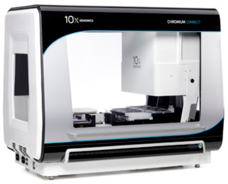
CG000384 Rev B



USER GUIDE

Chromium Next GEM Automated Single Cell 5' Reagent Kits v2

FOR USE WITH

Chromium Next GEM Automated Single Cell 5' Kit v2, 24 rxns PN-1000290
Chromium Next GEM Automated Single Cell 5' Kit v2, 4 rxns PN-1000298
Chromium Automated Single Cell Human TCR Amplification & Library Construction Kit, 24 rxns PN-1000300
Chromium Automated Single Cell Mouse TCR Amplification & Library Construction Kit, 24 rxns PN-1000310
Chromium Automated Single Cell Human BCR Amplification & Library Construction Kit, 24 rxns PN-1000305
Chromium Automated Single Cell Mouse BCR Amplification & Library Construction Kit, 24 rxns PN-1000311
Chromium Next GEM Chip K Automated Single Cell Kit, 48 rxns PN-1000289
Chromium Next GEM Chip K Automated Single Cell Kit, 16 rxns PN-1000297
Dual Index Kit TT Set A, 96 rxns PN-1000215

Notices

Document Number

CG000384 • Rev B

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Document Revision Summary

Document Number	CG000384
Title	Chromium Next GEM Automated Single Cell 5' Reagent Kits v2 User Guide
Revision	Rev B
Revision Date	December 2021

Specific Changes:

Updated to include information on Chromium Automated modular workflow compatibility.

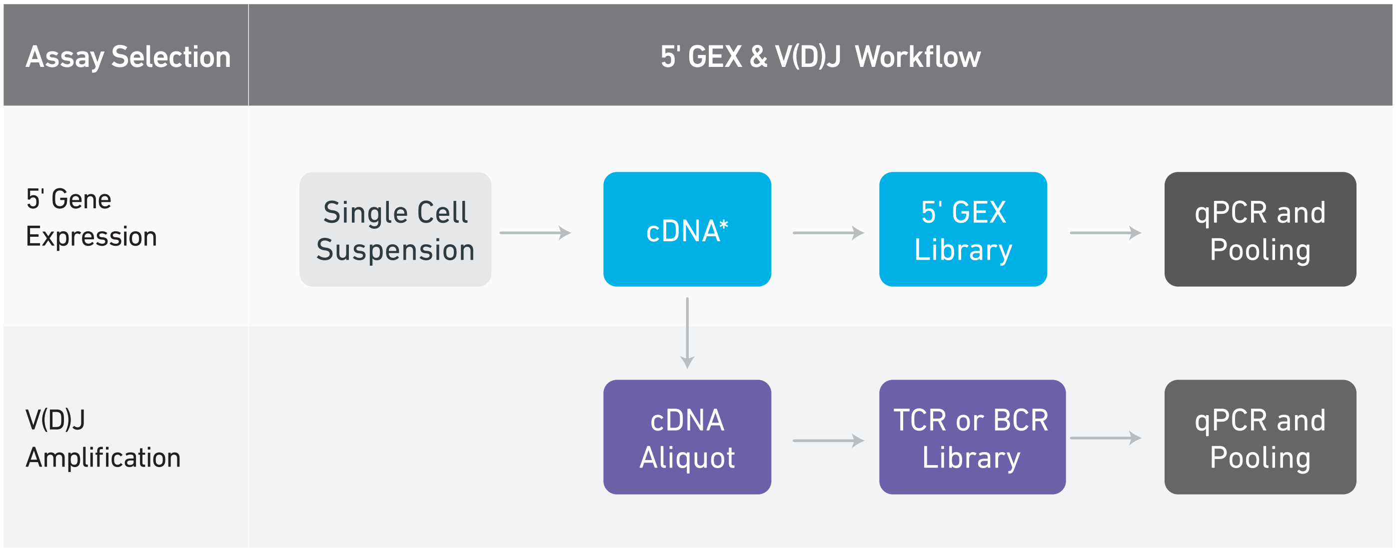
General Changes:

Updated for general minor consistency of language and terms throughout.

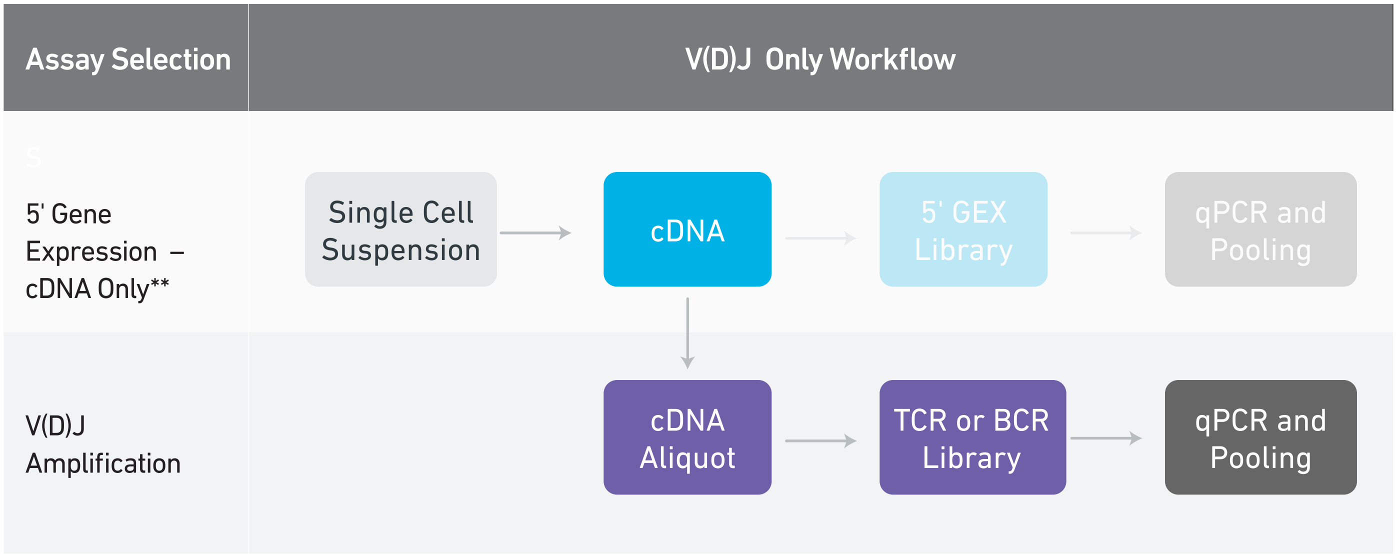
Table of Contents

Introduction

Chromium Automated Single Cell 5' Workflows



*For Automated Gene Expression flexible workflow with cDNA storage option, refer to Chromium Next GEM Single Cell 5' cDNA Kit v2 User Guide Supplement (CG000473) and Automated Library Construction User Guide (CG000474).



**Unused 5' gene expression library construction reagents will be lost, if the 5' Gene Expression – cDNA Only assay is selected. A more suitable option in this case would be to use the Chromium Next GEM Single Cell 5' cDNA Kit (PN-1000425) for cDNA generation.

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 5' protocol. DO NOT substitute any of the listed materials.

Supplier	Description	Part Number (US)
Plastics		
Hamilton	CO-RE Tips 50 µl Filtered Tips CO-RE Tips 300 µl Filtered Tips 60 ml Reagent Reservoir Self-Standing Hamilton PCR ComfortLid	235948 235903 194051 814300
Eppendorf	96-well Full-Skirted Plate* 96-well Semi-Skirted Plate *(Blue color listed; other colors are acceptable)* **Alternatively, use Amplifyt 96-Well PCR Plates, Full-Skirted, Clear from Thomas Scientific (485096/1149K05) or Fisher Scientific (NC1959287 for 25/case, NC1959288 for 100/case)*	951020460 951020362
Thermo Fisher Scientific	MicroAmp 8-Tube Strip, 0.2 ml MicroAmp 8-Cap Strip, clear	N8010580 4323032
Kits & Reagents		
Thermo Fisher Scientific	Nuclease-free Water	AM9937
Millipore Sigma	Ethanol, Pure (200 Proof, anhydrous)	E7023-500ML
Qiagen	Qiagen Buffer EB	19086
Equipment		
10x Genomics	10x Vortex Adapter	330002
-	Benchtop Vortex	-
-	Benchtop Centrifuge	-
-	Plate Centrifuge	-
-	Benchtop Thermal Cycler	-
Additional materials ONLY for optional assays – qPCR and pooling		
Bio-Rad	10% Tween 20 96-well PCR Plates	1662404 HSP9665
Thermo Fisher Scientific	2 ml-Screw-cap Tubes, NonKnurl 0.5 ml-Screw-cap Tubes, NonKnurl	3488NK 3472NK
KAPA Biosystems	KAPA Library Quantification Kit for Illumina Platforms	KK4824
Qiagen	Qiagen Buffer EB	19086
Additional materials for Chromium Connect maintenance		
Thor Labs	Lens tissues	MC-5
VWR	Microcide SQ Broad Spectrum Disinfectan	25099
Contec	70% Isopropanol (alternative to VWR disinfectant))	SB167030IR
	Use only indicated cleaning agents. DO NOT use bleach or organic oxidizers.	

Additional Kits, Reagents & Equipment

Supplier	Description	Part Number (US)
Quantification & Quality Control		
Agilent	2100 Bioanalyzer Laptop Bundle (discontinued) (Replacement 2100 Bioanalyzer Instrument/ 2100 Expert Laptop Bundle	G2943CA G2939BA/G2953CA
Choose Bioanalyzer, TapeStation, LabChip, or Qubit based on availability & preference.	High Sensitivity DNA Kit 4200 TapeStation High Sensitivity D1000 ScreenTape/Reagents High Sensitivity D5000 ScreenTape/Reagents	5067-4626 G2991AA 5067-5592/ 5067-5593 5067-5584/ 5067-5585
Thermo Fisher Scientific	Qubit 4.0 Fluorometer Qubit dsDNA HS Assay Kit	Q33238 Q32854
PerkinElmer	LabChip GX Touch HT Nucleic Acid Analyzer DNA High Sensitivity Reagent Kit	CLS137031 CLS760672

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 (discontinued)	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 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

		Steps	Timing
3 h	MANUAL	Cell Preparation (Dependent on Cell Type)	~1-1.5 h
		Gather & Load Reagents and Consumables	~60 min
	AUTOMATED	Master Mix Preparation	~3.5 h Walk-away time
		Chromium Automated Controller Loading	
		GEM Generation	
6 h		OPTIONAL Confirm GEM Generation (Manual, 5 min) ~45 min after starting	
		Post GEM RT-Cleanup – Dynabead	
		cDNA Amplification	
		cDNA Cleanup – SPRIselect	
9 h	MANUAL	cDNA QC & Quantification	~60 min
	AUTOMATED	**5' Gene Expression Library Construction**	~4.5 h Walk-away time
		Fragmentation, End Repair & A-tailing	
		Post Fragmentation, End Repair & A-tailing Double Sided Size Selection – SPRIselect	
		Adaptor Ligation	
		Post Ligation Cleanup – SPRIselect	
		Sample Index PCR	
12 h plus		Post Sample Index PCR Double Sided Size Selection – SPRIselect	
	MANUAL	Post Library Construction QC	~60 min
	MANUAL	**V(D)J Amplification & Library Construction**	~45 min
		Gather & Load Reagents and Consumables	
	AUTOMATED	V(D)J Amplification 1	~3.5 h Walk-away time
		V(D)J Amplification 1 Double Sided Size Selection – SPRIselect	
		V(D)J Amplification 2	
		V(D)J Amplification 2 Double Sided Size Selection – SPRIselect	
	MANUAL	V(D)J Amplification QC & Quantification	~60 min
	AUTOMATED	Fragmentation, End Repair & A-tailing	~4.5 h Walk-away time
		Adaptor Ligation	
		Post Ligation Cleanup – SPRIselect	
		Sample Index PCR	
		Post Sample Index PCR Cleanup – SPRIselect	
	MANUAL	Post Library Construction QC	~60 min

OPTIONAL
Library Quantification qPCR & Library Pooling

Stepwise Objectives

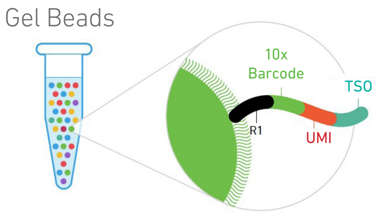
Chromium Connect automates the preparation of sequencing-ready, single cell libraries from input samples with walk-away convenience. Generation of Chromium Single Cell 5′ Gene Expression and V(D)J libraries on the Chromium Connect instrument includes automated Gel Beads-in-emulsion (GEM) generation, barcoding, and library preparation from single cell suspensions, along with additional functionalities for library quantification and pooling.

