

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

# Notices

## Document Number

CG000286 • Rev A

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Email: [support@10xgenomics.com](mailto:support@10xgenomics.com)

10x Genomics

6230 Stoneridge Road

Pleasanton, CA 94588 USA

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Document  
Revision  
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# Introduction

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

Additional Kits, Reagents & Equipment

Recommended Thermal Cyclers

Protocol Steps & Timing

Stepwise Objectives

## 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

	#
<input checked="" type="radio"/> Module 1	12 tube strips
<input type="radio"/> Dynabeads™ MyOne™ SILANE	6 tubes (PN-2000048)

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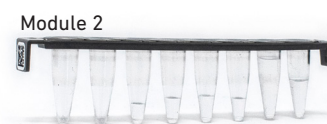
#### Chromium Next GEM Automated Single Cell 3' Library Kit v3.1, Module 2, 24 rxns PN-1000139 (store at -20°C)

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

	#
<input checked="" type="radio"/> Module 2	12 tube strips

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#### 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

	#
<input checked="" type="radio"/> Module 3	12 tube strips
Template Switch Oligo	6 tubes (PN-3000228)

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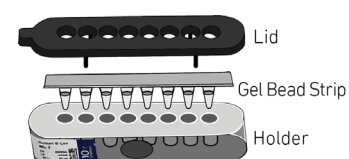
**Chromium Next GEM Automated Single Cell 3' Library and Gel Bead Kit v3.1, 24 rxns PN-1000141****Chromium Next GEM Automated Single Cell 3' Gel Bead Kit v3.1,  
24 rxns PN-1000137 (store at -80°C)**

**Chromium**  
Next GEM Automated  
Single Cell 3' Gel Bead Kit v3.1, 24 rxns

#

Single Cell 3' v3.1 Gel Beads    3 tube strips

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## 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	1 tube (PN-2000048)



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### 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)

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### 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

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

Chromium Partitioning Oil			
	#	PN	
<input checked="" type="radio"/> Partitioning Oil	6	2000190	

Chromium 50% Glycerol			
	#	PN	
<input type="radio"/> 50% Glycerol	6	2000109	

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

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

Chromium Partitioning Oil			
	#	PN	
<input checked="" type="radio"/> Partitioning Oil	2	2000190	

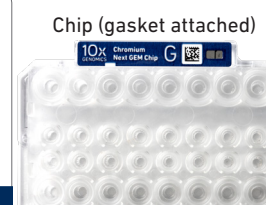
Chromium 50% Glycerol			
	#	PN	
<input type="radio"/> 50% Glycerol	2	2000109	

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

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## 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	

## 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)
<b>Plastics</b>		
Hamilton	CO-RE Tips 50 µl Filtered Tips	235948
	CO-RE Tips 300 µl Filtered Tips	235903
	60 ml Reagent Reservoir Self-Standing	194051
	Hamilton PCR ComfortLid	814300
Eppendorf	96-well Full-Skirted Plate	951020460
	96-well Semi-Skirted Plate	951020362
Thermo Fisher Scientific	MicroAmp 8-Tube Strip, 0.2 ml	N8010580
	MicroAmp 8-Cap Strip, clear	4323032
<b>Kits &amp; Reagents</b>		
Thermo Fisher Scientific	Nuclease-free Water	AM9937
	Low TE Buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA)	12090-015
Millipore Sigma	Ethanol, Pure (200 Proof, anhydrous)	E7023-500ML
Qiagen	Qiagen Buffer EB	19086
<b>Equipment</b>		
10x Genomics	10x Vortex Adapter	330002
-	Benchtop Vortex	-
-	Benchtop Centrifuge	-
-	Plate Centrifuge	-
-	Benchtop Thermal Cycler	-
<b>Additional materials ONLY for optional assays – qPCR and pooling</b>		
Bio-Rad	10% Tween 20	1662404
	96-well PCR Plates	HSP9665
Qiagen	Qiagen Buffer EB	19086
Thermo Fisher Scientific	2 ml-Screw-cap Tube	3488NK
	0.5 ml-Screw-cap Tubes	3472NK
KAPA Biosystems	KAPA Library Quantification Kit for Illumina Platforms	KK4824

## Additional Kits, Reagents & Equipment

Supplier	Description	Part Number (US)
<b>Quantification &amp; Quality Control</b>		
Agilent	2100 Bioanalyzer Laptop Bundle	G2943CA
	High Sensitivity DNA Kit	5067-4626
	4200 TapeStation	G2991AA
	High Sensitivity D1000 ScreenTape/Reagents	5067-5592/ 5067-5593
Thermo Fisher Scientific	High Sensitivity D5000 ScreenTape/Reagents	5067-5584/ 5067-5585
	Qubit 4.0 Fluorometer	Q33226
Advanced Analytical	Qubit dsDNA HS Assay Kit	Q32854
	Fragment Analyzer Automated CE System - 12 cap	FSv2-CE2F
	Fragment Analyzer Automated CE System - 48/96 cap	FSv2-CE10F
High Sensitivity NGS Fragment Analysis Kit		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)
		Gather & Load Reagents and Consumables
		Master Mix Preparation
6 h		Chromium Automated Controller Loading
		GEM Generation
		<div> <b>OPTIONAL</b>            Confirm GEM Generation (Manual, 5 min)         </div>
9 h		Post GEM RT-Cleanup – Dynabead
		cDNA Amplification
		cDNA Cleanup – SPRIselect
12 h	AUTOMATED	<div> <b>OPTIONAL</b>            cDNA QC &amp; Quantification (Manual, 50 min; best practice)         </div>
		Fragmentation, End Repair & A-tailing
		Post Fragmentation, End Repair & A-tailing Double Sided Size Selection – SPRIselect
12 h		Adaptor Ligation
		Post Ligation Cleanup- SPRIselect
		Sample Index PCR
12 h		Post Sample Index PCR Double Sided Size Selection- SPRIselect
	MANUAL	Post Library Construction QC
		<div> <b>OPTIONAL</b>            Library Quantification qPCR &amp; Library Pooling         </div>

