

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 F

Legal Notices

© 2023 10x Genomics, Inc. All rights reserved. Duplication and/or reproduction of all or any portion of this document without the express written consent of 10x Genomics, is strictly forbidden. Nothing contained herein shall constitute any warranty, express or implied, as to the performance of any products described herein. Any and all warranties applicable to any products are set forth in the applicable terms and conditions of sale accompanying the purchase of such product. 10x Genomics provides no warranty and hereby disclaims any and all warranties as to the use of any third-party products or protocols described herein. The use of products described herein is subject to certain restrictions as set forth in the applicable terms and conditions of sale accompanying the purchase of such product. A non-exhaustive list of 10x Genomics' marks, many of which are registered in the United States and other countries can be viewed at: www.10xgenomics.com/trademarks. 10x Genomics may refer to the products or services offered by other companies by their brand name or company name solely for clarity, and does not claim any rights in those third party marks or names. 10x Genomics products may be covered by one or more of the patents as indicated at: www.10xgenomics.com/patents. The use of products described herein is subject to 10x Genomics Terms and Conditions of Sale, available at www.10xgenomics.com/legal-notices, or such other terms that have been agreed to in writing between 10x Genomics and user. All products and services described herein are intended FOR RESEARCH USE ONLY and NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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 E to Rev F

Revision Date May 2023

Specific Changes:

• Updated to include information regarding two different sample input volumes (pages 19, 21, 26-27, 30, 33-35). Includes an updated Cell Suspension Volume Calculator table for 10 µl input volume and an additional table for 32 µl input volume.

• Updated Dynabead resuspension instructions (pages 19, 30).

General Changes:

• Updated for general minor consistency of language and terms throughout.

Table of Contents

Notices	2
Document Revision Summary	3
Introduction	6
Chromium Automated Single Cell 3' Workflow	7
Additional Kits, Reagents & Equipment	8
Recommended Thermal Cyclers	9
Protocol Steps & Timing	10
Stepwise Objectives	11
Chromium Next GEM Automated Single Cell 3' Reagent Kits v3.1	15
Tips & Best Practices	21
Consumables	22
Cell Concentration	22
Cell Preparation	22
Reagent Handling	23
Chromium Automated Chip Handling	23
Chromium Connect	24
Instrument Orientation	25
Deck Orientation	26
CSV Setup	27
Sample CSV File	28
Uploading Sample Information Using a CSV File	28
Items & Reagents	30
Gather Items & Reagents	31
Thaw & Prep Reagents	32
Sample Preparation Guidelines	34
Sample Preparation Guidelines	35
10 μl Sample Input – Cell Suspension Volume Calculator Table	36
32 μl Sample Input – Cell Suspension Volume Calculator Table	37
Carrier Loading Guidelines	38
Carrier Loading Guidelines	39

Additional Protocol Guidelines	41
Confirm GEM Generation	42
cDNA Amplification Cycle Number	42
cDNA QC & Quantification	43
Sample Index PCR	44
Post Library Construction QC	45
Post Library Construction Quantification & Pooling	46
Deck Orientation – Library Quantification	47
Gather Items & Reagents	48
Post Library Construction Quantification	49
Post Library Construction Quantification	50
Deck Orientation – Library Pooling	51
Gather Items & Reagents	52
Library Pooling	52
Sequencing	53
Sequencing Libraries	54
Illumina Sequencer Compatibility	55
Sample Indices	55
3' Gene Expression Library Sequencing Depth & Run Parameters	56
Library Loading	56
Library Pooling	56
Appendix	57
Agilent TapeStation Traces	58
Oligonucleotide Sequences	59

Introduction

Chromium Automated Single Cell 3' Workflow
Additional Kits, Reagents & Equipment
Recommended Thermal Cyclers
Recommended Real Time qPCR System
Protocol Steps & Timing
Stepwise Objectives
Chromium Next GEM Automated Single Cell 3' Reagent Kits v3.1
Chromium Next GEM Automated Single Cell 3' Quick Planner Card

Chromium Automated Single Cell 3' Workflow



*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).

Additional Kits, Reagents & Equipment

The items in the table below have been tested 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/CO-RE II Tips 50 µl Filtered Tips CO-RE/CO-RE II 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 (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 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 fo	or 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 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	Bioanalyzer, TapeStation, or Oubit based on	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

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 Bio-Rad 96-well PCR Plates, P/N HSP9665 and with the KAPA Library Quantification Kit dye. Refer to manufacturer's recommendation.

Protocol Steps & Timing

	9		
		Steps	Timing
	MANUAL	Cell Preparation (Dependent on Cell Type)	~1-1.5 h
	MANOAL	Gather & Load Reagents and Consumables	~60 min
3 h		Master Mix Preparation	
		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	
	AUTOMATED	OPTIONAL cDNA QC & Quantification (Manual, 50 min; best practice)	~8.5 h Walk-away time
9 h		Fragmentation, End Repair & A-tailing	
		Post Fragmentation, End Repair & A-tailing Double Sided Size Selection – SPRIselect	
		Adaptor Ligation	
		Post Ligation Cleanup- SPRIselect	
12 h		Sample Index PCR	
		Post Sample Index PCR Double Sided Size Selection- SPRIselect	
	MANUAL	Post Library Construction QC	50 min
		OPTIONAL	

OPTIONAL

Library Quantification qPCR & Library Pooling

Stepwise Objectives

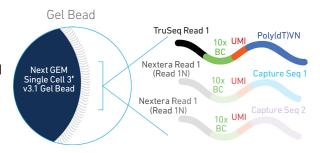
Chromium Connect automates the preparation of sequencing-ready, single cell libraries from input samples with walk-away convenience. Generation of Chromium Single Cell 3' Gene Expression libraries on the Chromium Connect instrument includes automated Gel Beads-in-emulsion (GEM) generation, barcoding, 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 $\sim 3,500,000$ 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.