The Chromium Connect platform for 5' digital gene expression profiles 500-10,000 individual cells per sample. A pool of ~750,000 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 5' Gene Expression and V(D)J libraries.

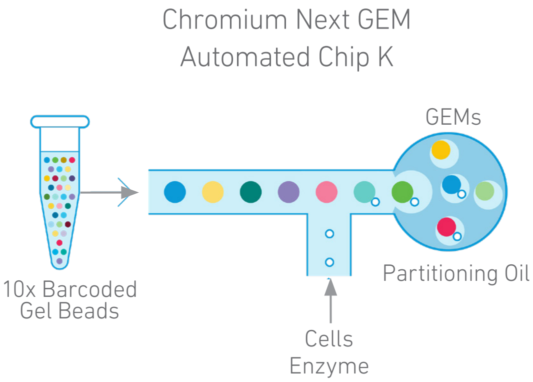
Single Cell 5' Gel Beads

The Single Cell VDJ 5' Gel Beads primer enables the production of barcoded, full-length cDNA from poly-adenylated mRNA, for generating Single Cell 5' Gene Expression and V(D)J libraries.



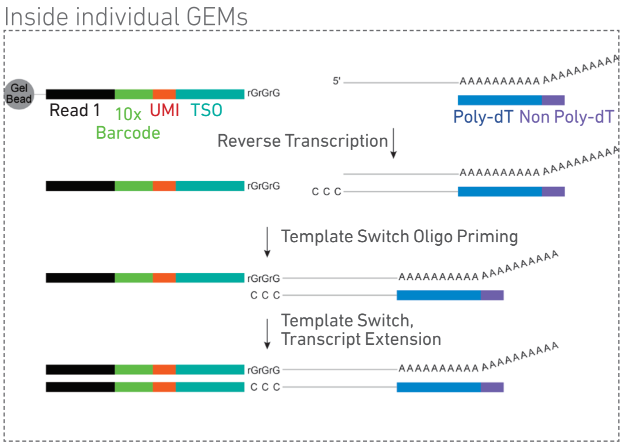
Automated GEM Generation & Barcoding

Automated GEM generation is done by combining barcoded Single Cell VDJ 5' Gel Beads, a Master Mix containing cells and enzymes, and Partitioning Oil onto Chromium Next GEM Automated Chip K. 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

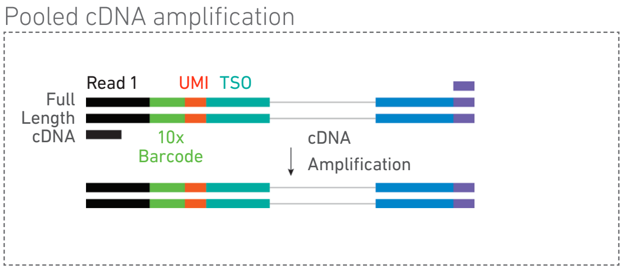
Immediately following GEM generation, the Gel Bead is dissolved and any co-partitioned cell is lysed. Oligonucleotides containing (i) an Illumina R1 sequence (read 1 sequencing primer), (ii) a 16 nt 10x Barcode, (iii) a 10 nt unique molecular identifier (UMI), and (iv) 13 nt template switch oligo (TSO) are released and mixed with the cell lysate and a Master Mix containing reverse transcription (RT) reagents and poly(dT) RT primers. Incubation of the GEMs produces 10x Barcoded, full-length cDNA from poly-adenylated mRNA.



Automated Post GEM-RT Cleanup & cDNA Amplification

GEMs are broken and pooled after GEM-RT reaction mixtures are recovered. Silane magnetic beads are used to purify the 10x Barcoded first-strand cDNA from the post GEM-RT reaction mixture, which includes leftover biochemical reagents and primers.

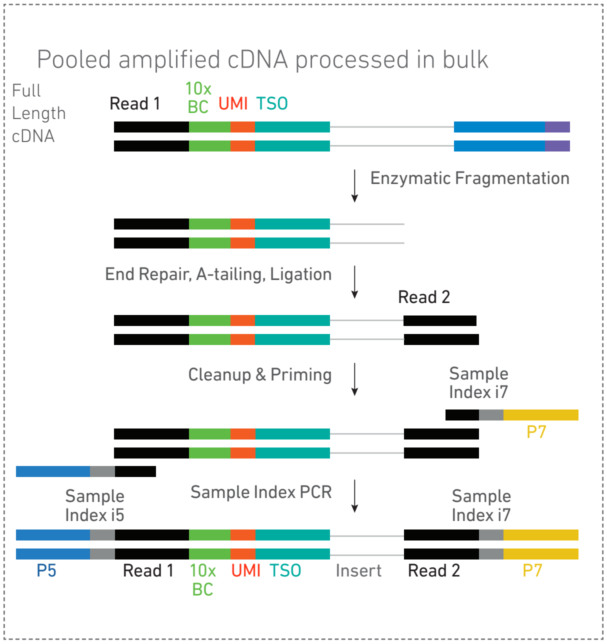
10x Barcoded, full-length cDNA is amplified via PCR with primers against common 5' and 3' ends added during GEM-RT. Amplification generates sufficient material to construct multiple libraries from the same cells, e.g. both T cell and/or B cell libraries and 5' Gene Expression libraries.



If 5' Gene Expression libraries are not desired, stop the automated protocol after cDNA amplification and proceed directly to V(D)J amplification. Unused 5' Gene Expression library construction reagents will be lost, if the Gene Expression Library Construction is not performed as the next step. A more suitable option in this case would be to use the Chromium Next GEM Single Cell 5' cDNA Kit (PN-1000425) for cDNA generation. Refer to Chromium Next GEM Single Cell 5' cDNA Kit v2 User Guide Supplement (CG000473) and Automated Library Construction User Guide (CG000474) for more details.

Automated 5' Gene Expression Library Construction

Amplified full-length cDNA from poly-adenylated mRNA is used to generate 5' Gene Expression library. Enzymatic fragmentation and size selection are used to optimize the cDNA amplicon size prior to 5' gene expression library construction. P5, P7, i5 and i7 sample indexes, and Illumina R2 sequence (read 2 primer sequence) are added via End Repair, A-tailing, Adaptor Ligation, and Sample Index PCR. The final libraries contain the P5 and P7 priming sites used in Illumina sequencers.

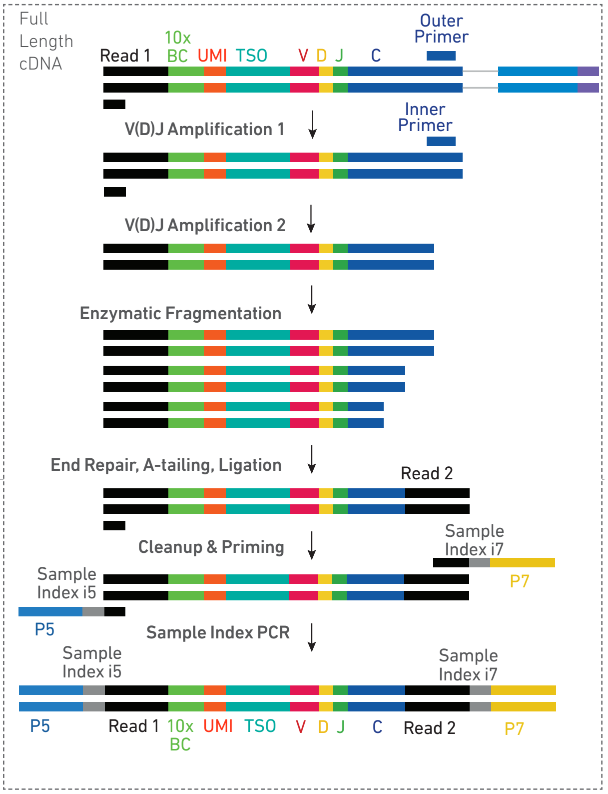


Automated V(D)J Amplification from cDNA

Amplified full-length cDNA from poly-adenylated mRNA is used to amplify full-length V(D)J segments (10x Barcoded) via PCR amplification with primers specific to either the TCR or BCR constant regions. If both T and B cells are expected to be present in the partitioned cell population, TCR and BCR transcripts can be amplified in separate reactions from the same amplified cDNA material.

Automated V(D)J Library Construction

Enzymatic fragmentation and size selection are used to generate variable length fragments that collectively span the V(D)J segments of the amplified TCR or BCR transcripts prior to library construction.

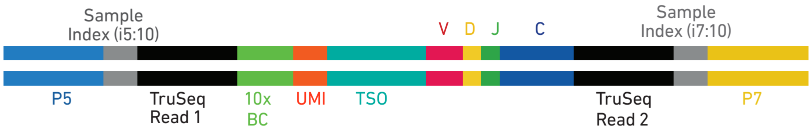


P5, P7, i5 and i7 sample indexes, and an Illumina R2 sequence (read 2 primer sequence) are added via End Repair, A-tailing, Adaptor Ligation, and Sample Index PCR. The final libraries contain the P5 and P7 priming sites used in Illumina sequencing.

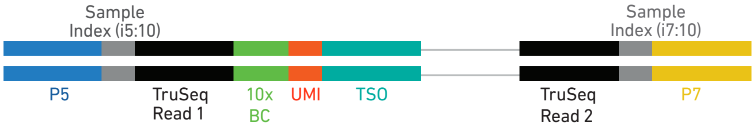
Sequencing

Illumina-ready dual index libraries can be sequenced at the recommended depth & run parameters. Illumina sequencer compatibility, sample indices, library loading and pooling for sequencing are summarized in the Sequencing chapter.

