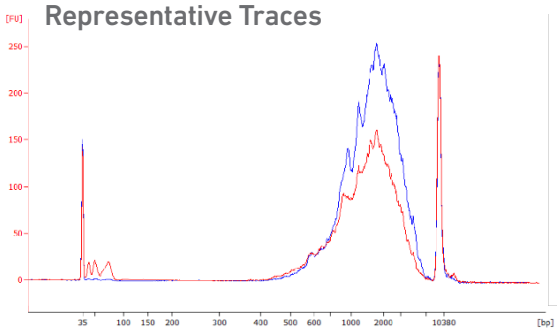
Cycle number selected for one sample will apply to all the other samples in the run.

cDNA QC & Quantification

a. Follow the instruction on the touchscreen for cDNA QC & quantification.

b. Run sample on an Agilent Bioanalyzer High Sensitivity chip.

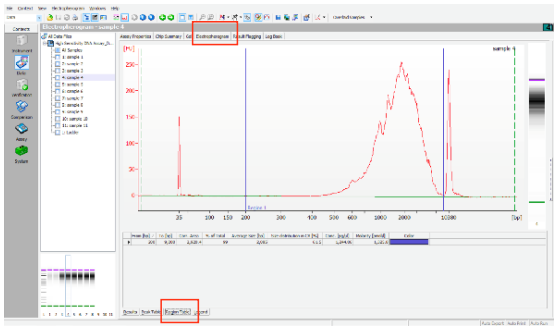
For input cells with low RNA content (<1pg total RNA/cell), 1 µl undiluted product may be run.
Lower molecular weight product (35 – 150 bp) may be present. This is normal and does not affect sequencing or application performance.



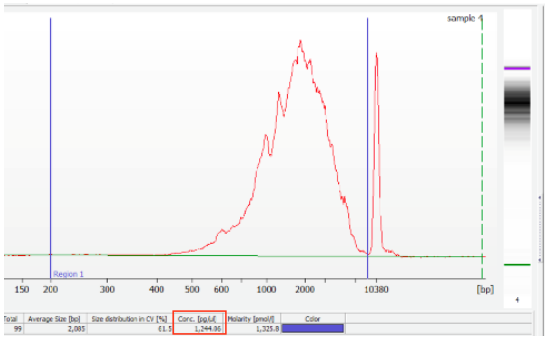
EXAMPLE CALCULATION

i. Select Region

Under the "Electropherogram" view choose the "Region Table". Manually select the region of ~200 – ~9000 bp



ii. Note Concentration [pg/µl]



Alternate Quantification Methods:

- Agilent TapeStation. See Appendix for representative traces

Agilent Bioanalyzer or Agilent TapeStation are the recommended methods for accurate quantification.

Sample Index PCR

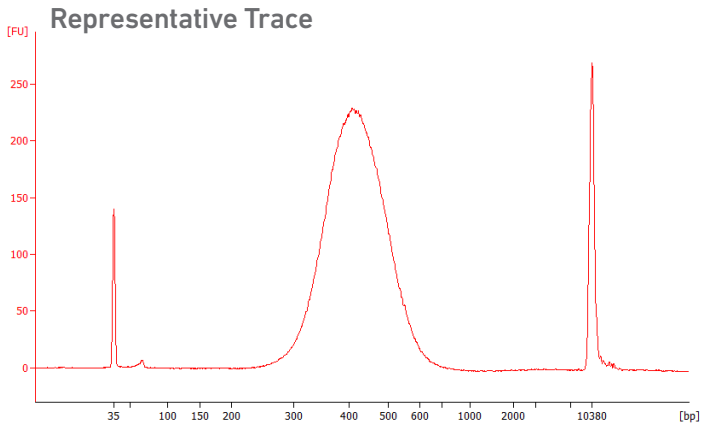
- The cycle numbers can be manually selected based on cDNA input.
- Recommended guidelines for selecting optimal Sample Index PCR cycle number. Carry forward 15 µl of cDNA if using the Automated workflow and 10 µl of cDNA if using the Manual workflow.

cDNA Concentration (pg/µl)	Total Cycles
<2,500	14
2,500-15,000	12
15,000-50,000	10
50,000-100,000	8
>100,000	6

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.