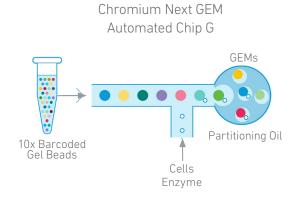
Single Cell 3' v3.1 Gel Beads

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.



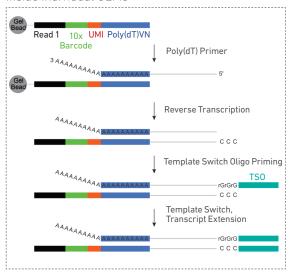
Automated GEM Generation & Barcoding

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

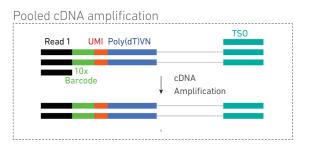
are mixed with the cell lysate and a Master Mix containing reverse transcription (RT) reagents. Incubation of the GEMs produces barcoded, full-length cDNA from poly-adenylated mRNA.

Inside individual GEMs



Automated Post GEM-RT Cleanup & cDNA Amplification

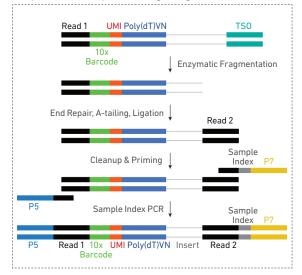
After incubation, GEMs are broken and pooled fractions are recovered. Silane magnetic beads are used to purify the first-strand cDNA from the post GEM-RT reaction mixture, which includes leftover biochemical reagents and primers. Barcoded, full-length cDNA is amplified via PCR to generate sufficient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



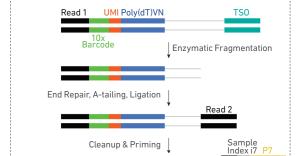
Automated 3' Gene Expression Library Construction

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.



Sample Index PCR

Read 1 10x UMI Poly(dT)VN Barcode

Sample Index (i5)

Amplified cDNA processing (dual index)

Sample Index i7

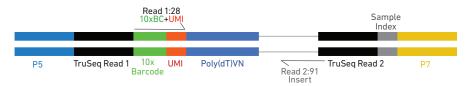
Read 2

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 sequencing are summarized in the Sequencing chapter.

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

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

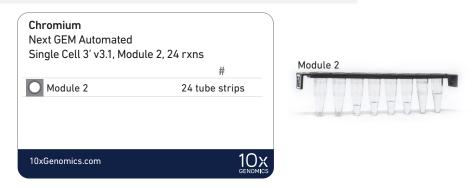
Chromium Next GEM Automated Single Cell 3' Library and Gel Bead Kit v3.1, 24 rxns PN-1000141

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

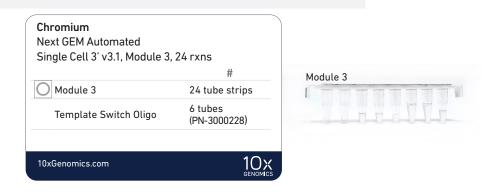
Chromium Next GEM Automated Single Cell 3' Library Kit v3.1, Module 1, 24 rxns PN-1000138 (store at 4°C)



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



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' 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)



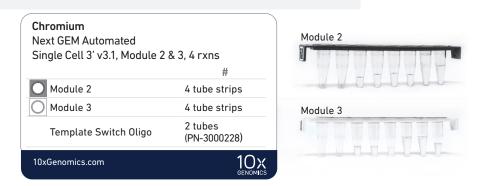
Chromium Next GEM Automated Single Cell 3' Library and Gel Bead Kit v3.1, 4 rxns PN-1000147

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)



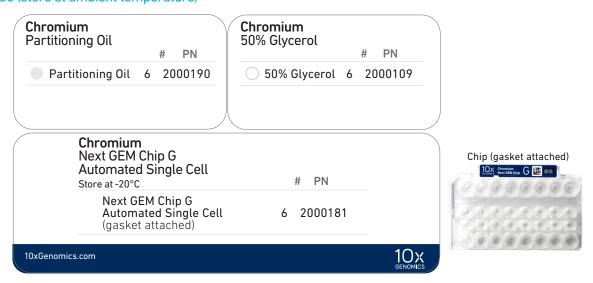
Chromium Next GEM Automated Single Cell 3' Library Kit v3.1, Module 2 & 3, 4 rxns PN-1000150 (store at -20°C)