~8.5 h  
Walk-away  
time

## Stepwise Objectives

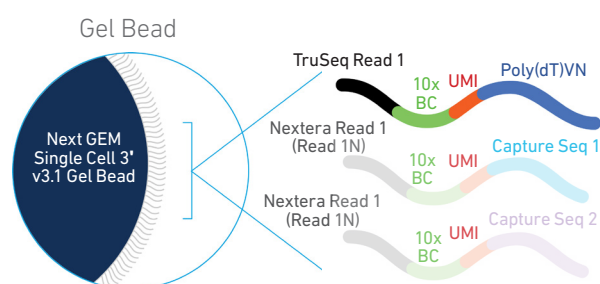
Chromium Connect automates the preparation of sequencing-ready, single cell libraries from input samples with walk-away convenience. Generation of Chromium Single Cell 3' Gene Expression libraries on the Chromium Connect instrument includes automated Gel Beads-in-emulsion (GEM) generation, barcoding,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

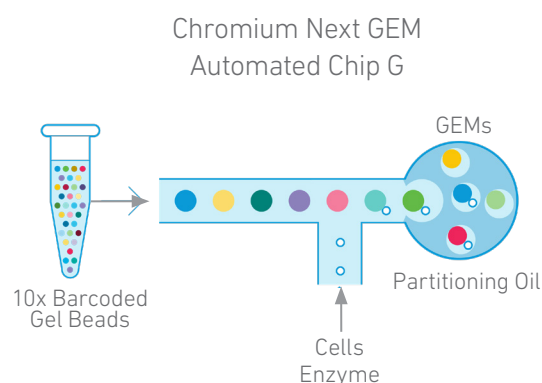
## Single Cell 3' v3.1 Gel Beads

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.



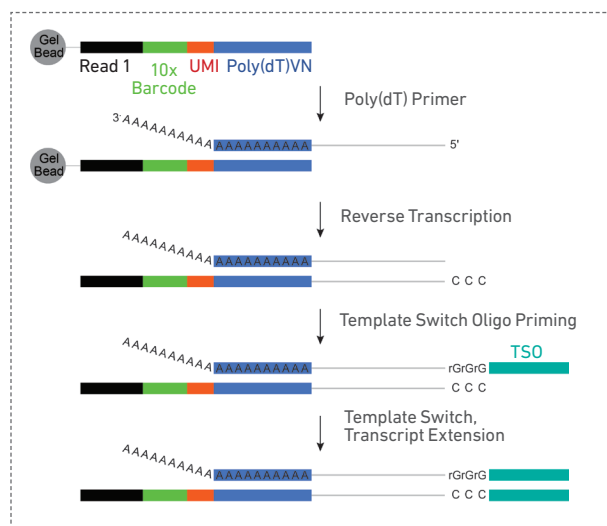
## Automated GEM Generation & Barcoding

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.

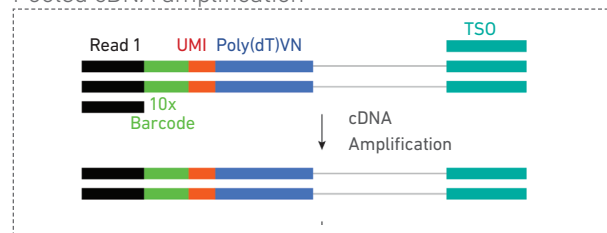
### Inside individual GEMs



## 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.

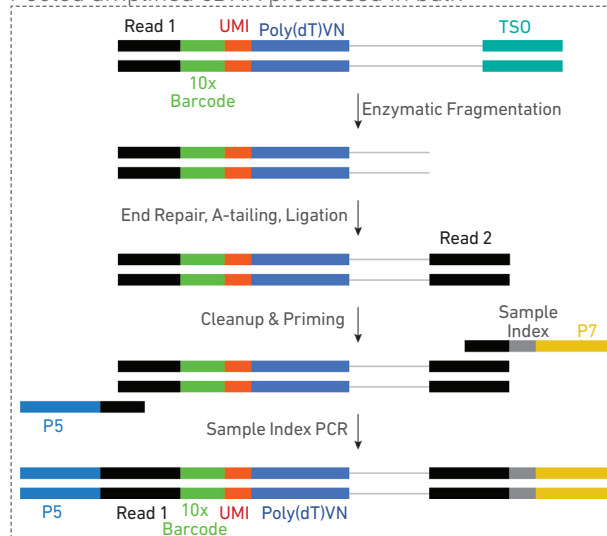
### Pooled cDNA amplification



## Automated 3' Gene Expression Library Construction

Enzymatic fragmentation and size selection are used to optimize the cDNA amplicon size. TruSeq Read 1 (read 1 primer sequence) was added to the molecules during GEM incubation. 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.

### Pooled amplified cDNA processed in bulk

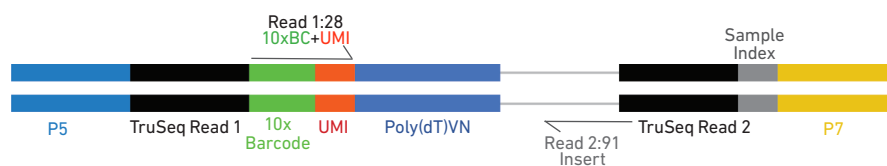


## Sequencing

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. 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



[See Appendix for Oligonucleotide Sequences](#)

# Tips & Best Practices



TIPS





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  $\mu$ L**.
- 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

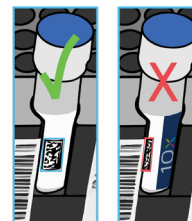
## Cell Preparation

- 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.