Chromium Single Cell V(D)J Dual Index Library



Chromium Single Cell 5' Gene Expression Dual Index Library



See Appendix for Oligonucleotide Sequences

Chromium Next GEM Automated Single Cell 5' Reagent Kits v2

Chromium Next GEM Automated Single Cell 5' Kit v2, 24 rxns PN-1000290

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

Chromium Next GEM Automated Single Cell 5' Kit v2, Module 1, 24 rxns PN-1000292 (store at 4°C)

Chromium
Next GEM Automated
Single Cell 5’ v2, Module 1, 24 rxns

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

10xGenomics.com

Module 1

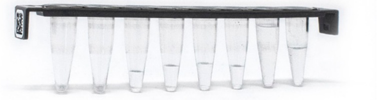
Chromium Next GEM Automated Single Cell 5' Kit v2, Module 2, 24 rxns PN-1000293 (store at -20°C)

Chromium
Next GEM Automated
Single Cell 5’ v2, Module 2, 24 rxns

		#
○	Module 2	24 tube strips

10xGenomics.com

Module 2



Chromium Next GEM Automated Single Cell 5' Kit v2, Module 3, 24 rxns PN-1000294 (store at -20°C)

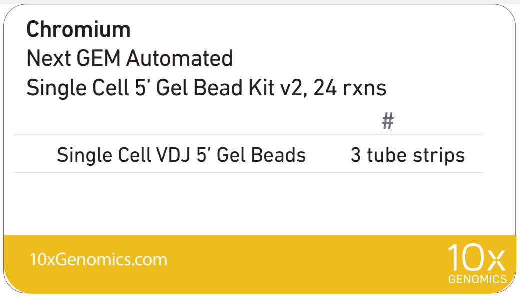
Chromium
Next GEM Automated
Single Cell 5’ v2, Module 3, 24 rxns

		#
○	Module 3	24 tube strips
	Poly-dT RT Primer	6 tubes (PN-2000007)

10xGenomics.com

Module 3

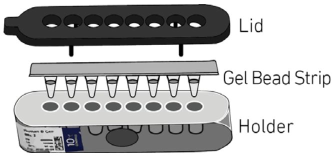
Chromium Next GEM Automated Single Cell 5' Gel Bead Kit v2, 24 rxns PN-1000291 (store at -80°C)



Chromium
Next GEM Automated
Single Cell 5' Gel Bead Kit v2, 24 rxns

	#
Single Cell VDJ 5' Gel Beads	3 tube strips

10xGenomics.com



Chromium Next GEM Automated Single Cell 5' Kit v2, 4 rxns PN-1000298

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

Chromium Next GEM Automated Single Cell 5' Kit v2, Module 1, 4 rxns PN-1000295 (store at 4°C)

Chromium
Next GEM Automated
Single Cell 5' v2, Module 1, 4 rxns

		#
■	Module 1	4 tube strips
○	Dynabeads^{TM} MyOne^{TM} SILANE	2 tubes (PN-2000048)

10xGenomics.com

Module 1

Chromium Next GEM Automated Single Cell 5' Kit v2, Modules 2 & 3, 4 rxns PN-1000296 (store at -20°C)

Chromium
Next GEM Automated
Single Cell 5' v2, Modules 2 & 3, 4 rxns

		#
▨	Module 2	4 tube strips
☐	Module 3	4 tube strips
	Poly-dT RT Primer	2 tubes (PN-2000007)

10xGenomics.com

Module 2

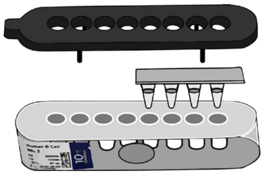
Module 3

Chromium Next GEM Automated Single Cell 5' Gel Bead Kit v2, 4 rxns PN-1000299 (store at -80°C)

Chromium
Next GEM Automated
Single Cell 5' Gel Bead Kit v2, 4 rxns

	#
Single Cell VDJ 5' Gel Beads	4 rxn-tube strip

10xGenomics.com



Chromium Automated Single Cell Human TCR Amplification & Library Construction Kit, 24 rxns PN-1000300

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

Chromium Automated Single Cell Human TCR Amplification & Library Construction, V(D)J Module 1, 24 rxns PN-1000301 (store at 4°C)

Chromium
Automated Single Cell Human TCR
Amplification & Library Construction,
V(D)J Module 1, 24 rxns

	#
● V(D)J Module 1	24 tube strips

10xGenomics.com

V(D)J Module 1

Chromium Automated Single Cell Human TCR Amplification & Library Construction, V(D)J Module 2, 24 rxns PN-1000302 (store at -20°C)

Chromium
Automated Single Cell Human TCR
Amplification & Library Construction,
V(D)J Module 2, 24 rxns

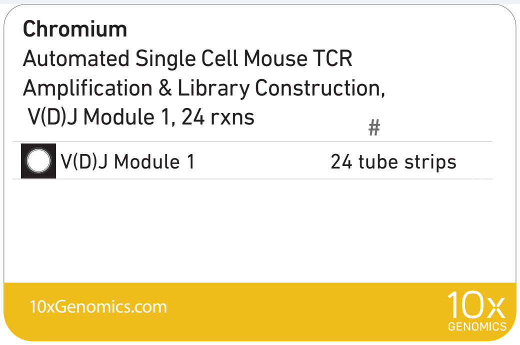
	#
○ V(D)J Module 2	24 tube strips
Human T Cell Primer Mix 1 v2	6 tubes (PN-2000242)
Human T Cell Primer Mix 2 v2	6 tubes (PN-2000246)

10xGenomics.com

V(D)J Module 2

Chromium Automated Single Cell Mouse TCR Amplification & Library Construction Kit, 24 rxns PN-1000310

Chromium Automated Single Cell Mouse TCR Amplification & Library Construction, V(D)J Module 1, 24 rxns PN-1000303 (store at 4°C)

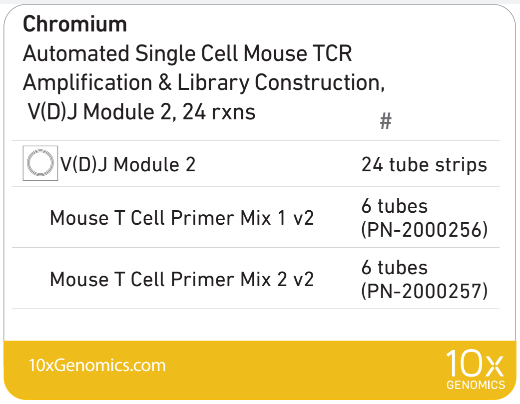


Chromium
Automated Single Cell Mouse TCR
Amplification & Library Construction,
V(D)J Module 1, 24 rxns

		#
●	V(D)J Module 1	24 tube strips

V(D)J Module 1

Chromium Automated Single Cell Mouse TCR Amplification & Library Construction, V(D)J Module 2, 24 rxns PN-1000304 (store at -20°C)



Chromium
Automated Single Cell Mouse TCR
Amplification & Library Construction,
V(D)J Module 2, 24 rxns

		#
○	V(D)J Module 2	24 tube strips
	Mouse T Cell Primer Mix 1 v2	6 tubes (PN-2000256)
	Mouse T Cell Primer Mix 2 v2	6 tubes (PN-2000257)

V(D)J Module 2

Chromium Automated Single Cell Human BCR Amplification & Library Construction Kit, 24 rxns PN-1000305

Chromium Automated Single Cell Human BCR Amplification & Library Construction, V(D)J Module 1, 24 rxns PN-1000306 (store at 4°C)

Chromium
Automated Single Cell Human BCR
Amplification & Library Construction,
V(D)J Module 1, 24 rxns

	#
○ V(D)J Module 1	24 tube strips

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V(D)J Module 1

Chromium Automated Single Cell Human BCR Amplification & Library Construction, V(D)J Module 2, 24 rxns PN-1000307 (store at -20°C)

Chromium
Automated Single Cell Human BCR
Amplification & Library Construction,
V(D)J Module 2, 24 rxns

	#
○ V(D)J Module 2	24 tube strips
Human B Cell Primer Mix 1 v2	6 tubes (PN-2000254)
Human B Cell Primer Mix 2 v2	6 tubes (PN-2000255)

10xGenomics.com

V(D)J Module 2

Chromium Automated Single Cell Mouse BCR Amplification & Library Construction Kit, 24 rxns PN-1000311

Chromium Automated Single Cell Mouse BCR Amplification & Library Construction Kit, V(D)J Module 1, 24 rxns PN-1000308 (store at 4°C)

Chromium
Automated Single Cell Mouse BCR
Amplification & Library Construction,
V(D)J Module 1, 24 rxns

	#
○ V(D)J Module 1	24 tube strips

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V(D)J Module 1

Chromium Automated Single Cell Mouse BCR Amplification & Library Construction Kit, V(D)J Module 2, 24 rxns PN-1000309 (store at -20°C)

Chromium
Automated Single Cell Mouse BCR
Amplification & Library Construction,
V(D)J Module 2, 24 rxns

	#
○ V(D)J Module 2	24 tube strips
Mouse B Cell Primer Mix 1 v2	6 tubes (PN-2000258)
Mouse B Cell Primer Mix 2 v2	6 tubes (PN-2000259)

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V(D)J Module 2

Chromium Next GEM Chip K Automated Single Cell Kit, 48 rxns PN-1000289 (store at ambient temperature)

Chromium
Partitioning Oil

	#	PN
● Partitioning Oil	6	2000190

Chromium
50% Glycerol

	#	PN
○ 50% Glycerol	6	2000109

Chromium
Next GEM Chip K
Automated Single Cell

	#	PN
Next GEM Chip K Automated Single Cell (gasket attached)	6	2000371

10xGenomics.com

Chip (gasket attached)

Chromium Next GEM Chip K Automated Single Cell Kit, 16 rxns PN-1000297 (store at ambient temperature)

Chromium
Partitioning Oil

	#	PN
● Partitioning Oil	2	2000190

Chromium
50% Glycerol

	#	PN
○ 50% Glycerol	2	2000109

Chromium
Next GEM Chip K
Automated Single Cell

	#	PN
Next GEM Chip K Automated Single Cell (gasket attached)	2	2000371

10xGenomics.com

Chip (gasket attached)

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

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 µl**.
- Based on cell stock 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.
- Refer to the 10x Genomics Support website for more information regarding cell type specific sample preparation.

Multiplet Rate (%)	# of Cells Loaded	# of Cells Recovered
~0.4%	~870	~500
~0.8%	~1,700	~1,000
~1.6%	~3,500	~2,000
~2.3%	~5,300	~3,000
~3.1%	~7,000	~4,000
~3.9%	~8,700	~5,000
~4.6%	~10,500	~6,000
~5.4%	~12,200	~7,000
~6.1%	~14,000	~8,000
~6.9%	~15,700	~9,000
~7.6%	~17,400	~10,000

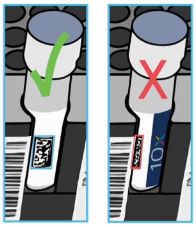
Cell Preparation

- Ensure cell counts are accurate.
- Based on cell stock 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 targeted cell recovery. 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.
- Resuspend Dynabeads and Poly-dT RT Primers at the end of loading.
- Ensure there are no air bubbles at the bottoms of reagent tubes.
- Follow the prompts on the touchscreen for handling Library Modules 1, 2, and 3 during setup and use.
- Follow the promps on the touchscreen for handling V(D)J Modules 1 and 2.
- 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 indicated, promptly move reagents back to the recommended storage.