Alternate QC Method:

- Agilent TapeStation. See Appendix for representative traces

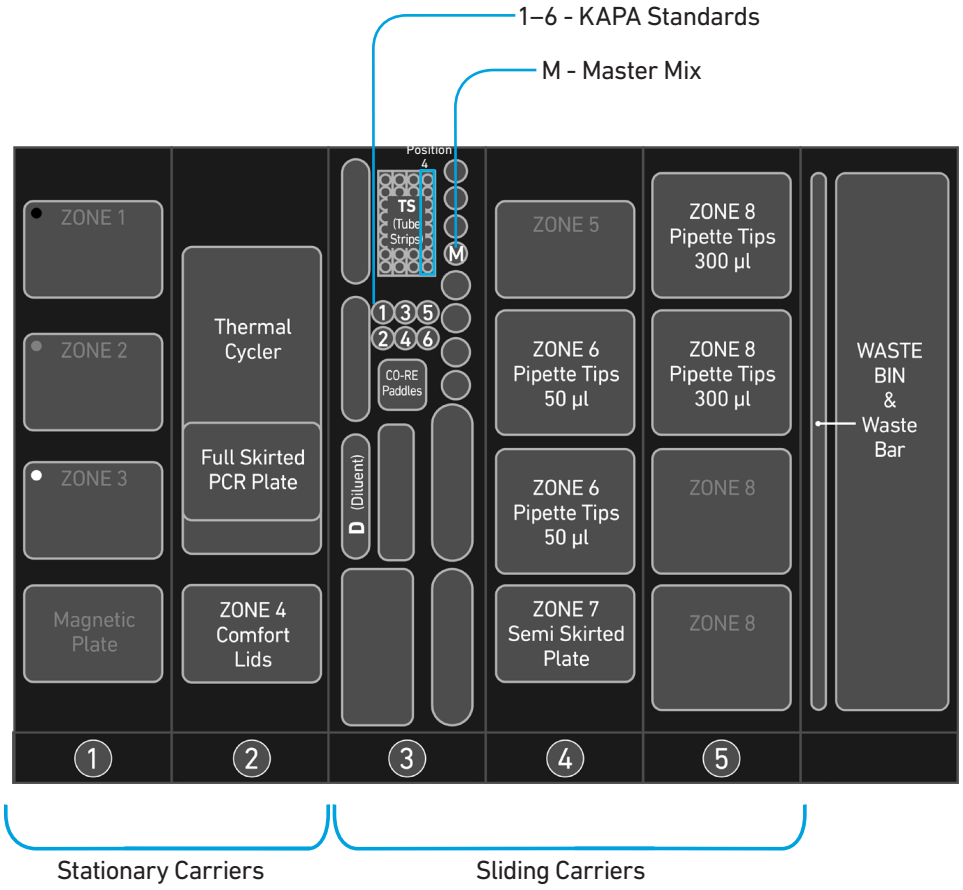
Post Library Construction Quantification & Pooling

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 Pipette Tips, with filter (Black, Conductive)	2 racks
300 µl CO-RE Pipette Tips, with filter (Black, Conductive)	2 racks
60-ml Reagent Reservoir	1
Eppendorf	
96-well Semi Skirted Plate	1
Thermo Fisher Scientific	
2-ml Tube with Screw Cap	1
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 an 8-tube strip)	1-8
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

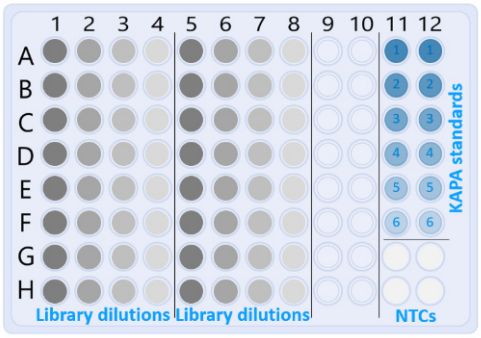
Post Library Construction Quantification

- 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.