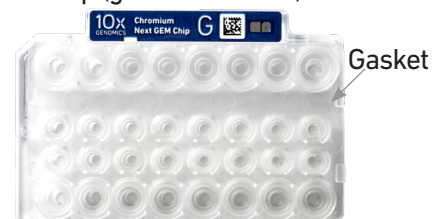
Barcode Orientation



## 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  $\leq 24$  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)



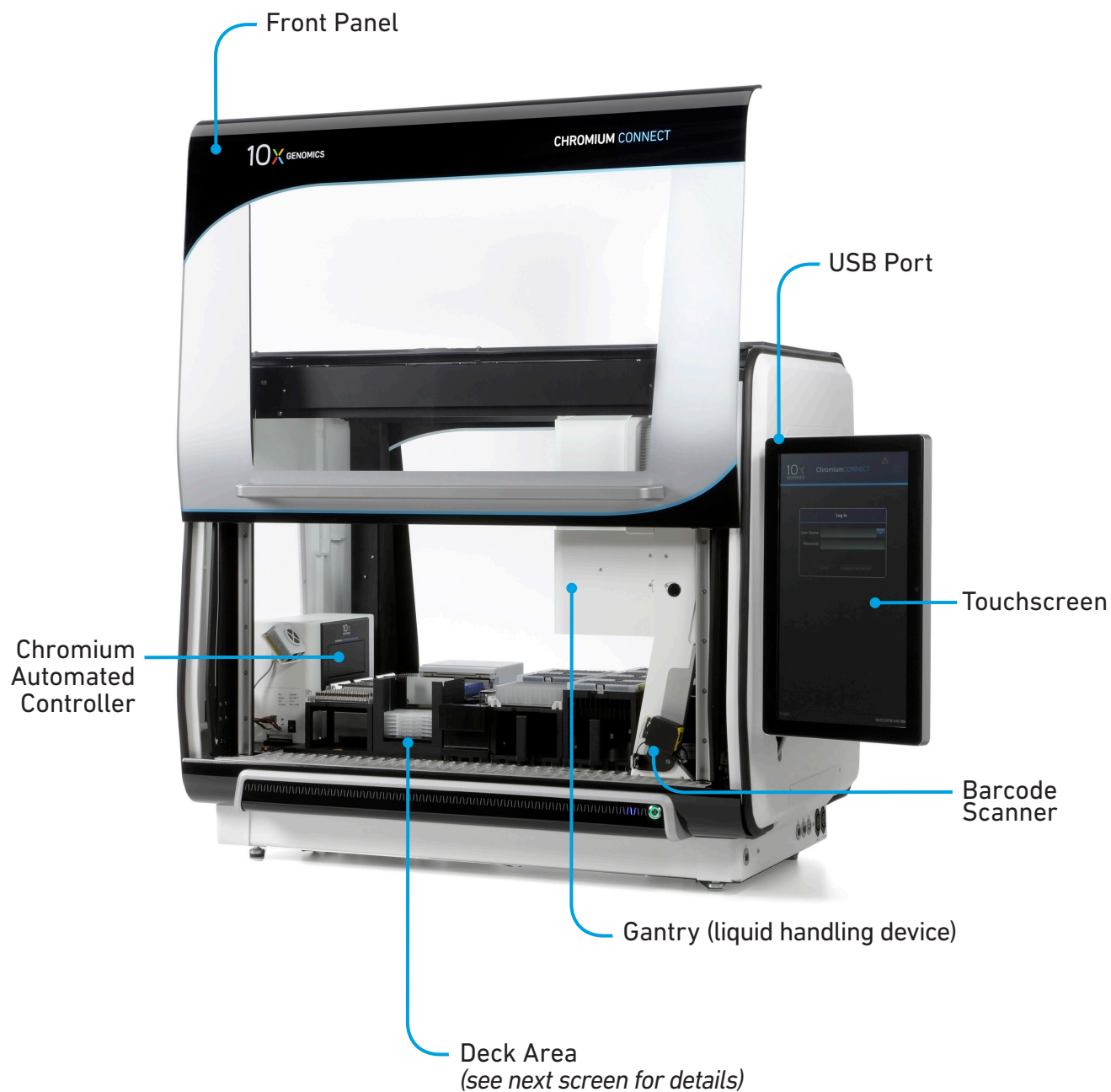
# Chromium Connect

Instrument Orientation

Deck Orientation

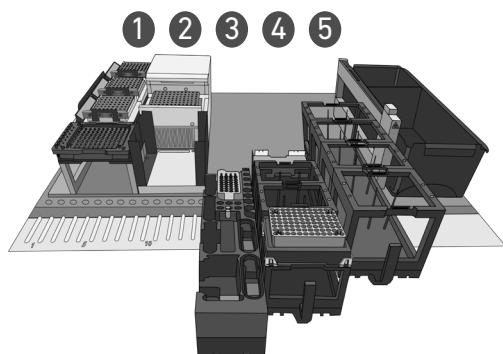
CSV Setup

## Instrument Orientation

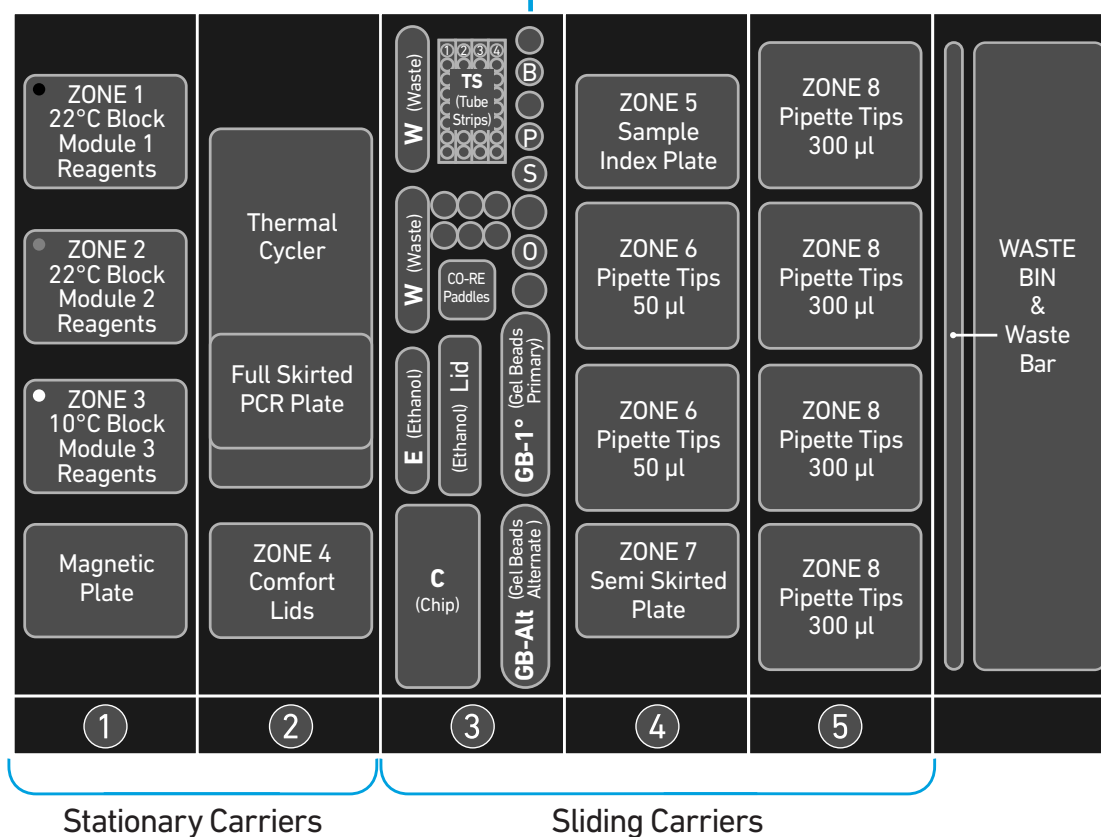


Refer to the Chromium Connect Instrument User Guide (CG000180) and Quick Reference Cards (CG000256) for more information.

## Deck Orientation



B - Dynabeads MyOne SILANE  
 P - Template Switch Oligo  
 S - 50% Glycerol  
 O - Partitioning Oil



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

## CSV Setup

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

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

## Run Set-up Screen

The image displays two side-by-side screenshots of the Chromium Connect software interface, specifically the 'Run Set-up Screen'.

**Left Screenshot:** The interface shows the 'CHROMIUM CONNECT' header with the 10x Genomics logo. Below the header is a progress bar with buttons: 'SC3' GEX', 'Setup' (highlighted), 'Load', 'Run', and 'Complete'. The 'Experiment Name' field is labeled 'Enter Experiment Name' and has a folder icon button to its right, which is highlighted with a yellow box. Below this are several dropdown menus for configuration: 'Instruction Level' (Standard), 'GEM Check?' (Yes), 'Mid-run QC?' (Yes), 'qPCR Setup?' (No), and 'Pooling?' (No). At the bottom are 'CANCEL' and 'NEXT' buttons.

**Right Screenshot:** This screenshot shows the 'Select File' dialog box. It has a title bar 'Select File' and a list of files. The 'File Name' field is empty, and the file type is set to 'CSV files (\*.csv)'. There are 'SELECT' and 'CANCEL' buttons at the bottom of the dialog. The 'CANCEL' and 'NEXT' buttons from the previous screen are also visible at the bottom of the main window.

## 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
SI Index	Location on single index plate to be used for each sample during SI PCR
Cell Expression	User defined field for tracking <u>Example:</u> 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 <a href="#">cDNA Amplification Cycle Number</a> 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 &amp; Reagents

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

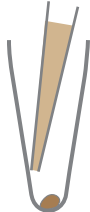