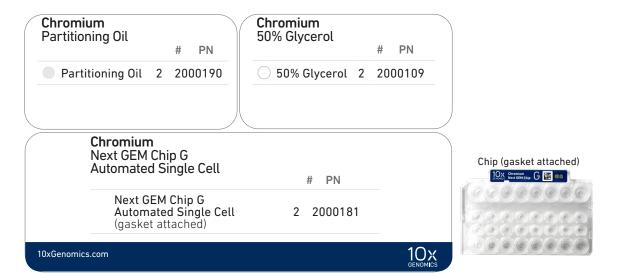
Chromium Next GEM Automated Single Cell 3' Gel Bead Kit v3.1, 4 rxns PN-1000148 (store at -80°C)



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



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





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)



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



Dual Index Kit TT Set A	
	# PN
Dual Index Plate TT Set A	1 3000431

Chromium Next GEM Automated Single Cell 3' Quick Planner Card

Gather the listed items & reagents before running the assay. Follow touchscreen prompts for detailed information.

Gather indicated items prior to running the assay	
$\hfill \Box$ Set thermal cycler to 37°C and lid to 50°C	□ Eppendorf 96-well Semi Skirted plate, 96 well – 1 per run
□ Nuclease free water – 10 ml	□ Eppendorf 96-well Full Skirted plate, 96 well – 1 per run
□ Ethanol, Pure (200 Proof, anhydrous) – 40 ml Combine 40 ml EtOH and 10 ml nuclease free water to prepare 80% EtOH	 50 μl Black CO-RE/CO-RE II Pipette Tips, with filter 7-8 samples: 2 racks 4-6 samples: 2 racks 1-3 samples: 1 rack
□ ComfortLids – 6 per run	
☐ MicroAmp 8-tube strips, 0.2 ml − 1 per run (optional- 2 per run)	 300 μl Black CO-RE/ CO-RE II Pipette Tips, with filter 7-8 samples: 4 racks
☐ Reagent reservoirs, 60 ml – 3 per run	4-6 samples: 3 racks1-3 samples: 2 rack
□ Low TE Buffer – 2 ml	

10	x Reagents	Storage	Preparation & Handling
	Next GEM Chip G Automated 1 per run	Room temp.	Set aside, keep sealed. Follow the touchscreen prompts to load on deck.
	Partitioning oil, 50% Glycerol 1 tube each per run	Room temp. (Chip box)	Keep capped. Follow the touchscreen prompts to remove the cap after cells are loaded on the deck.
	Library Module 1 (black tube strip) 1 tube strip per sample	4°C	Use a thermal cycler (lid temp 50°C) to thaw for 30 min at 37°C. Vortex at 15 min and again at 30 min, centrifuge at 300 rcf for 1 min.
	Dynabeads MyOne Silane - 1 tube per run DO NOT save excess 2 tubes/4rxn kit; 6 tubes/24rxn kit	4°C (Module 1 Box)	Equilibrate to room temperature. Immediately before use, vortex (≥30 sec). Aspirate the full liquid volume with a pipette tip to verify that the beads have not settled in the bottom of the tube. Using a 200 μl pipettor (set to 150 μl), pipette mix at least 20X to fully resuspend clumps. If clumps are still present, repeat vortex ≥30 sec; pipette mix 20X or until fully resuspended. DO NOT centrifuge. DO NOT remove cap until prompted on touchscreen.
	Library Module 2 (grey tube strip) 1 tube strip per sample	-20°C	Thaw at room temperature for 30 min . Vortex, centrifuge at 300 rcf for 1 min .
	Library Module 3 (white strip tube) 1 tube strip per sample	-20°C	Thaw at 4°C or on ice. Maintain on ice until ready to load. Before loading, invert mix (DO NOT vortex), centrifuge at 300 rcf for 1 min.
	Template Switch Oligo - 1 tube per run DO NOT save excess 2 tubes/4rxn kit; 6 tubes/24rxn kit	-20°C (Module 3 box)	Centrifuge briefly. For 10 µl sample input: Resuspend in 1,120 µl Low TE Buffer. For 32 µl sample input: Resuspend in 385 µl Low TE Buffer. Vortex at maximum speed, centrifuge briefly.
	Single Index Kit T Set A/Dual Index TT Set A Plate (SI Plate) 1 plate per run, 1 well per sample	-20°C	Thaw at room temperature for 30 min . Vortex, centrifuge briefly.
	Gel Beads Strip(s) – 1 well per sample	-80°C	Thaw at room temperature ≥ 30 min. Vortex 30 sec, centrifuge 5 sec.

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 can be either 10 µl or 32 µl (must be the same volumes for all samples in a given run).
- · 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. Based on sample input volume (10 μ l or 32 μ l), refer to the applicable 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)