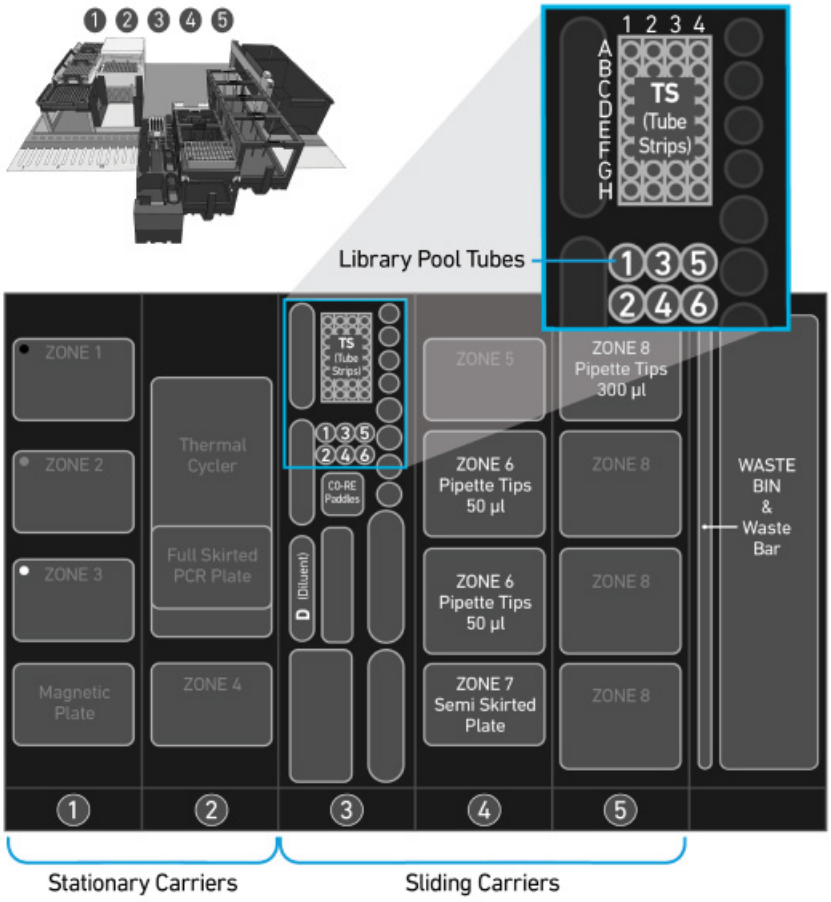
Step	Temperature	Run Time
1	95°C	00:05:00
2	95°C	00:00:30
3	60°C	00:00:45
4	Go to Step 2, 29X (Total 30 cycles)	

- 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 strip)	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 strip.
- 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.

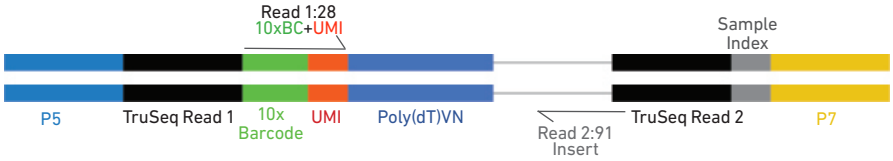
Sequencing

Sequencing Libraries

Single Index

Single Cell 3' 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 8 bp sample index sequences are incorporated as the i7 sample index read. 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 (Single Index)

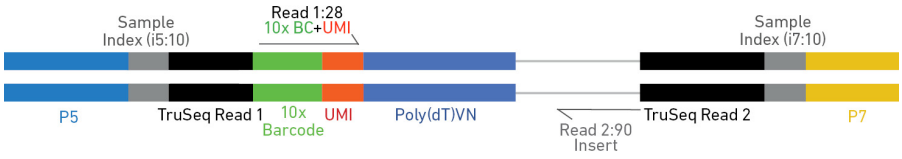


Dual Index

Single Cell 3' 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.

DUAL INDEX

Chromium Single Cell 3' 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 2500 (Rapid Run)
- HiSeq 3000/4000
- NovaSeq

Sample Indices

Single index

Each sample index in the Single Index Kit T (PN-1000213) is a mix of 4 different sequences to balance across all 4 nucleotides. If multiple samples are pooled in a sequence lane, the sample index name (i.e. the Sample Index plate T well ID, SI-GA-) 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.

DUAL INDEX

Dual Index

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.

3' Gene Expression Library Sequencing Depth & Run Parameters

Sequencing Depth	Minimum 20,000 read pairs per cell
Sequencing Type	Paired-end, single/dual indexing (depending on library type)
Sequencing Read	Recommended Number of Cycles
For single index	
Read 1	28 cycles
i7 Index	8 cycles
i5 Index	0 cycles
Read 2	91 cycles
For dual index	
Read 1	28 cycles
i7 index	10 cycles
i5 index	10 cycles
Read 2	90 cycles

Choose the sequencing parameters based on library type

DUAL INDEX

Library Loading

Once quantified and normalized, the 3' 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.

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

* Use 150 pM loading concentration for Illumina XP workflow.

Library Pooling

The 3' 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.