Gather the quantities specified for each of the items and reagents.

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) <i>Partitioning Oil</i> <i>50% Glycerol</i> <i>Chip G (keep chip sealed)</i>	1
Chromium Next GEM Automated Single Cell 3' Gel Bead Kit v3.1 (stored at -80°C) <i>Single Cell 3' Gel Bead Kit v3.1</i>	1 tube/sample
Chromium Next GEM Automated Single Cell 3' Library Kit v3.1	
Library Module 1 (stored at 4°C) <i>Black tube strip</i> <i>Dynabeads</i>	1 tube strip/sample 1 tube/run
Library Module 2 (stored at -20°C) <i>Gray tube strip</i>	1 tube strip/sample
Library Module 3 (stored at -20°C) <i>White tube strip</i> <i>Template Switch Oligo</i>	1 tube strip/sample 1 tube/run
Single Index Plate T Set A (stored at -20°C)	1 plate

See [Additional Kits, Reagents & Equipment](#) list for performing optional assays and/or QC.

## Thaw &amp; Prep Reagents

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

ACTION	GUIDELINES <i>Follow touchscreen prompts for specifics and timing</i>
Thaw Reagents	<ul style="list-style-type: none"> <li>Thaw reagents as indicated on the touchscreen. Verify no precipitate is present.</li> <li>Ensure that the correct thawing locations and temperatures are used.</li> <li>During reagent thaw load the consumables following touchscreen prompts.</li> </ul>
Prepare Ethanol	<ul style="list-style-type: none"> <li>Prepare <b>50 ml</b> 80% Ethanol in Nuclease-free water and dispense in Ethanol Reservoir when prompted.</li> </ul>
Resuspend TSO	<ul style="list-style-type: none"> <li>Resuspend only when prompted on the touchscreen.</li> <li>Centrifuge TSO briefly. Add <b>1,120 µl</b> Low TE Buffer to resuspend. Vortex <b>15 sec</b> at maximum speed, centrifuge briefly.</li> </ul>
Dynabeads	<ul style="list-style-type: none"> <li>Vortex Dynabeads for <b>≥30 sec</b>.</li> <li>Pipette mix to resuspend completely. <b>DO NOT</b> centrifuge.</li> <li>Confirm there are no bubbles at the bottom of the tube.</li> </ul> <div data-bbox="1230 999 1450 1241">  <p>Resuspend Clump</p> </div>
Library Modules	<ul style="list-style-type: none"> <li>Thaw Library Modules as prompted on the touchscreen.</li> <li>After reagent thaw, invert rack holding Module tube strips and vortex Library Modules 1 and 2 for <b>30 sec</b>; verify no precipitate.</li> <li>Centrifuge Library Modules 1 and 2 at <b>300 rcf</b> for <b>1 min</b> at <b>22°C</b>.</li> <li>Retrieve Library Module 3 from <b>4°C</b> storage. Invert-mix;</li> </ul>