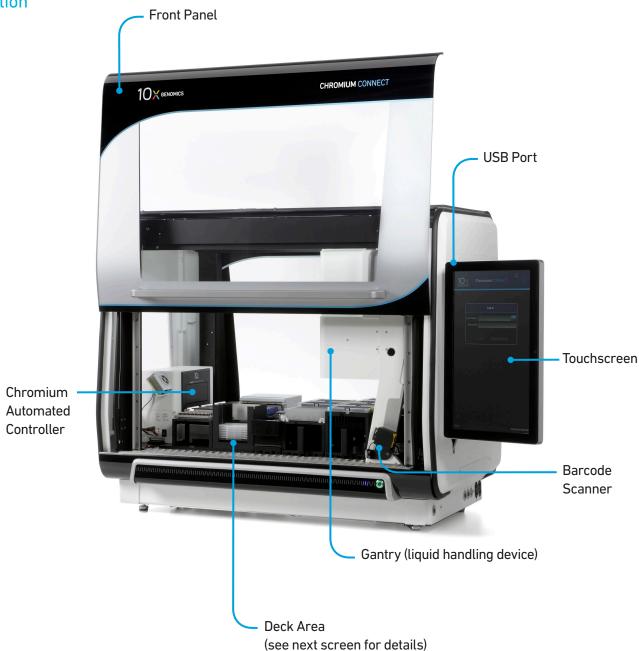


Gaske

Chromium Connect

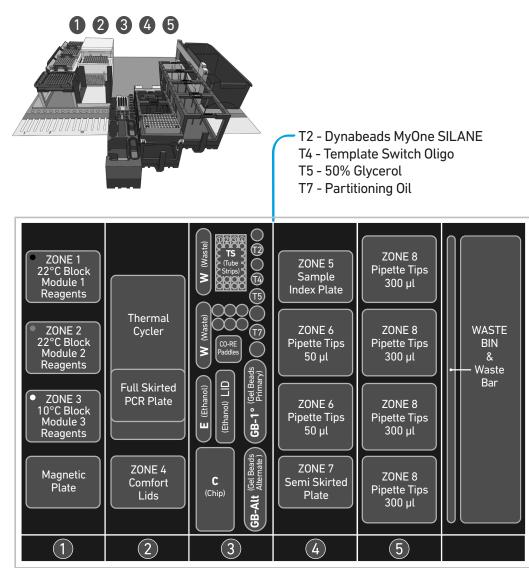
Instrument Orientation
Deck Orientation
CSV Setup

Instrument Orientation



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

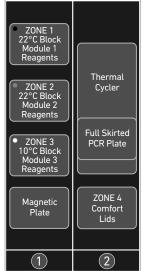
Deck Orientation



Stationary Carriers

Sliding Carriers

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









Deck Layout Reagents/Consumables Chromium Next GEM Automated Single Cell 5' Gene Expression v2 Assay		
Carrier	Zone	Item
	Zone 1 (Black)	22°C Block, Reagent Strips, Module 1
1	Zone 2 (Gray)	22°C Block, Reagent Strips, Module 2
Stationary	Zone 3 (White)	10°C Block, Reagent Strips, Module 3
	-	Magnetic Plate
2	-	Thermal Cycler
2 Stationary	-	Full Skirted PCR Plate (within Thermal Cycler)
	Zone 4	ComfortLids
	Position W	Waste Reservoirs
	Position TS	Tube Strips (TS-1, TS-2, TS-3 & TS-4)
	Position T2	Dynabeads [™] MyOne [™] SILANE
	Position T4	Template Switch Oligo
3*	Position T5	50% Glycerol
Sliding Deck Rails: 15-18	Position T7	Partitioning Oil
Number of Lights: 4	-	CO-RE Paddles
	Position E	Ethanol Reservoir
	Position LID	Lid for Ethanol Reservoir
	Position GB-1°	Gel Beads Primary
*Assay choices determine items loaded in Carrier 3	Position GB-Alt	Gel Beads Alternate
loaded in Carrier 3	Position C	Chip
4	Zone 5	Sample Index Plate
Sliding Deck Rails: 19-24	Zone 6	Pipette Tips 50 μl
Number of Lights: 6	Zone 7	Semi Skirted Plate
5 Sliding Deck Rails: 25-30 Number of Lights: 6	Zone 8	Pipette Tips 300 μl

CSV Setup

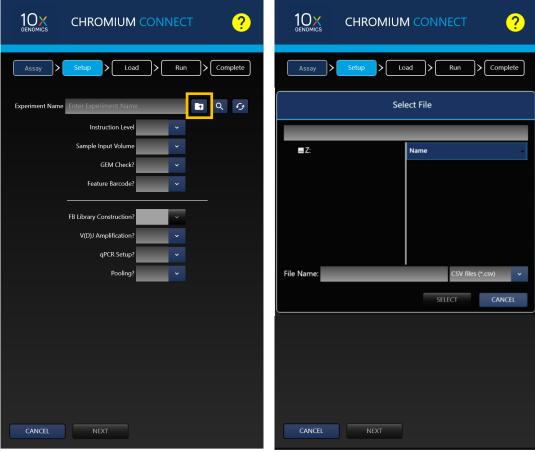
Sample information can also be uploaded using a CSV file at the run set-up screen. Use the folder icon to search a network file system or USB drive. Navigate to the appropriate CSV file and click "SELECT".

The Chromium Connect Input Sample Info Template (CG000309) is available on the 10x Genomics support website.



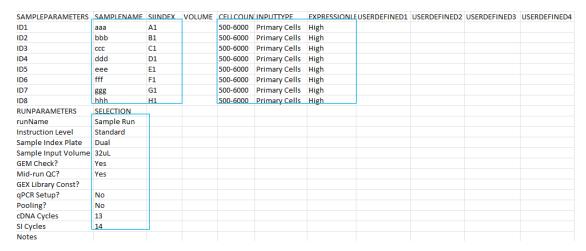
Select either single index or dual index in the sample index plate drop down menu

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.