Barcode Orientation



Chromium Automated Chip Handling

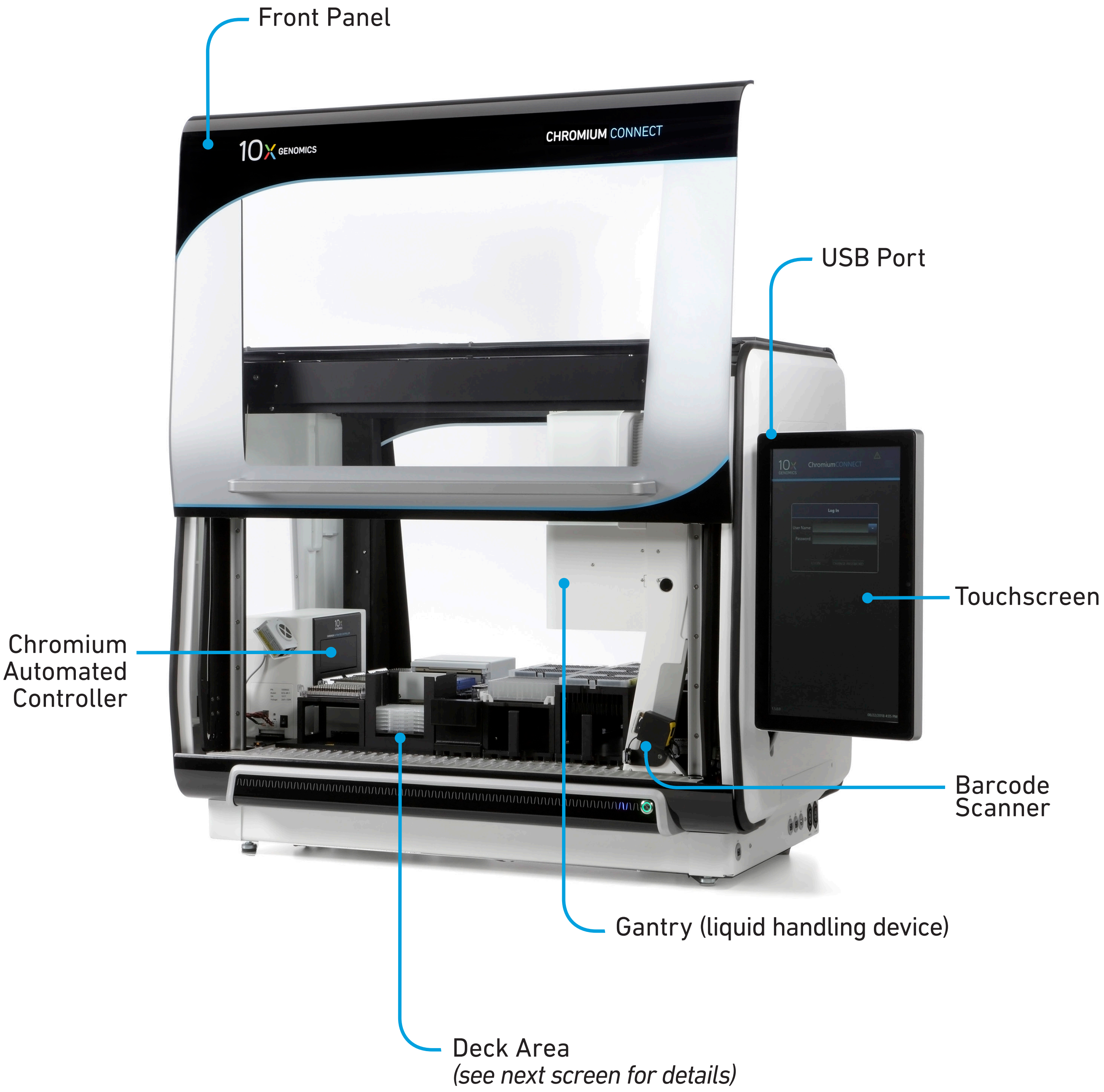
- The automated chip includes a pre-installed gasket.
- Minimize exposure of reagents and chips to sources of particles and fibers, laboratory wipes, frequently opened flip-cap tubes, clothing that sheds fibers, and dusty surfaces.
- Keep chip and gasket in sealed package until prompted to load.
- After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

Chip (gasket attached)

Chromium Connect

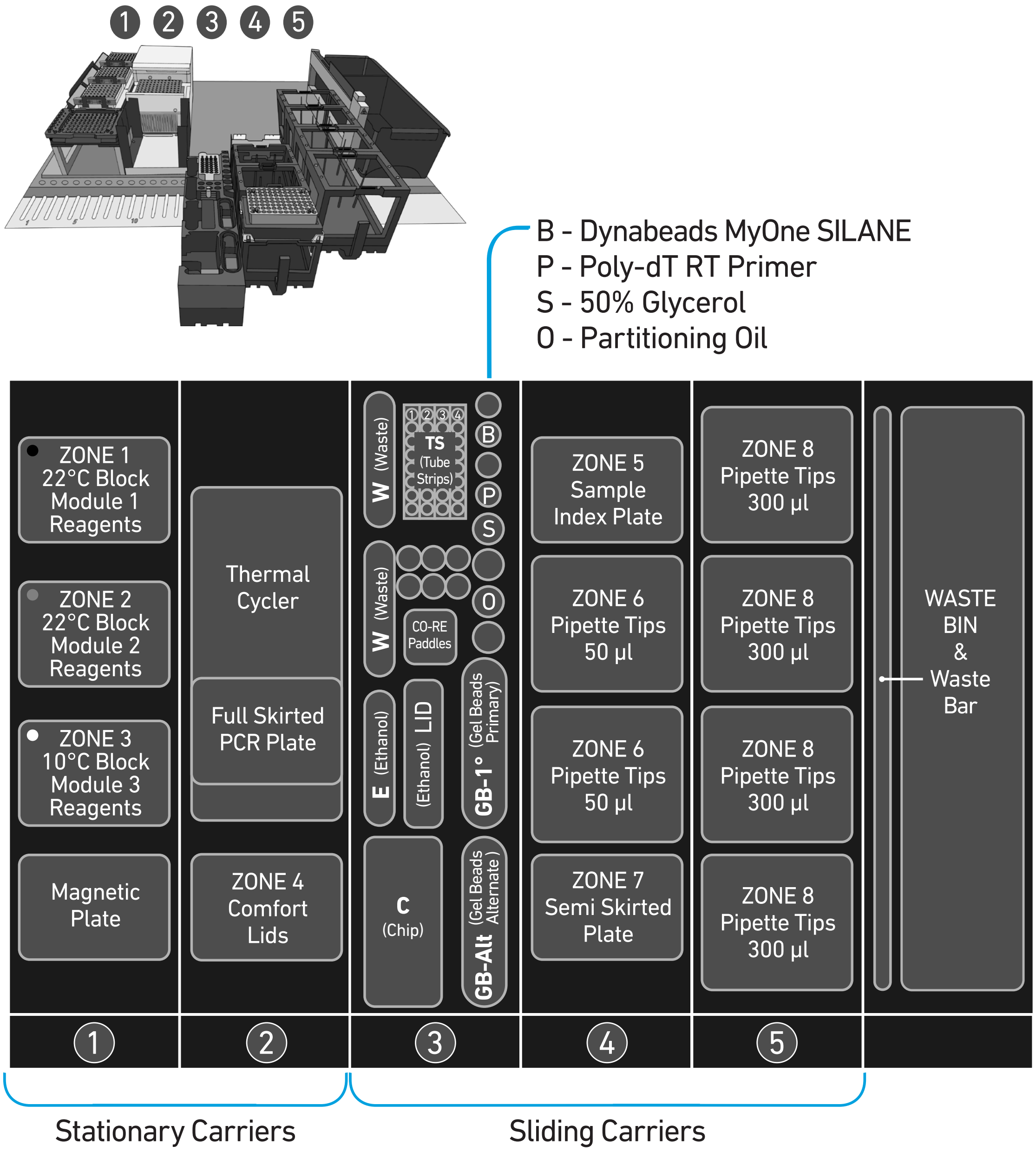
- Instrument Orientation
- Deck Orientation
- CSV Setup

Instrument Orientation



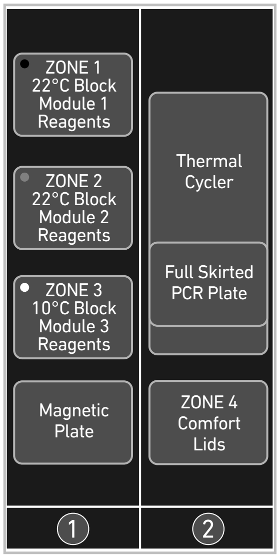
Refer to the Chromium Connect Instrument User Guide (CG000180) and Quick Reference Cards (CG000256) for more information.

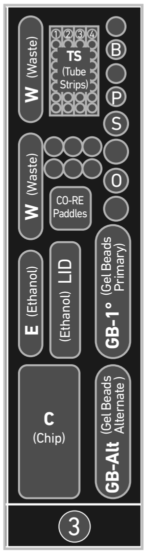
Deck Orientation

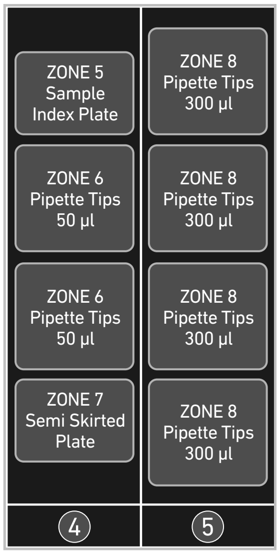


B - Dynabeads MyOne SILANE
P - Poly-dT RT Primer
S - 50% Glycerol
O - Partitioning Oil

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
1 Stationary	Zone 1 (Black)	22°C Block, Reagent Strips, Module 1
	Zone 2 (Gray)	22°C Block, Reagent Strips, Module 2
	Zone 3 (White)	10°C Block, Reagent Strips, Module 3
	-	Magnetic Plate
2 Stationary	-	Thermal Cycler
	-	Full Skirted PCR Plate (within Thermal Cycler)
	Zone 4	Comfort Lids
3* Sliding Deck Rails: 15-18 Number of Lights: 4 *Assay choices determine items loaded in Carrier 3	Position W	Waste Reservoirs
	Position TS	Tube Strips (positions 1 & 4)
	Position B	Dynabeads^{TM} MyOne^{TM} SILANE
	Position P	Poly-dT RT Primer
	Position S	50% Glycerol
	Position O	Partitioning Oil
	Position CP	CO-RE Paddles
	Position E	Ethanol Reservoir
	Position Lid	Lid for Ethanol Reservoir
	Position GB-1°	Gel Beads Primary
	Position GB-Alt	Gel Beads Alternate
	Position C	Chip
4 Sliding Deck Rails: 19-24 Number of Lights: 6	Zone 5	Sample Index Plate
	Zone 6	Pipette Tips 50 µl
	Zone 7	Semi Skirted Plate
5 Sliding Deck Rails: 25-30 Number of Lights: 6	Zone 8	Pipette Tips 300 µl