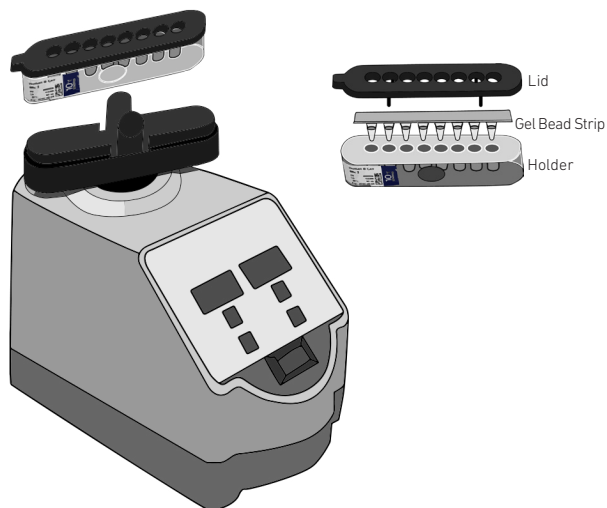
## Thaw &amp; Prep Reagents

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

ACTION	GUIDELINES
	<i>Follow touchscreen prompts for specifics and timing</i>

## 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  $\mu$ l**.
- 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 $\mu$ l	10 $\mu$ l
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
<b>100</b>	8.25 1.75	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
<b>200</b>	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
<b>300</b>	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
<b>400</b>	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
<b>500</b>	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
<b>600</b>	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
<b>700</b>	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
<b>800</b>	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
<b>900</b>	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
<b>1000</b>	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
<b>1100</b>	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
<b>1200</b>	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
<b>1300</b>	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
<b>1400</b>	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
<b>1500</b>	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
<b>1600</b>	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
<b>1700</b>	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
<b>1800</b>	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
<b>1900</b>	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

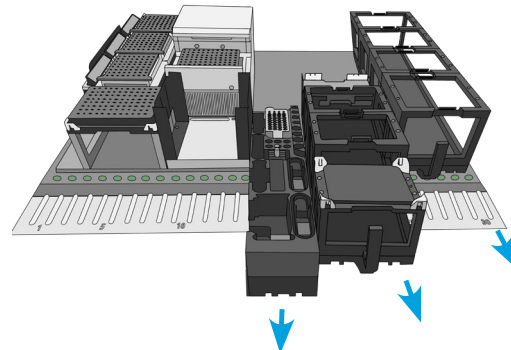
## Carrier Loading Guidelines

Follow the instructions on the touchscreen to load the carriers.

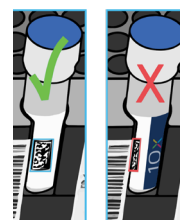
### Carriers

- 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.

Carriers: ① ② ③ ④ ⑤

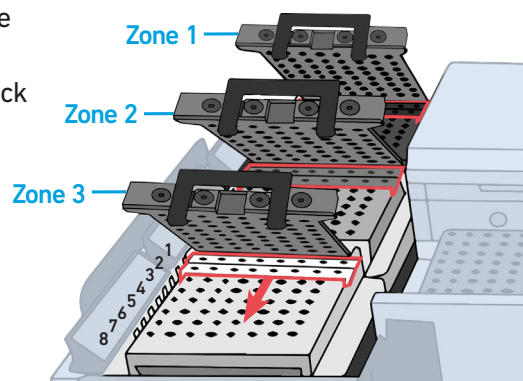


Barcode Orientation



### Modules

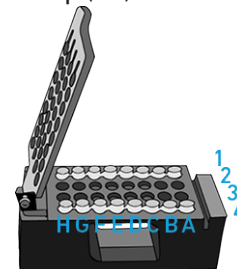
- Load one tube strip/sample of each of the indicated modules in the corresponding positions on the Carrier, starting from back to front row.
- DO NOT skip any rows when loading.
- Use pinhole alignment to place module tube strips in the correct orientation (as shown on the touchscreen).



### 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



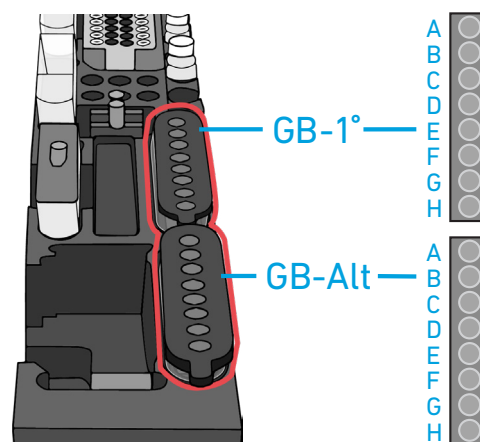
Consult the Chromium Connect User Guide (CG000180) for more information.



## Carrier Loading Guidelines

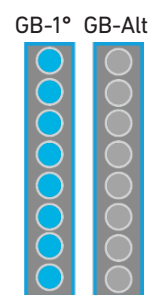
### Load Gel Beads

- 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.



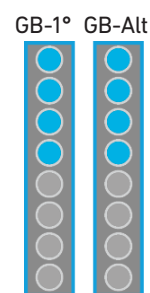
### Example 1

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



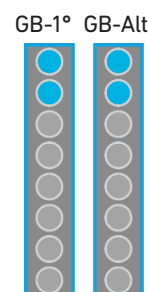
### Example 2

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

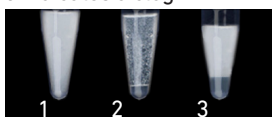
Confirm GEM Generation  
cDNA Amplification Cycles  
cDNA QC & Quantification  
Sample Index PCR

## 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.

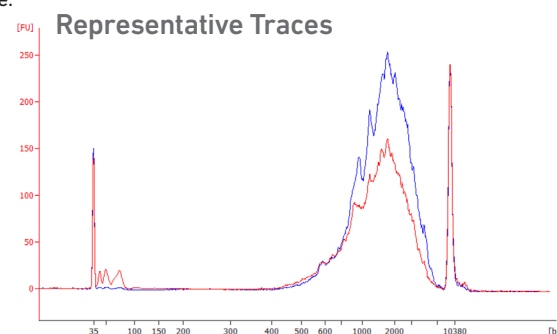
## cDNA QC &amp; Quantification

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.