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 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 D0 NOT use commas. > 6000 Space between symbol & number required.	

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
Sample Index Plate	Single or Dual
Sample Input Volume	10 μl or 32 μl

Run Parameters	Information
Run Name	Alphanumeric and up to 32 characters
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
cDNA Cycles	User defined field. Refer to cDNA Amplification Cycle Number for guidance on optimal cycles.
SI Cycles	User defined field. Refer to Sample Index PCR for guidance.

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	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 Library Module 2 (stored at -20°C) Gray tube strip Library Module 3 (stored at -20°C) White tube strip Template Switch Oligo	1 tube strip/sample 1 tube/run 1 tube strip/sample 1 tube strip/sample 1 tube/run
Single Index Plate T Set A (stored at -20°C) Dual Index Plate TT Set A (stored at -20°C)	1 plate 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.

	GUIDELINES
ACTION	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. For 10 μl sample input: Add 1,120 μl Low TE Buffer to resuspend. For 32 μl sample input: Add 385 μl Low TE Buffer to resuspend.
	 Vortex 15 sec at maximum speed, centrifuge briefly.
Dynabeads	Equilibrate to room temperature. Immediately before use:
	 Vortex Dynabeads for ≥30 sec. Aspirate the full liquid volume with a pipette tip to verify that the beads have not settled in the bottom of the tube.
	 Using a 200 µl pipettor (set to 150 µl), pipette mix at least 20X to fully resuspend clumps. If clumps are still present, repeat vortex ≥30 sec; pipette mix 20X or until fully resuspended. DO NOT centrifuge. Resuspend Clump DO NOT remove cap until prompted on touchscreen.
	 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,



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

to avoid reagent precipitation).

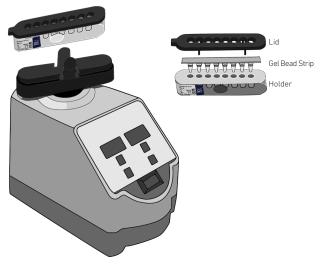
Thaw & Prep Reagents

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. Total volume loaded per sample onto the sample plate is either 10 μ l or 32 μ l (must be the same volumes for all samples in a given run).



- Based on the sample input volume (10 μl or 32 μl), refer to the applicable Cell Suspension Volume Calculator Table for the cell suspension and buffer volumes.
- 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 Demonstra QC	ated Protocols for cell prep and
		10 μl or 32 μl
Sample Input Volume	Up to 43 μl	Refer to the applicable Cell Suspension Volume Calculator Table
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
Sample 1 B C D G G Sample 8	4 5 6 7 8 9 10 11 12 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Samples are loaded in column 1, starting at A1. DO NOT add glycerol in unused sample wells when running <8 samples.

10 μl Sample Input – 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											
Conc. (cells/ µl)	500	1000	2000	3000	4000	5000	6000	7000	8000	9000	10000
100	8.3	n/a									
100	1.8	n/a									
200	4.1	8.3	n/a								
200	5.9	1.8	n/a								
300	2.8	5.5	n/a								
	7.3	4.5	n/a								
400	2.1	4.1	8.3	n/a							
	7.9	5.9	1.8	n/a							
500	1.7	3.3	6.6	9.9	n/a						
	8.4	6.7	3.4	0.1	n/a						
600	1.4	2.8	5.5	8.3	n/a						
	8.6	7.3	4.5	1.8	n/a						
700	1.2	2.4	4.7	7.1	9.4	n/a	n/a	n/a	n/a	n/a	n/a
	8.8	7.6	5.3	2.9	0.6	n/a	n/a	n/a	n/a	n/a	n/a
800	1.0	2.1	4.1	6.2	8.3	n/a	n/a	n/a	n/a	n/a	n/a
	9.0	7.9	5.9	3.8	1.8	n/a	n/a	n/a	n/a	n/a	n/a
900	0.9	1.8	3.7	5.5	7.3	9.2	n/a	n/a	n/a	n/a	n/a
	9.1	8.2	6.3	4.5	2.7	8.0	n/a	n/a	n/a	n/a	n/a
1000	0.8	1.7	3.3	5.0	6.6	8.3	9.9	n/a	n/a	n/a	n/a
	9.2	8.4	6.7	5.1	3.4	1.8	0.1	n/a	n/a	n/a	n/a
1100	0.8	1.5	3.0	4.5	6.0	7.5	9.0	n/a	n/a	n/a	n/a
	9.3	8.5	7.0	5.5	4.0	2.5	1.0	n/a	n/a	n/a	n/a
1200	0.7	1.4	2.8	4.1	5.5	6.9	8.3	9.6	n/a	n/a	n/a
	9.3	8.6	7.3	5.9	4.5	3.1	1.8	0.4	n/a	n/a	n/a
1300	0.6	1.3	2.5	3.8	5.1	6.3	7.6	8.9	n/a	n/a	n/a
	9.4	8.7	7.5	6.2	4.9	3.7	2.4	1.1	n/a	n/a	n/a
1400	0.6	1.2	2.4	3.5	4.7	5.9	7.1	8.3	9.4	n/a	n/a
	9.4	8.8	7.6	6.5	5.3	4.1	2.9	1.8	0.6	n/a	n/a
1500	0.6	1.1	2.2	3.3	4.4	5.5	6.6	7.7	8.8	9.9	n/a
	9.5	8.9	7.8	6.7	5.6	4.5	3.4	2.3	1.2	0.1	n/a
1600	0.5	1.0	2.1	3.1	4.1	5.2	6.2	7.2	8.3	9.3	n/a
	9.5	9.0	7.9	6.9	5.9	4.8	3.8	2.8	1.8	0.7	n/a
1700	0.5	1.0	1.9	2.9	3.9	4.9	5.8	6.8	7.8	8.7	9.7
	9.5	9.0	8.1	7.1	6.1	5.1	4.2	3.2	2.2	1.3	0.3
1800	0.5	0.9	1.8	2.8	3.7	4.6	5.5	6.4	7.3	8.3	9.2
	9.5	9.1	8.2	7.3	6.3	5.4	4.5	3.6	2.7	1.8	0.8
1900	0.4	0.9	1.7	2.6	3.5	4.3	5.2	6.1	6.9	7.8	8.7
	9.6	9.1	8.3	7.4	6.5	5.7	4.8	3.9	3.1	2.2	1.3
2000	0.4	0.8	1.7	2.5	3.3	4.1	5.0	5.8	6.6	7.4	8.3
	9.6	9.2	8.4	7.5	6.7	5.9	5.1	4.2	3.4	2.6	1.8