Appendix

Agilent TapeStation Traces

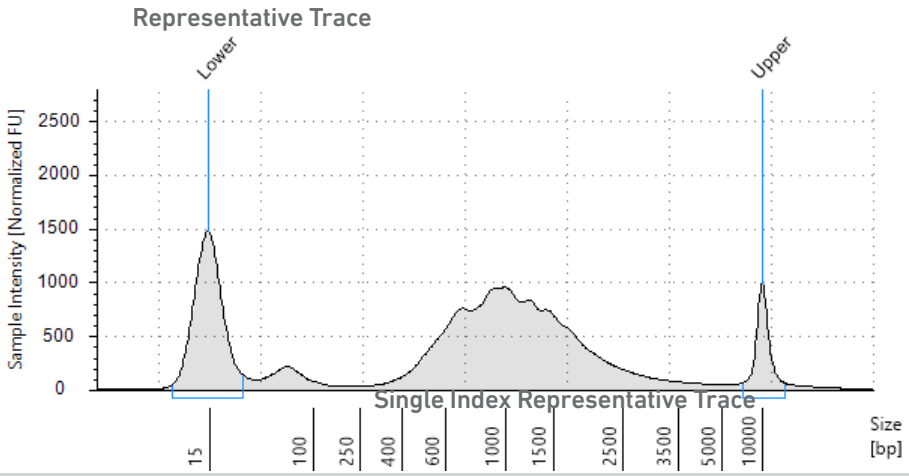
Oligonucleotide Sequences

Agilent TapeStation Traces

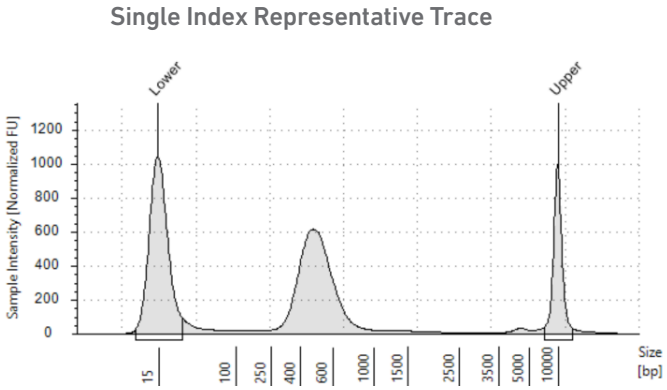
Agilent TapeStation Traces

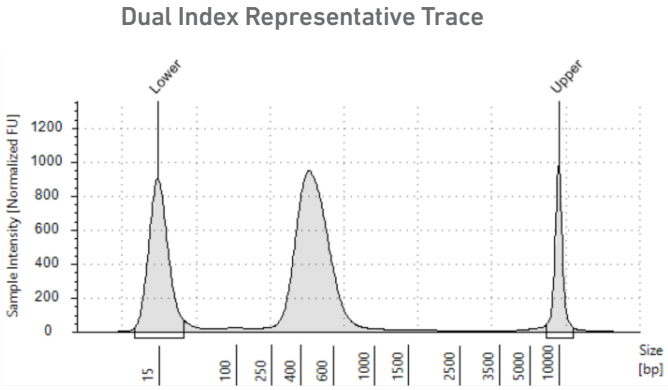
Agilent TapeStation High Sensitivity D5000 ScreenTape was used.

cDNA QC & Quantification



Post Library Construction QC





Alternate QC Method:

Qubit Fluorometer and Qubit dsDNA HS Assay Kit

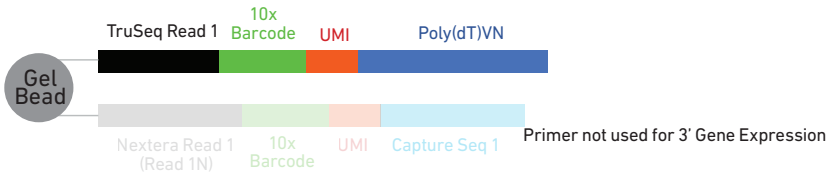
Oligonucleotide Sequences

Protocol steps correspond to the Chromium Next GEM Automated Single Cell 3' Reagent Kits v3.1 protocol.