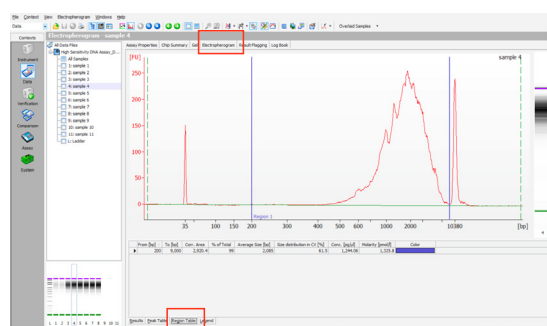
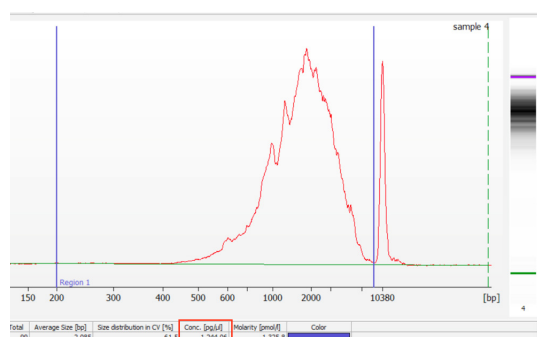
Lower molecular weight product (35 – 150 bp) may be present. This is normal and does not affect sequencing or application performance.



## 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 cDNA amplification cycle number.

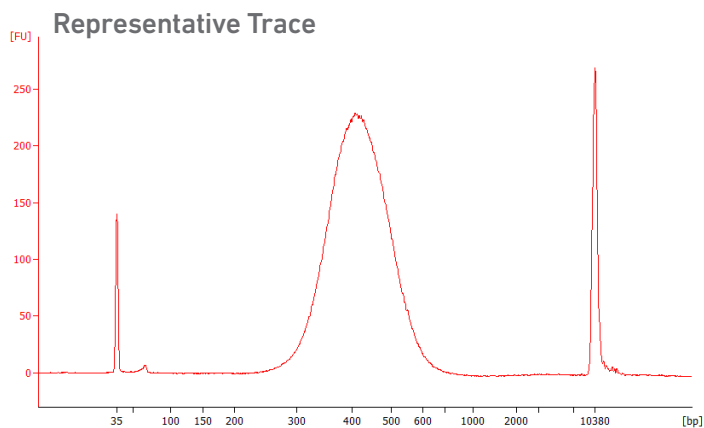
cDNA Concentration (pg/μl)	Total Cycles
<2,500	14
2,500-15,000	12
15,000-50,000	10
50,000-100,000	8
>100,000	6



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

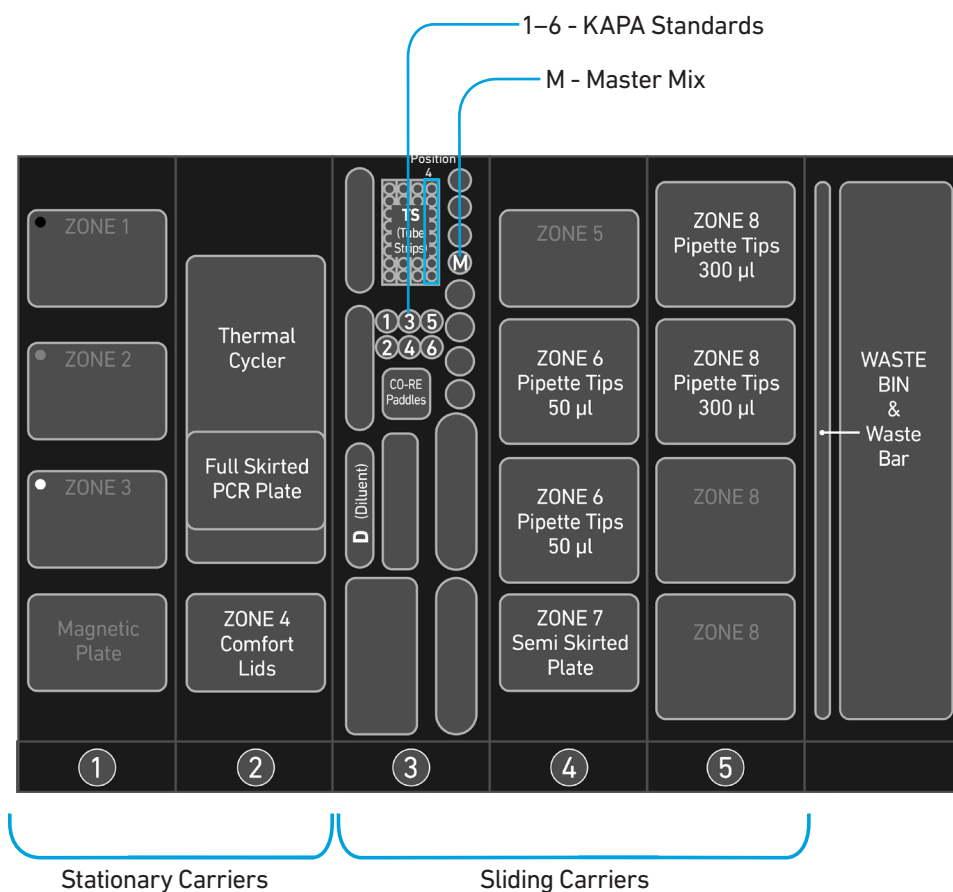
Deck Orientation – Library Pooling

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  $\mu\text{L}$ . Only 6  $\mu\text{L}$  of the sample will be used for qPCR plate setup.

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





## Gather Items &amp; 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	5 ml
Primer Mix	1 ml
Standards	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

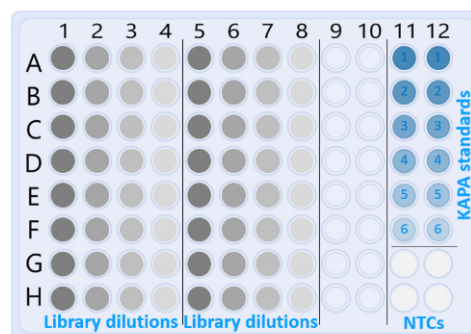
## Post Library Construction Quantification

- 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  $\mu$ l)=  
 16  $\mu$ l Master Mix  
 +  
 4  $\mu$ l 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.