Grey boxes: Yellow boxes: Volumes that would exceed the allowable buffer volume in each reaction Indicate a low transfer volume that may result in higher cell load variability

Optimal range of cell stock concentration to maximize the likelihood of achieving the desired cell recovery target Blue boxes:

32 µl Sample Input – 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											
Conc. (cells/ µl)	500	1000	2000	3000	4000	5000	6000	7000	8000	9000	10000
100	8.3	16.5	n/a								
100	23.8	15.5	n/a								
200	4.1	8.3	16.5	24.8	n/a						
	27.9	23.8	15.5	7.3	n/a						
300	2.8	5.5	11.0	16.5	22.0	27.5	n/a	n/a	n/a	n/a	n/a
	29.3	26.5	21.0	15.5	10.0	4.5	n/a	n/a	n/a	n/a	n/a
400	2.1	4.1	8.3	12.4	16.5	20.6	24.8	28.9	n/a	n/a	n/a
	29.9	27.9	23.8	19.6	15.5	11.4	7.3	3.1	n/a	n/a	n/a
500	1.7	3.3	6.6	9.9	13.2	16.5	19.8	23.1	26.4	29.7	n/a
	30.4	28.7	25.4	22.1	18.8	15.5	12.2	8.9	5.6	2.3	n/a
600	1.4	2.8	5.5	8.3	11.0	13.8	16.5	19.3	22.0	24.8	27.5
	30.6	29.3	26.5	23.8	21.0	18.3	15.5	12.8	10.0	7.3	4.5
700	1.2	2.4	4.7	7.1	9.4	11.8	14.1	16.5	18.9	21.2	23.6
	30.8	29.6	27.3	24.9	22.6	20.2	17.9	15.5	13.1	10.8	8.4
800	1.0	2.1	4.1	6.2	8.3	10.3	12.4	14.4	16.5	18.6	20.6
	31.0	29.9	27.9	25.8	23.8	21.7	19.6	17.6	15.5	13.4	11.4
900	0.9	1.8	3.7	5.5	7.3	9.2	11.0	12.8	14.7	16.5	18.3
	31.1	30.2	28.3	26.5	24.7	22.8	21.0	19.2	17.3	15.5	13.7
1000	0.8	1.7	3.3	5.0	6.6	8.3	9.9	11.6	13.2	14.9	16.5
	31.2	30.4	28.7	27.1	25.4	23.8	22.1	20.5	18.8	17.2	15.5
1100	0.8	1.5	3.0	4.5	6.0	7.5	9.0	10.5	12.0	13.5	15.0
	31.3	30.5	29.0	27.5	26.0	24.5	23.0	21.5	20.0	18.5	17.0
1200	0.7	1.4	2.8	4.1	5.5	6.9	8.3	9.6	11.0	12.4	13.8
	31.3	30.6	29.3	27.9	26.5	25.1	23.8	22.4	21.0	19.6	18.3
1300	0.6	1.3	2.5	3.8	5.1	6.3	7.6	8.9	10.2	11.4	12.7
	31.4	30.7	29.5	28.2	26.9	25.7	24.4	23.1	21.8	20.6	19.3
1400	0.6	1.2	2.4	3.5	4.7	5.9	7.1	8.3	9.4	10.6	11.8
	31.4	30.8	29.6	28.5	27.3	26.1	24.9	23.8	22.6	21.4	20.2
1500	31.5	30.9	2.2	3.3 28.7	27.6	5.5 26.5	6.6 25.4	7.7 24.3	8.8 23.2	9.9 22.1	11.0 21.0
1600	31.5	31.0	2.1	3.1	4.1	5.2	6.2	7.2	8.3	9.3	10.3
	0.5	1.0	29.9 1.9	28.9	27.9 3.9	26.8	25.8 5.8	24.8 6.8	23.8 7.8	22.7 8.7	9.7
1700	31.5	31.0							24.2		22.3
	0.5	0.9	30.1 1.8	29.1	3.7	27.1 4.6	26.2 5.5	25.2 6.4	7.3	23.3 8.3	9.2
1800	31.5	31.1						25.6			22.8
	0.4	0.9	30.2 1.7	29.3	28.3 3.5	27.4 4.3	26.5 5.2	6.1	6.9	23.8 7.8	8.7
1900	31.6					27.7					
	0.4	31.1 0.8	30.3 1.7	29.4	28.5 3.3		26.8 5.0	25.9 5.8	25.1	7.4	23.3 8.3
2000						4.1			6.6		
	31.6	31.2	30.4	29.5	28.7	27.9	27.1	26.2	25.4	24.6	23.8