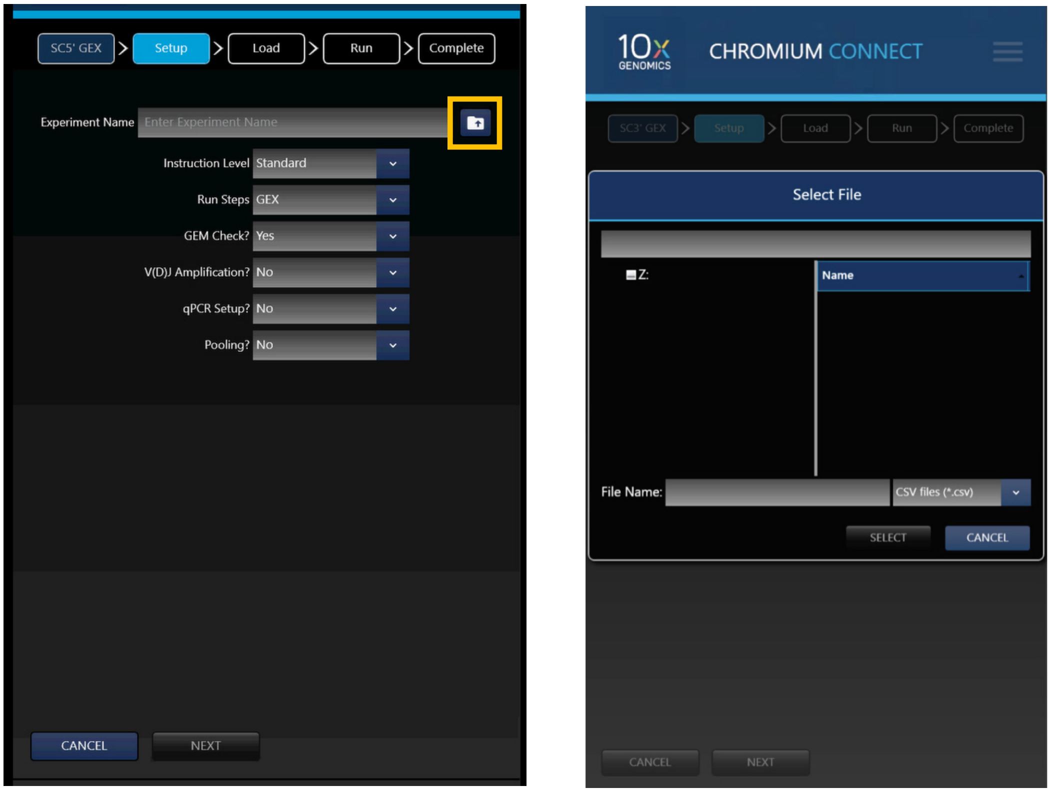
CSV Setup

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

For 5' Gene Expression Library construction, use Chromium Connect Single Cell 5' Gene Expression Input File (CG000430) and for V(D)J Library construction, use Chromium Connect Single Cell 5' V(D)J Input File (CG000432). All the files are available on the 10x Genomics support website.

Alternatively, customer's CSV files can also be generated using the customer's LIMS system. If using a LIMS system to generate CSV files, use ChromiumConnect_SC5-GEX_InputSampleInfo_Template file (CG000429) and for V(D)J Library construction, use ChromiumConnect_SC5-VDJ_InputSampleInfo_Template file (CG000431).

Run Setup Screen



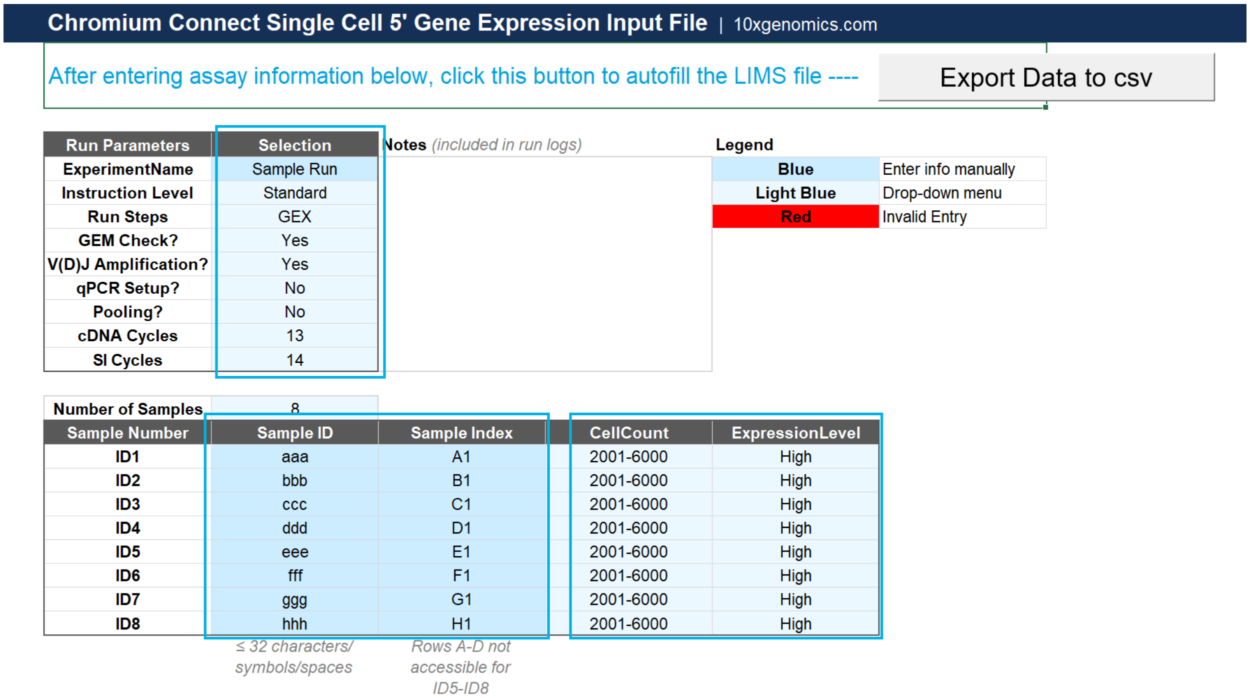
Experiment Name: Enter Experiment Name
Instruction Level: Standard
Run Steps: GEX
GEM Check?: Yes
V(D)J Amplification?: No
qPCR Setup?: No
Pooling?: No

File Name:
CSV files (*.csv)

Sample Input Files

Sample input files for Gene Expression and V(D)J Amplification are shown below, refer to Chromium Connect SC5'-GEX Input File (CG000430) for more info. 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.

Gene Expression Sample Input File



Chromium Connect Single Cell 5' Gene Expression Input File | 10xgenomics.com

After entering assay information below, click this button to autofill the LIMS file ----

Run Parameters	Selection
ExperimentName	Sample Run
Instruction Level	Standard
Run Steps	GEX
GEM Check?	Yes
V(D)J Amplification?	Yes
qPCR Setup?	No
Pooling?	No
cDNA Cycles	13
SI Cycles	14

Notes *(included in run logs)*

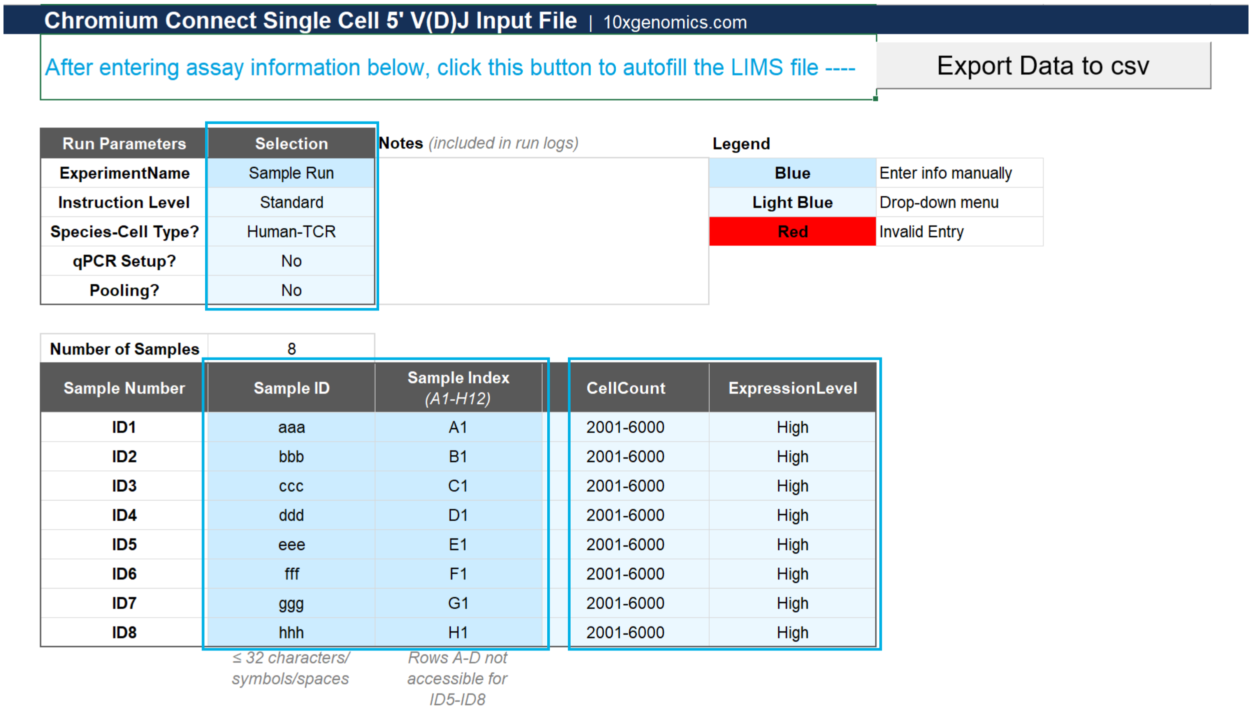
Legend

Blue	Enter info manually
Light Blue	Drop-down menu
Red	Invalid Entry

Number of Samples 8

Sample Number	Sample ID	Sample Index	CellCount	ExpressionLevel
ID1	aaa	A1	2001-6000	High
ID2	bbb	B1	2001-6000	High
ID3	ccc	C1	2001-6000	High
ID4	ddd	D1	2001-6000	High
ID5	eee	E1	2001-6000	High
ID6	fff	F1	2001-6000	High
ID7	ggg	G1	2001-6000	High
ID8	hhh	H1	2001-6000	High
	≤ 32 characters/ symbols/spaces	*Rows A-D not accessible for ID5-ID8*		

V(D)J Sample Input File



Chromium Connect Single Cell 5' V(D)J Input File | 10xgenomics.com

After entering assay information below, click this button to autofill the LIMS file ----

Run Parameters	Selection
ExperimentName	Sample Run
Instruction Level	Standard
Species-Cell Type?	Human-TCR
qPCR Setup?	No
Pooling?	No