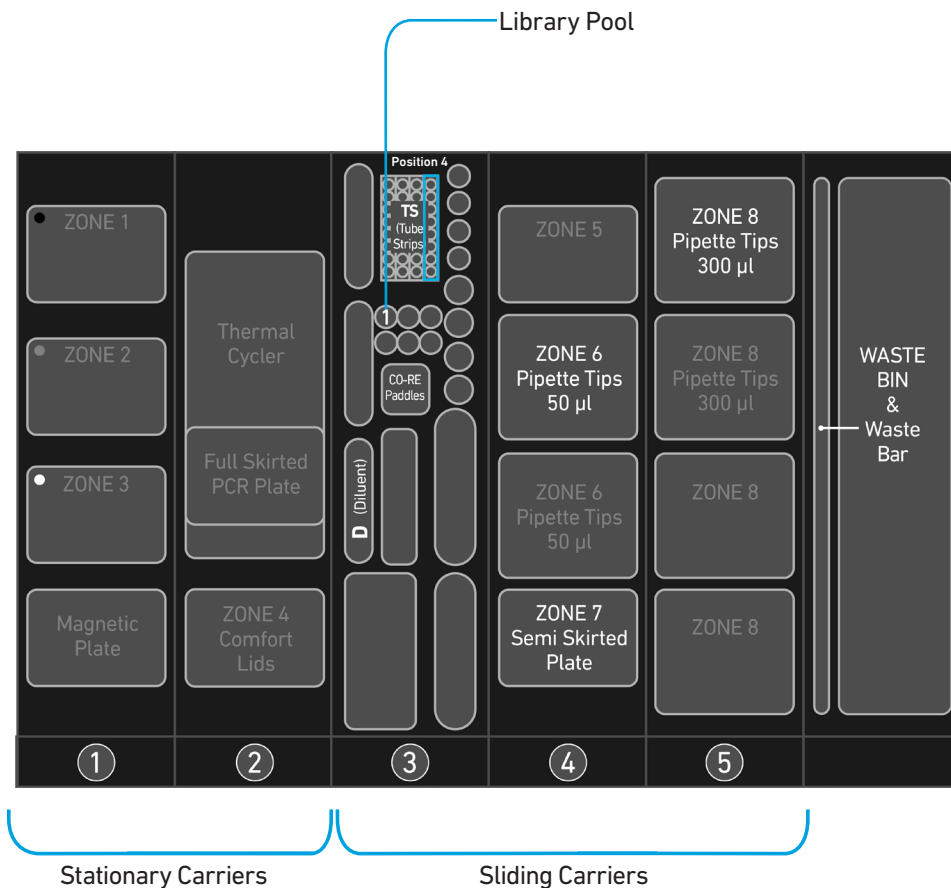
Step	Temperature	Run Time
1	95°C	00:05:00
2	95°C	00:00:30
3	60°C	00:00:45
4	Go to Step 2, 29X (Total 30 cycles)	

- **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 Quantification is shown below.



## Gather Items &amp; 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 racks
300 µl CO-RE Pipette Tips, with filter (Black, Conductive)	1 racks
Reagent Reservoir, 60 ml	1
Eppendorf	
96-well Semi Skirted Plate	1
Thermo Fisher Scientific	
0.5-ml Tube with Screw Cap	1
Reagent	Qty
Qiagen Buffer EB	50 ml
Libraries (in an 8-tube strip)	1-8

## Library Pooling

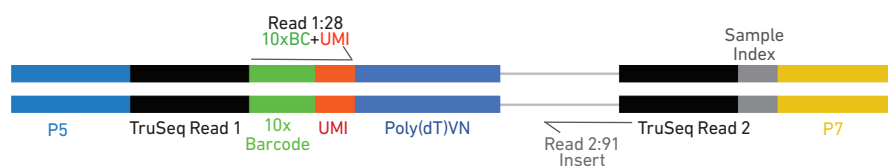
- Follow the touchscreen prompts for loading, scanning, and executing the run.
- Briefly vortex and centrifuge libraries in the 8-tube strip.
- Ensure a minimum **25 µl** library volume is available in the tubes.
- After run is complete, follow touchscreen prompts to unload and store the libraries.
- Unload remaining items and clean as prompted on the touchscreen.
- **Resource:**  
Use the Chromium Connect Library Pooling Worksheet (CG000156) provided on the 10x Genomics Support website to calculate volumes to be pooled. The calculated volumes can be input into the instrument via the Chromium Connect Library Pooling Input CSV Template (CG000310).

# Sequencing

## Sequencing Libraries

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 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



## 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
- HiSeq 2500 (Rapid Run)
- HiSeq 3000/4000
- NovaSeq

## Sample Indices

Each sample index in the Sample 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.

### 3' Gene Expression Library Sequencing Depth & Run Parameters

<b>Sequencing Depth</b>	Minimum 20,000 read pairs per cell
<b>Sequencing Type</b>	Paired-end, single indexing
<b>Sequencing Read</b>	Recommended Number of Cycles
Read 1	28 cycles
i7 Index	8 cycles
i5 Index	0 cycles
Read 2	91 cycles

### 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	1.8	1
HiSeq 2500 (RR)	11	1
HiSeq 4000	240	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

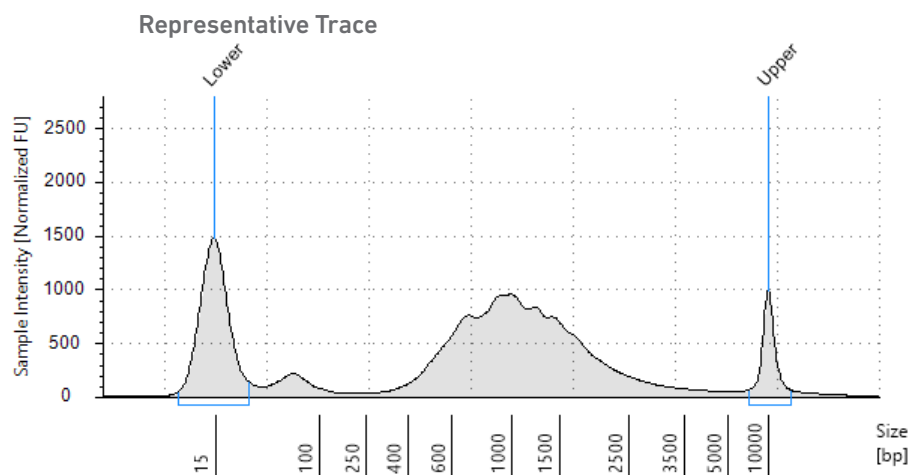
Agilent TapeStation Traces  
Oligonucleotide Sequences