Grey boxes: Yellow boxes:

Volumes that would exceed the allowable buffer volume in each reaction 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

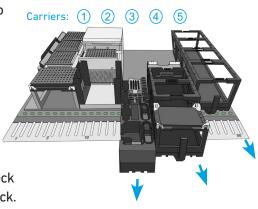
- · Handle the carriers as prompted.
- Ensure that Carriers 3, 4, and 5 are completely slid out and placed on an offdeck workspace before loading.
- Align the carriers to the corresponding Deck Rails when sliding them in or out of the deck.
- Ensure correct orientation of tube labels with barcodes to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

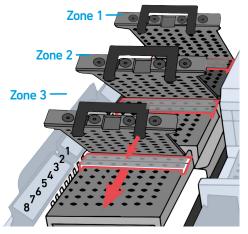
Label Tube Strip Orientation

- The 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.



Barcode Orientation





Tube Strip (TS) Holder



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

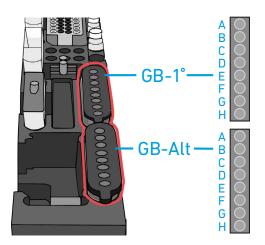
Carrier Loading Guidelines

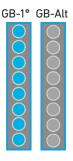
Load Gel Beads

- 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

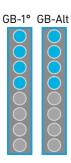
8 samples run with 1 Gel Bead tube strip loaded in GB-1° location.





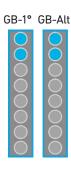
Example 2

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).



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

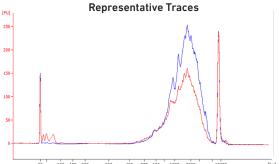
 The optimal number of cycles is a trade-off between generating sufficient final mass for library construction and minimizing PCR amplification artifacts. The number of cDNA cycles should also be reduced if large numbers of cells are sampled.



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

cDNA QC & Quantification

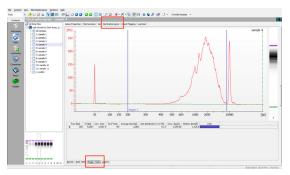
- a. Follow the instruction on the touchscreen for cDNA QC & quantification.
- a. Run sample on an Agilent Bioanalyzer High Sensitivity chip.
 For input cells with low RNA content (<1pg total RNA/cell), 1 µl undiluted product may be run.
 Lower molecular weight product (35 150 bp) may be present. This is normal and does not affect sequencing or application performance.



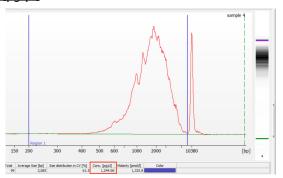
EXAMPLE CALCULATION

i. Select Region

Under the " $\bar{\text{E}}$ lectropherogram" 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 μ l of cDNA if using the Automated workflow and 10 μ l of cDNA if using the Manual workflow.

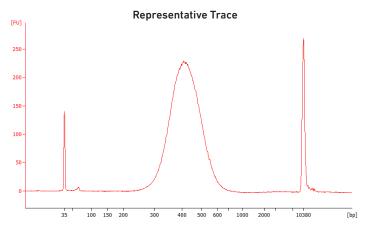
cDNA Concentration (pg/µl)	Total Cycles
<2,500	14
2,500-15,000	12
15,000-50,000	10
50,000-100,000	8
>100,000	6



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

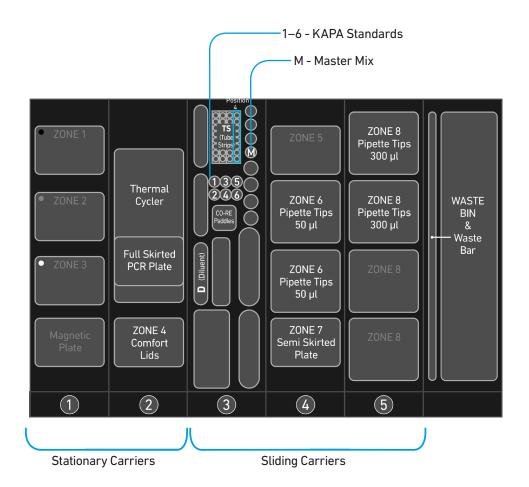
Post Library Construction Quantification & Pooling

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 set-up, 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 $25~\mu l$. Only $6~\mu l$ 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.