Notes *(included in run logs)*

Legend

Blue	Enter info manually
Light Blue	Drop-down menu
Red	Invalid Entry

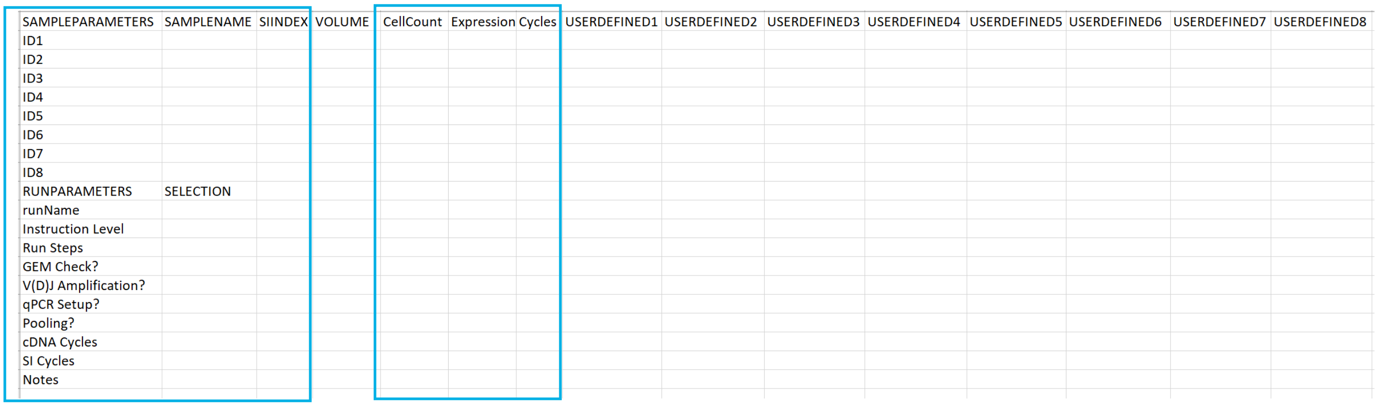
Number of Samples 8

Sample Number	Sample ID	Sample Index *(A1-H12)*	CellCount	ExpressionLevel
ID1	aaa	A1	2001-6000	High
ID2	bbb	B1	2001-6000	High
ID3	ccc	C1	2001-6000	High
ID4	ddd	D1	2001-6000	High
ID5	eee	E1	2001-6000	High
ID6	fff	F1	2001-6000	High
ID7	ggg	G1	2001-6000	High
ID8	hhh	H1	2001-6000	High
	≤ 32 characters/ symbols/spaces	*Rows A-D not accessible for ID5-ID8*		

Sample Input Template Files

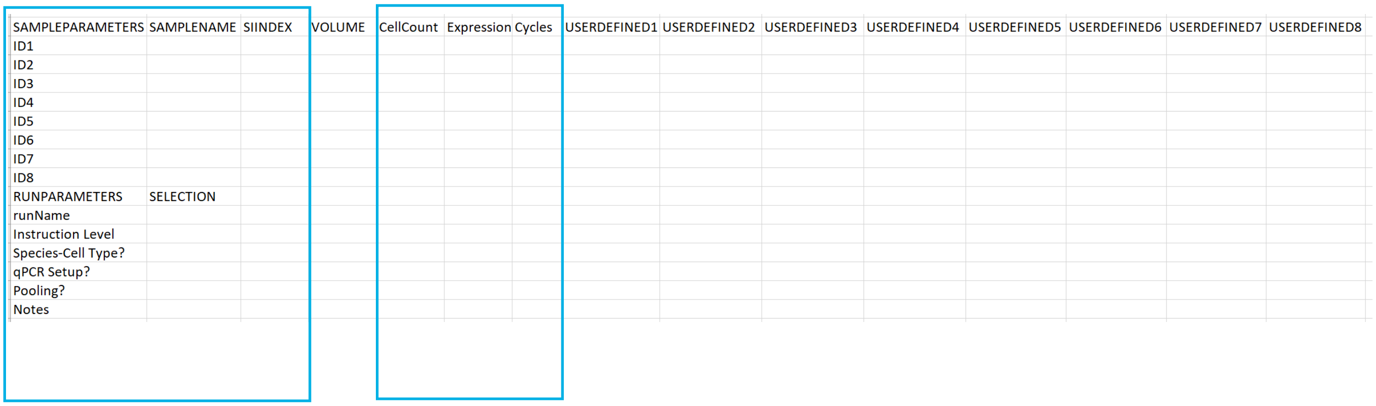
Sample input template files for Gene Expression and V(D)J Amplification are shown below, refer to Chromium Connect SC5'-GEX Input Sample Info Template (CG000429) for more info . The columns highlighted in blue are mandatory to start a run. Any missing fields/corrections can be added during sample information setup. Final selections will be recorded in the final run report CSV file.

Gene Expression Sample Input Template File



SAMPLEPARAMETERS	SAMPLENAME	SIINDEX	VOLUME	CellCount	Expression Cycles	USERDEFINED1	USERDEFINED2	USERDEFINED3	USERDEFINED4	USERDEFINED5	USERDEFINED6	USERDEFINED7	USERDEFINED8
ID1													
ID2													
ID3													
ID4													
ID5													
ID6													
ID7													
ID8													
RUNPARAMETERS	SELECTION												
runName													
Instruction Level													
Run Steps													
GEM Check?													
V(D)J Amplification?													
qPCR Setup?													
Pooling?													
cDNA Cycles													
SI Cycles													
Notes													

V(D)J Sample Input Template File



SAMPLEPARAMETERS	SAMPLENAME	SIINDEX	VOLUME	CellCount	Expression Cycles	USERDEFINED1	USERDEFINED2	USERDEFINED3	USERDEFINED4	USERDEFINED5	USERDEFINED6	USERDEFINED7	USERDEFINED8
ID1													
ID2													
ID3													
ID4													
ID5													
ID6													
ID7													
ID8													
RUNPARAMETERS	SELECTION												
runName													
Instruction Level													
Species-Cell Type?													
qPCR Setup?													
Pooling?													
Notes													

Uploading Sample Information Using a Template File

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

Sample Parameters	Information
Sample Name	Alphanumeric and up to 32 characters
SI Index	Location on sample index plate to be used for each sample during SI PCR
Expression Level	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) **500-2000** **2001-6000** **6001-10000** DO NOT use commas. 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
Run Steps	GEX/cDNA only
GEM Check	Opt-in for optional QC step: Yes/No
V(D)J Amplification	Opt-in for optional assay step: Yes/No
qPCR Setup	Opt-in for optional assay step: Yes/No
Pooling	Opt-in for optional assay step: Yes/No
Species-Cell Type	Human-TCR/Human-BCR Mouse-TCR/Mouse-BCR

Items & Reagents for cDNA Amplification and 5' GEX Library Construction

Gather Items & Reagents

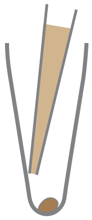
Follow prompts on the Chromium Connect touchscreen to gather the listed items and reagents for loading the Deck Carriers.
Gather the quantities specified for each of the items and reagents.

Item	Qty
Nuclease-free Water	10 ml
Ethanol, Pure (200 Proof, anhydrous)	40 ml
Hamilton	
Comfort Lids	6
50 µl CO-RE Pipette Tips, with filter (Black, Conductive)	2 racks
300 µl CO-RE Pipette Tips, with filter (Black, Conductive)	4 racks
Reagent Reservoir, 60 ml	3
Eppendorf	
96-well Semi Skirted Plate	1
96-well Full Skirted Plate	1
Thermo Fisher Scientific	
MicroAmp 8-Tube Strip, 0.2 ml	2
10x Genomics	
Chromium Next GEM Chip K Automated Single Cell Kit (stored at room temperature) *Partitioning Oil* *50% Glycerol* *Chip K (keep chip sealed)*	1
Chromium Next GEM Automated Single Cell 5' Gel Bead Kit v2 (stored at -80°C) *Single Cell VDJ 5' Gel Bead v2*	1 tube/sample
Chromium Next GEM Automated Single Cell 5' Kit v2	
Module 1 (stored at 4°C) *Black tube strip* *Dynabeads*	1 tube strip/sample 1 tube/run
Module 2 (stored at -20°C) *Gray tube strip*	1 tube strip/sample
Module 3 (stored at -20°C) *White tube strip* *Poly-dT RT Primer*	1 tube strip/sample 1 tube/run
Dual Index Plate TT Set A (stored at -20°C)	1 plate

See Additional Kits, Reagents & Equipment list for performing optional assays and/or QC.

Thaw & Prep Reagents

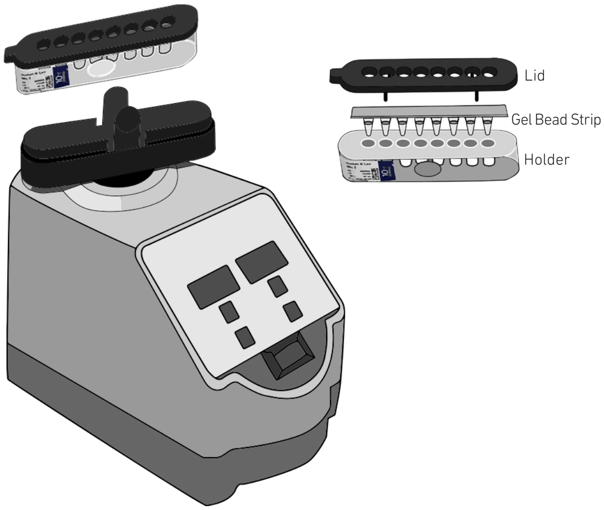
Follow prompts on the touchscreen to thaw and prepare reagents.
Some important guidelines are highlighted below.

ACTION	GUIDELINES *Follow touchscreen prompts for specifics and timing*
Thaw Reagents	• Thaw reagents as indicated on the touchscreen. Verify no precipitate is present. • Ensure that the correct thawing locations and temperatures are used. • During reagent thaw load the consumables following touchscreen prompts.
Prepare Ethanol	• Prepare **50 ml** 80% Ethanol in Nuclease-free water and dispense in Ethanol Reservoir when prompted.
Poly-dT RT Primer	• Vortex only when prompted on the touchscreen. • Centrifuge briefly before loading.
Dynabeads	• Vortex Dynabeads for **≥30 sec**. • Pipette mix to resuspend completely by using a 200 µl pipettor set to 150 µl; pipette mix at least 20 times until fully resuspended. DO NOT centrifuge. • Confirm there are no bubbles at the bottom of the tube.  Resuspend Clump
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** thaw. DO NOT vortex. Invert-mix and centrifuge at **300 rcf** for **1 min** at **22°C.**

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

Thaw & Prep Reagents

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

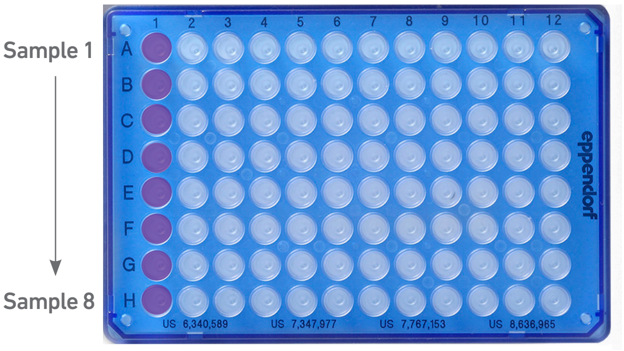
ACTION	GUIDELINES *Follow touchscreen prompts for specifics and timing*
Prepare Gel Beads	• Equilibrate the Gel Beads for **30 min** at **room temperature** before use. • Snap the tube strip holder with the Gel Bead strip into a 10x Vortex Adapter. Vortex **30 sec**. • Centrifuge the Gel Bead strip for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

Sample Preparation Guidelines

Sample Preparation Guidelines

- Resuspend samples in PBS + 0.04% BSA. Refer to Cell Suspension Volume Calculator Table for the cell suspension and buffer volumes. Total volume loaded per sample onto the sample plate is **10 µl**.
- Based on cell stock 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 38.7 µl	10 µl
Sample Loading	PCR strip tubes	96-well skirted plate
Samples per Chip	1-8	1-8
Samples Tested	Various	Human PBMCs, mouse PBMCs, mouse splenocytes, human melanoma



Samples are loaded in column 1, starting at A1. It is not necessary to add glycerol to unused sample wells when running <8 samples.