Automated Protocol Step – GEM-RT Incubation

Gel Bead Primers

5'-CTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNNNNN-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTVN-3'



5'-GTCAGATGTGTATAAGAGACAG-NNNNNNNNNNNNNNNN-NNNNNNNNNNNN-TTGCTAGGACCGGCCTTAAAGC-3'

Template Switch Oligonucleotide PN-3000228

5'-AAGCAGTGGTATCAACGCAGAGTACATrGrGrG-3'

PCR Product

5'-CTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNNNNN-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTVN-cDNA_Insert-CCCATGTACTCTGCGTTGATACCACTGCTT-3'

Automated Protocol Step – cDNA Amplification

cDNA Primers

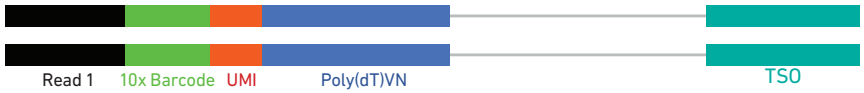
Forward Primer: Partial Read 1

5'-CTACACGACGCTCTTCCGATCT-3'

Reverse Primer: Partial TSO

5'-AAGCAGTGGTATCAACGCAGAG-3'

Amplification Products



5'-CTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNNNNN-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTVN-cDNA_Insert-CCCATGTACTCTGCGTTGATACCACTGCTT-3'

3'-GATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNNNNNN-NNNNNNNNNNNN-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAABN-cDNA_Insert-GGGTACATGAGACGCAACTATGGTGACGAA-5'

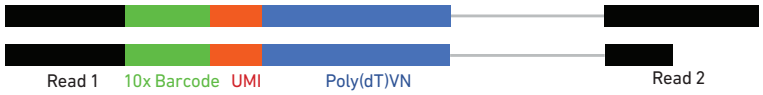
Automated Protocol Step – Adaptor Ligation

Adaptor Oligos

5'- GATCGGAAGAGCACACGTCTGAACTCCAGTCAC-3'

3'-TCTAGCCTTCTCG-5'

Ligation Product



5'-CTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNNNNN-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTVN-cDNA_Insert-AGATCGGAAGAGC ACACGTCTGAACTCCAGTCAC-3'

3'-GATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNNNNNN-NNNNNNNNNNNN-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAABN-cDNA_Insert-TCTAGCCTTCTCG-5'

Oligonucleotide Sequences

Automated Protocol Step – Single Index PCR

Sample Index PCR Primer

Single Index T Set A PN-2000240

5'-AATGATACGGCGACCACCGAGATCT-ACACTCTTTCCCTACACGACGCTC-3'

5'-CAAGCAGAAGACGGCATACGAGAT-NNNNNNNN GTGACTGGAGTTCAGACGTGT-3'

Sample Index PCR Product

5'-AATGATACGGCGACCACCGAGATCT-ACACTCTTTCCCTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNNNN-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTVN-cDNA_Insert-AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC-NNNNNNNN-ATCTCGTATGCCGTCTTCTGCTTG-3'

3'-TTACTATGCCGCTGGTGGCTCTAGA-TGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNNNNNN-NNNNNNNNNNN-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAABN-cDNA_Insert-TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG-NNNNNNNN-TAGAGCATACGGCAGAAGACGAAC-5'

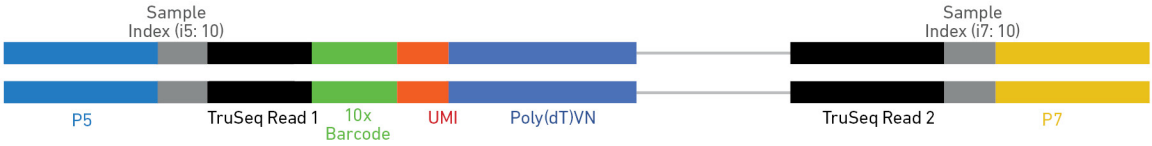
Automated Protocol Step – Dual Index PCR

Dual Index Plate TT Set A PN-1000215

5'-AATGATACGGCGACCACCGAGATCTACAC-N10-ACACTCTTTCCCTACACGACGCTC-3'

5'-CAAGCAGAAGACGGCATACGAGAT-N10-GTGACTGGAGTTCAGACGTGT-3'

Sample Index PCR Product



5'-AATGATACGGCGACCACCGAGATCTACAC-N10-ACACTCTTTCCCTACACGACGCTCTTCCGATCT-N16-N12-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTVN-cDNA_Insert-AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC-N10-ATCTCGTATGCCGTCTTCTGCTTG-3'

3'-TTACTATGCCGCTGGTGGCTCTAGATGTG-N10-TGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-N16-N12-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAABN-cDNA_Insert-TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG-N10-TAGAGCATACGGCAGAAGACGAAC-5'