·	3
Item	Qty
Hamilton	
ComfortLid	1
$50~\mu l$ 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

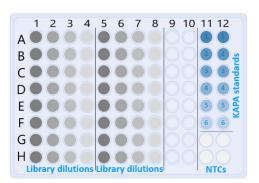
Dilutions:

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)		

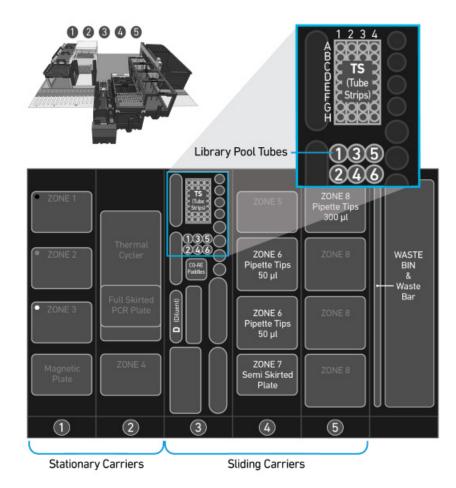
• Resource:

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.

ltem	Qty
Hamilton	
50 μl CO-RE Pipette Tips, with filter (Black, Conductive)	1-2 racks
$300~\mu l$ 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.

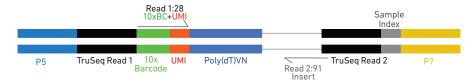
Sequencing

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)

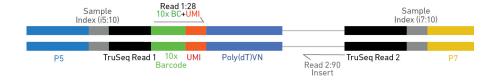


Dual Index

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.



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

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



Choose the sequencing parameters based on library type

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

28 cycles

8 cycles

0 cycles

91 cycles



For dual index	
Read 1	28 cycles
i7 index	10 cycles
i5 index	10 cycles
Read 2	90 cycles

For single index

Read 1

i7 Index

i5 Index Read 2

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

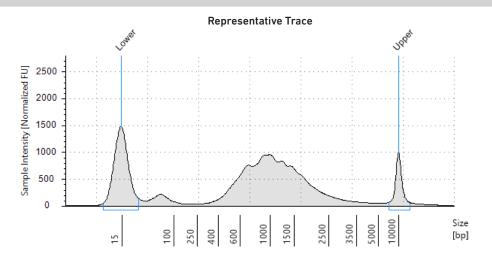
Agilent TapeStation Traces Oligonucleotide Sequences

Agilent TapeStation Traces

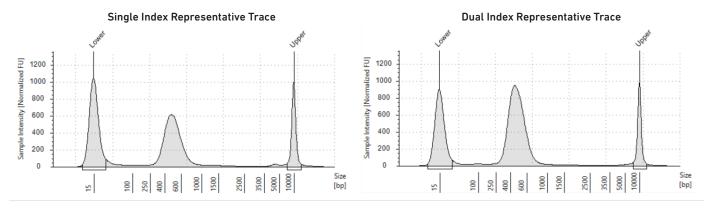
Agilent TapeStation Traces

Agilent TapeStation High Sensitivity D5000 ScreenTape was used.

cDNA QC & Quantification



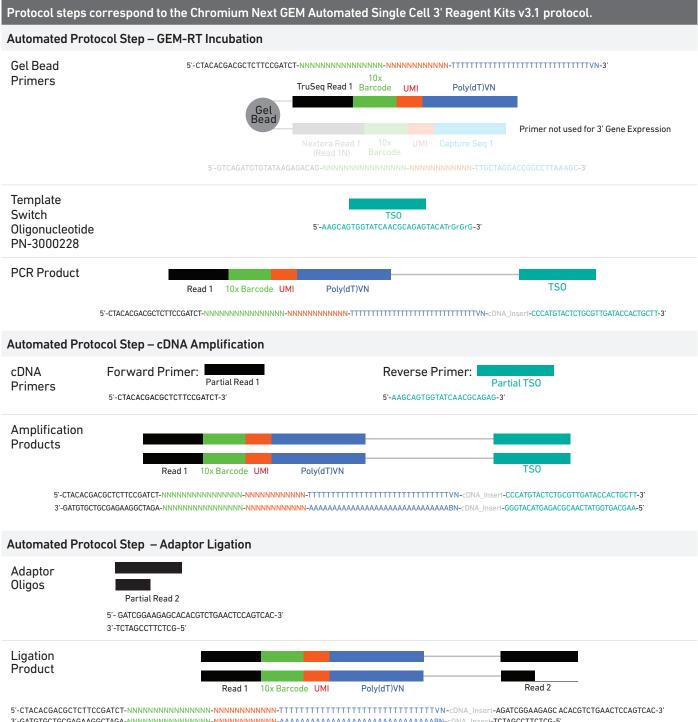
Post Library Construction QC



Alternate QC Method:

Qubit Fluorometer and Qubit dsDNA HS Assay Kit

Oligonucleotide Sequences



5'-CAAGCAGAAGACGGCATACGAGAT-NNNNNNNN GTGACTGGAGTTCAGACGTGT-3'

Read 2

Sample

Index



Poly(dT)VN

10x Barcode UMI

5'-AATGATACGGCGACCACCGAGATCT-ACACTCTTTCCCTACACGACGCTC-3'

Read 1

Sample Index

Dual Index Plate TT Set A PN-1000215

Sample Index PCR Product