Cell Suspension Volume Calculator Table

(Chromium Connect Automated Single Cell 5' v2 protocol)

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

Cell Stock Concentration (cells/ µl)	Targeted Cell Recovery										
	500	1000	2000	3000	4000	5000	6000	7000	8000	9000	10000
100	8.50 1.50	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
200	4.25 5.75	8.50 1.50	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
300	2.83 7.17	5.67 4.33	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
400	2.13 7.88	4.25 5.75	8.50 1.50	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
500	1.70 8.30	3.40 6.60	6.80 3.20	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
600	1.42 8.58	2.83 7.17	5.67 4.33	8.50 1.50	n/a	n/a	n/a	n/a	n/a	n/a	n/a
700	1.21 8.79	2.43 7.57	4.86 5.14	7.29 2.71	9.71 0.29	n/a	n/a	n/a	n/a	n/a	n/a
800	1.06 8.94	2.13 7.88	4.25 5.75	6.38 3.63	8.50 1.50	n/a	n/a	n/a	n/a	n/a	n/a
900	0.94 9.06	1.89 8.11	3.78 6.22	5.67 4.33	7.56 2.44	9.44 0.56	n/a	n/a	n/a	n/a	n/a
1000	0.85 9.15	1.70 8.30	3.40 6.60	5.10 4.90	6.80 3.20	8.50 1.50	n/a	n/a	n/a	n/a	n/a
1100	0.77 9.23	1.55 8.45	3.09 6.91	4.64 5.36	6.18 3.82	7.73 2.27	9.27 0.73	n/a	n/a	n/a	n/a
1200	0.71 9.29	1.42 8.58	2.83 7.17	4.25 5.75	5.67 4.33	7.08 2.92	8.50 1.50	9.92 0.08	n/a	n/a	n/a
1300	0.65 9.35	1.31 8.69	2.62 7.38	3.92 6.08	5.23 4.77	6.54 3.46	7.85 2.15	9.15 0.85	n/a	n/a	n/a
1400	0.61 9.39	1.21 8.79	2.43 7.57	3.64 6.36	4.86 5.14	6.07 3.93	7.29 2.71	8.50 1.50	9.71 0.29	n/a	n/a
1500	0.57 9.43	1.13 8.87	2.27 7.73	3.40 6.60	4.53 5.47	5.67 4.33	6.80 3.20	7.93 2.07	9.07 0.93	n/a	n/a
1600	0.53 9.47	1.06 8.94	2.13 7.88	3.19 6.81	4.25 5.75	5.31 4.69	6.38 3.63	7.44 2.56	8.50 1.50	9.56 0.44	n/a
1700	0.50 9.50	1.00 9.00	2.00 8.00	3.00 7.00	4.00 6.00	5.00 5.00	6.00 4.00	7.00 3.00	8.00 2.00	9.00 1.00	10.00 0.00
1800	0.47 9.53	0.94 9.06	1.89 8.11	2.83 7.17	3.78 6.22	4.72 5.28	5.67 4.33	6.61 3.39	7.56 2.44	8.50 1.50	9.44 0.56
1900	0.45 9.55	0.89 9.11	1.79 8.21	2.68 7.32	3.58 6.42	4.47 5.53	5.37 4.63	6.26 3.74	7.16 2.84	8.05 1.95	8.95 1.05
2000	0.43 9.58	0.85 9.15	1.70 8.30	2.55 7.45	3.40 6.60	4.25 5.75	5.10 4.90	5.95 4.05	6.80 3.20	7.65 2.35	8.50 1.50

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

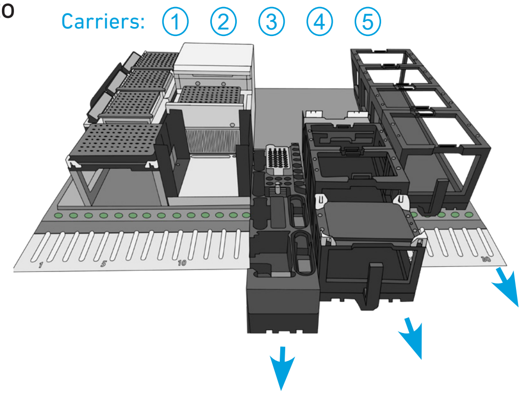
Carrier Loading Guidelines

Carrier Loading Guidelines

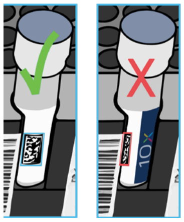
Follow the instructions on the touchscreen to load the carriers.

Carriers

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

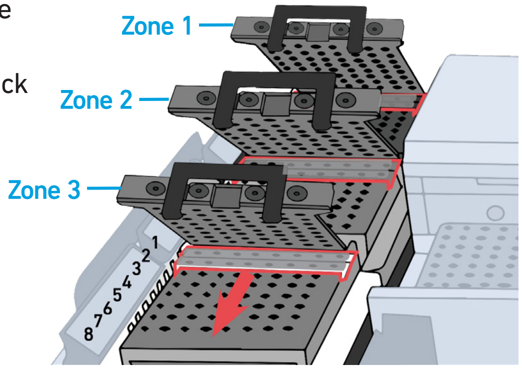


Barcode Orientation



Modules

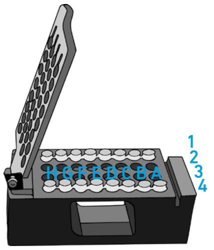
- Load one tube strip/sample of each of the indicated modules in the corresponding positions on the Carrier, starting from back to front row.
- DO NOT skip any rows when loading.
- Use pinhole alignment to place module tube strips in the correct orientation (as shown on the touchscreen).



Label Tube Strip Orientation

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

Tube Strip (TS) Holder

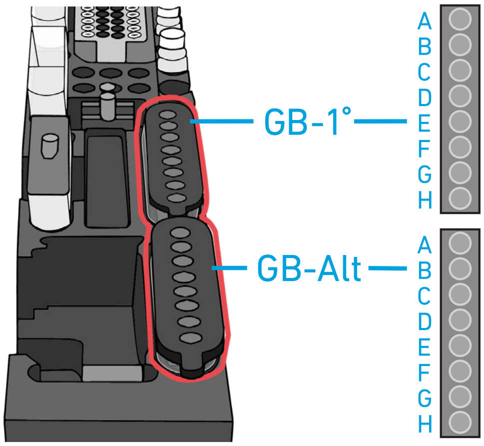


Consult the Chromium Connect User Guide (CG000180) for more information.

Carrier Loading Guidelines

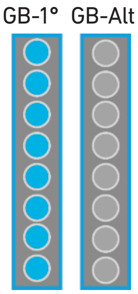
Load Gel Beads

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



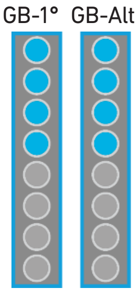
Example 1

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



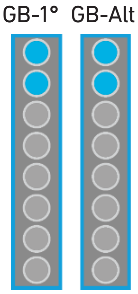
Example 2

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.

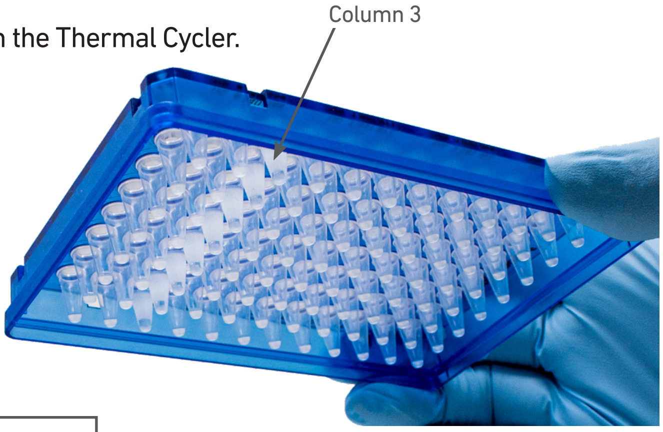


Additional Protocol Guidelines

Confirm GEM Generation
cDNA Amplification Cycles
cDNA QC & Quantification

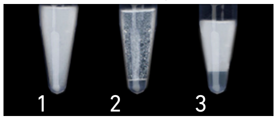
Confirm GEM Generation

- 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

Recommended starting point for cycle number optimization.

Targeted Cell Recovery	Low RNA Content Cells e.g., Primary Cells Total Cycles	High RNA Content Cells e.g., Cell Lines Total Cycles
500-2,000	16	14
2,001-6,000	14	12
6,001-10,000	13	11

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

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

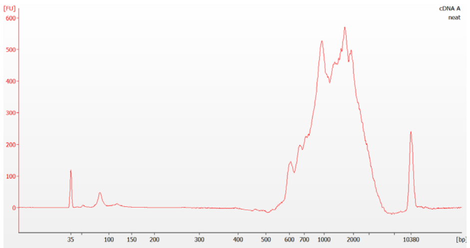
cDNA QC & Quantification

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

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

Run 1 µl undiluted product for input cells with low RNA content (<1 pg total RNA/cell), and 1 µl of 1:10 diluted product for input cells with high RNA content.

Representative Trace for PBMCs



c. If proceeding to 5' GEX Library Construction, determine cDNA yield for each sample. Example calculation below.

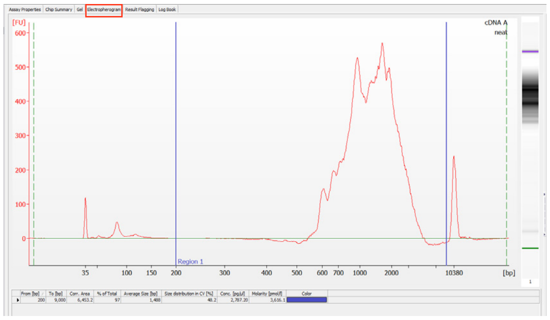
d. Enter the cDNA concentration (pg/µl) and the calculated input volume (µl) on the touchscreen to proceed with GEX library construction.

For V(D)J + GEX Library Construction proceed directly to GEX Library Construction first, followed by V(D)J Amplification and V(D)J Library Construction. If GEX library is not desired, proceed directly to V(D)J Amplification.

EXAMPLE CALCULATION

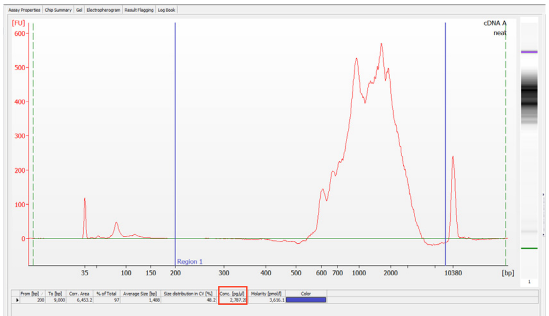
i. Select Region

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



	From [bp]	To [bp]	Corr. Area	% of Total	Average Size [bp]	Size distribution in CV [%]	Conc. [pg/µl]	Molarity [pmol/l]	Color
▸	200	9,000	6,453.2	97	1,488	48.2	2,787.20	3,616.1	▬

ii. Note Concentration [pg/µl]



	From [bp]	To [bp]	Corr. Area	% of Total	Average Size [bp]	Size distribution in CV [%]	Conc. [pg/µl]	Molarity [pmol/l]	Color
▸	200	9,000	6,453.2	97	1,488	48.2	2,787.20	3,616.1	▬

iii. Calculate

Concentration: 2787.20 pg/µl

Dilution Factor: 1

$$\text{cDNA Conc.} = \frac{\text{Conc. (pg/µl) x Dilution Factor}}{\text{1000 (pg/ng)}} = \frac{2787.20 \times 1}{1000} = \mathbf{2.79\ ng/µl}$$

Example Calculation for Carrying Forward 60 ng Sample for 5' GEX Library Construction

$$\text{Volume for 60 ng} = \frac{\text{60 ng}}{\text{2.79 (ng/µl)}} = 21.5\ \text{µl}$$

- If the volume for 60 ng exceeds 22 µl, carry ONLY 22 µl sample into library construction. The sample input volume should be in the 5-22 µl range.