## Agilent TapeStation Traces

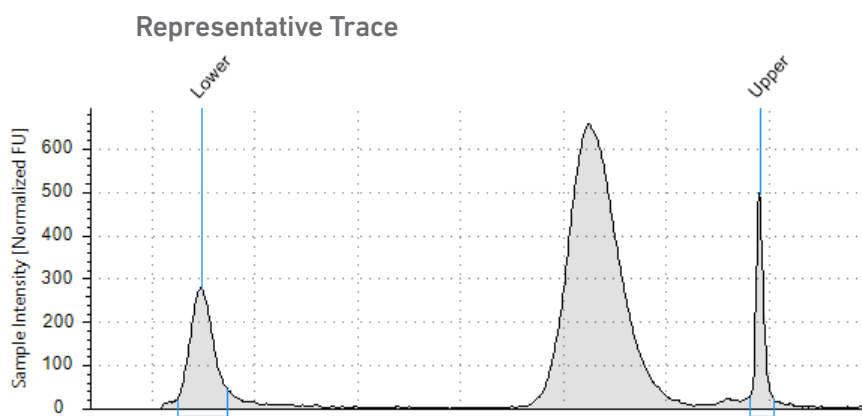
### Agilent TapeStation Traces

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](#)

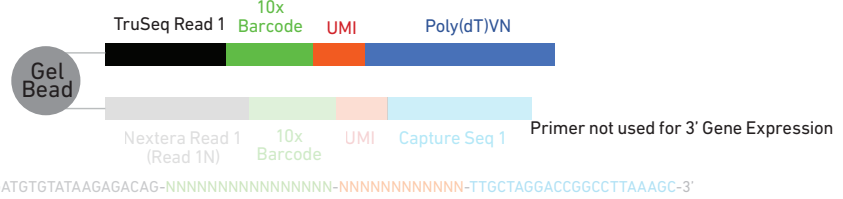
## Oligonucleotide Sequences

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

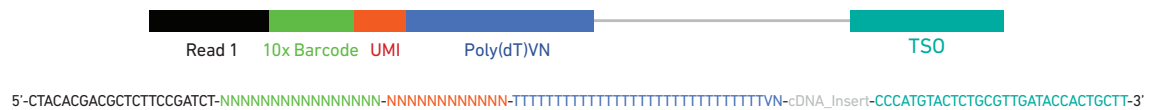
5'-CTACACGACGCTCTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNNNNN-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-3'



Template Switch  
Oligonucleotide  
PN-3000228

TSO  
5'-AAGCAGTGGTATCAACGCAGAGTACATrGrGrG-3'

PCR Product



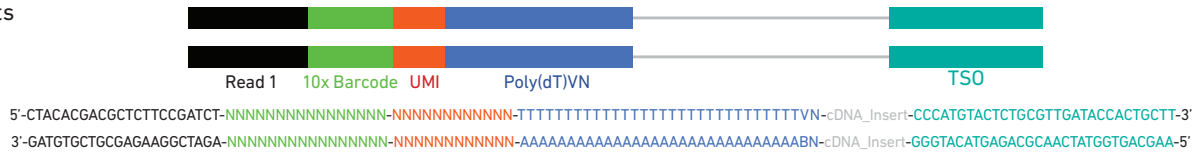
### Automated Protocol Step – cDNA Amplification

cDNA Primers

Forward Primer: Partial Read 1  
5'-CTACACGACGCTCTCCGATCT-3'

Reverse Primer: Partial TSO  
5'-AAGCAGTGGTATCAACGCAGAG-3'

Amplification  
Products

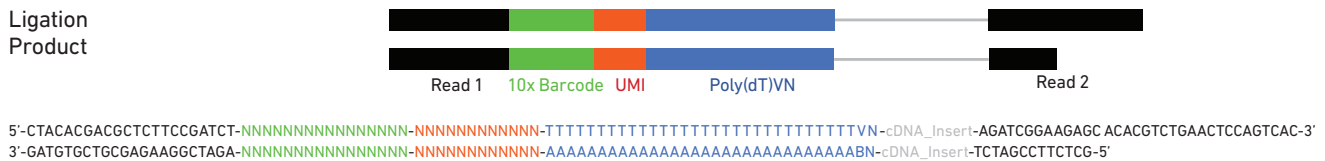


### Automated Protocol Step – Adaptor Ligation

Adaptor Oligos

Partial Read 2  
5'-GATCGGAAGAGCACACGTCTGAACTCCAGTCA-3'  
3'-TCTAGCCTTCTCG-5'

Ligation  
Product



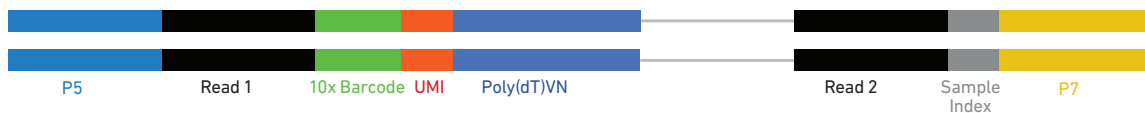
## Oligonucleotide Sequences

## Automated Protocol Step – Sample Index PCR

Sample Index  
PCR PrimerSingle Index T  
Set A  
PN-2000240

5'-AATGATACGGCGACCACCGAGATCT-ACACTCTTTCCCTACACGACGCTC-3'

5'-CAAGCAGAAGACGGCATACGAGAT-NNNNNNNN GTGACTGGAGTTCAGACGTGT-3'

Sample Index  
PCR Product

5'-AATGATACGGCGACCACCGAGATCT-ACACTCTTTCCCTACACGACGCTCTCCGATCT-NNNNNNNNNNNN-NNNNNNNNNN-TTTTTTTTTTTTTTTTTTTTTTTNN-cDNA\_Insert-AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC-NNNNNNNN-ATCTCGTATGCCGTCTTCTGCTTG-3'  
 3'-TTACTATGCCGCTGGTGGCTCTAGA-TGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNN-NNNNNNNNNN-AAAAAAAAAAAAAAAAAAAAAAAAAABN-cDNA\_Insert-TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAAGT-NNNNNNNN-TAGAGCATACGGCAGAAAGACGAAC-5'