Sample volume for library construction = 21.5 µl

If <60* ng available, carry forward 22 µl sample (2-60 ng) into 5' GEX Library Construction.

*Note that the intended sample amount differs from manual protocol to account for pipetting differences in automation.

DO NOT exceed a mass of 60 ng in the 22 µl carry forward volume.

Alternate Quantification Methods:

- Agilent TapeStation
- PerkinElmer LabChip (See Appendix for representative traces)

5' Gene Expression (GEX) Library Construction Guidelines

Sample Index PCR

Post Library Construction QC

Sample Index PCR

- The cycle numbers can be manually selected based on cDNA input.
- Recommended guidelines for selecting optimal Sample Index PCR cycle number.

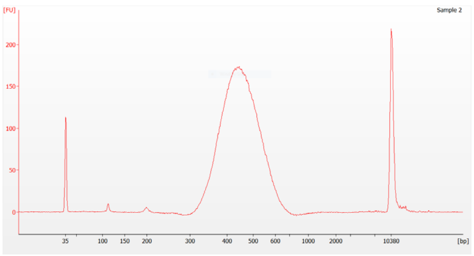
cDNA Input	Total Cycles
1-30 ng	16
31-60 ng	14

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.

Representative Trace



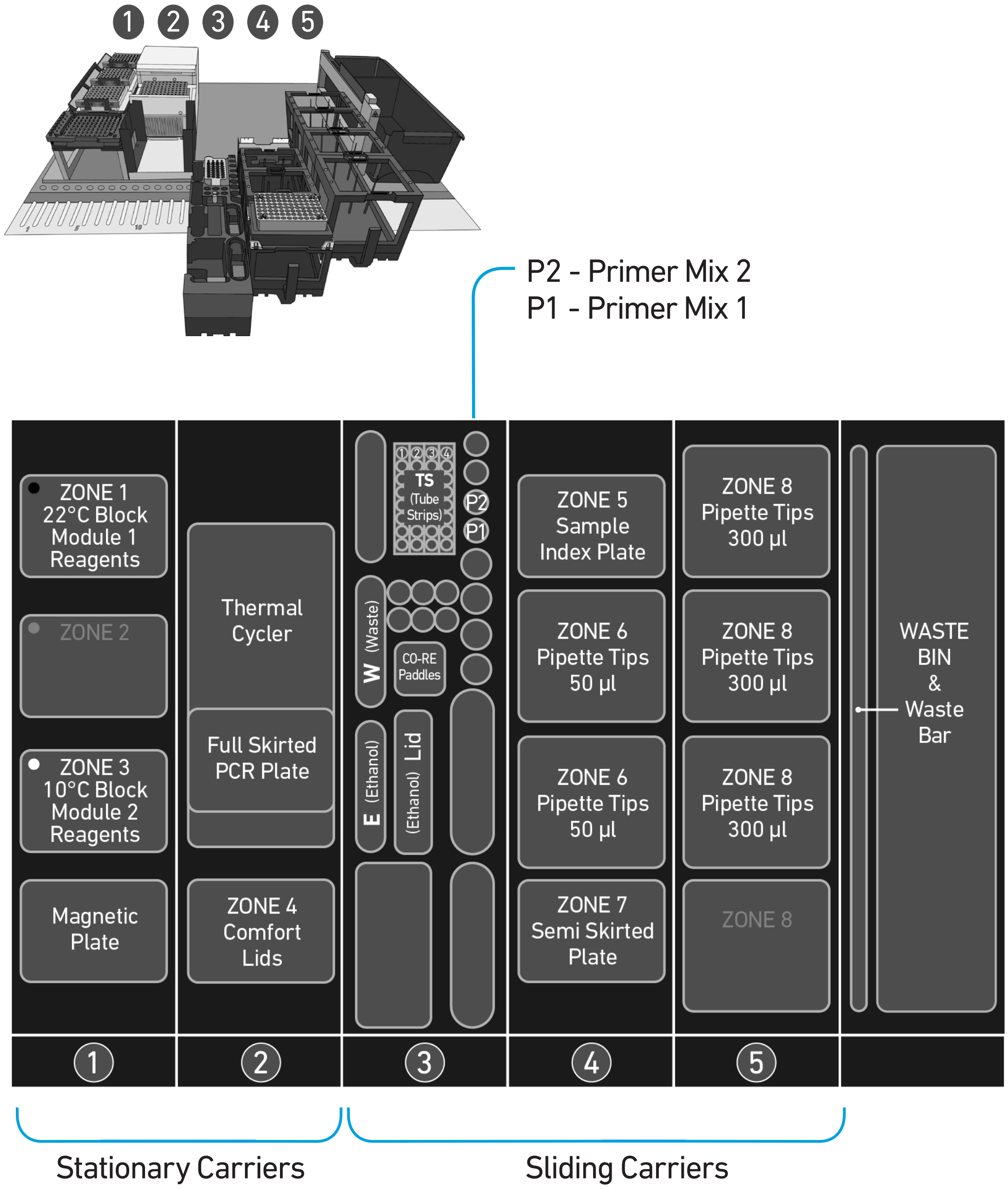
Determine the average fragment size from the Bioanalyzer trace. This will be used as the insert size for library quantification.

Alternate QC Method:

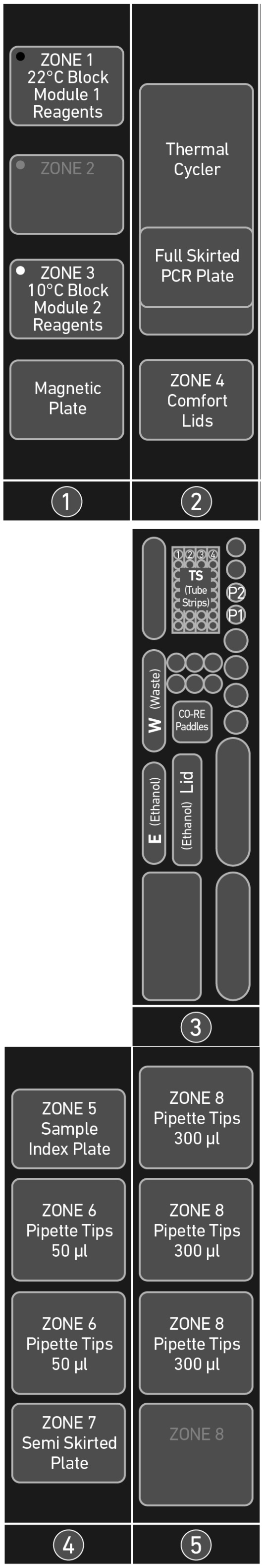
- Agilent TapeStation
- PerkinElmer LabChip (See Appendix for representative traces)

V(D)J Amplification & Library Construction Guidelines

Deck Orientation – V(D)J Amplification & Library Construction



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
1 Stationary	Zone 1 (Black)	22°C Block, Reagent Strips, Module 1
	Zone 3 (White)	10°C Block, Reagent Strips, Module 2
	-	Magnetic Plate
2 Stationary	-	Thermal Cycler
	-	Full Skirted PCR Plate (within Thermal Cycler)
	Zone 4	Comfort Lids
3* Sliding Deck Rails: 15-18 Number of Lights: 4 *Assay choices determine items loaded in Carrier 3	Position W	Waste Reservoirs
	Position TS	Tube Strips (positions 1 & 4)
	Position P2	Primer Mix 2
	Position P1	Primer Mix 1
	Position CP	CO-RE Paddles
	Position E	Ethanol Reservoir
	Position Lid	Lid for Ethanol Reservoir
4 Sliding Deck Rails: 19-24 Number of Lights: 6	Zone 5	Sample Index Plate
	Zone 6	Pipette Tips 50 µl
	Zone 7	Semi Skirted Plate
5 Sliding Deck Rails: 25-30 Number of Lights: 6	Zone 8	Pipette Tips 300 µl

Gather Items & Reagents for V(D)J Amplification and Library Construction

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)	3 racks
Reagent Reservoir, 60 ml	2
Eppendorf	
96-well Semi Skirted Plate	1
96-well Full Skirted Plate	1
Thermo Fisher Scientific	
MicroAmp 8-Tube Strip, 0.2 ml	2
10x Genomics	
Chromium Automated Single Cell Human TCR Amplification & Library Construction v2	
V(D)J Module 1 (stored at 4°C) *Black tube strip*	1 tube strip/sample
V(D)J Module 2 (stored at -20°C) *White tube strip* *Human T Cell Primer Mix 1 v2* *Human T Cell Primer Mix 2 v2*	1 tube strip/sample 1 tube/run 1 tube/run
Chromium Automated Single Cell Mouse TCR Amplification & Library Construction v2	
V(D)J Module 1 (stored at 4°C) *Black tube strip*	1 tube strip/sample
V(D)J Module 2 (stored at -20°C) *White tube strip* *Mouse T Cell Primer Mix 1 v2* *Mouse T Cell Primer Mix 2 v2*	1 tube strip/sample 1 tube/run 1 tube/run
Chromium Automated Single Cell Human BCR Amplification & Library Construction v2	
V(D)J Module 1 (stored at 4°C) *Black tube strip*	1 tube strip/sample
V(D)J Module 2 (stored at -20°C) *White tube strip* *Human B Cell Primer Mix 1 v2* *Human B Cell Primer Mix 2 v2*	1 tube strip/sample 1 tube/run 1 tube/run
Chromium Automated Single Cell Mouse BCR Amplification & Library Construction v2	
V(D)J Module 1(stored at 4°C) *Black tube strip*	1 tube strip/sample
V(D)J Module 2 (stored at -20°C) *White tube strip* *Mouse B Cell Primer Mix 1 v2* *Mouse B Cell Primer Mix 2 v2*	1 tube strip/sample 1 tube/run 1 tube/run
Dual Index Plate TT Set A (stored at -20°C)	1 plate

See Additional Kits, Reagents & Equipment list for performing optional assays and/or QC.

Thaw & Prep Reagents for V(D)J Amplification & Library Construction

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

ACTION	GUIDELINES *Follow touchscreen prompts for specifics and timing*
Thaw Reagents	• Thaw reagents as indicated on the touchscreen. Verify no precipitate is present. • Ensure that the correct thawing locations and temperatures are used. • During reagent thaw load the consumables following touchscreen prompts.
Prepare Ethanol	• Prepare **50 ml** 80% Ethanol in Nuclease-free water and dispense in Ethanol Reservoir when prompted.
V(D)J Modules	• Thaw V(D)J Modules as prompted on the touchscreen. • After reagent thaw, invert rack holding Module tube strips and vortex V(D)J Modules 1 for **30 sec**; verify no precipitate. • Confirm that there are no bubbles at the bottoms of any module tubes. • Centrifuge V(D)J Module 1 at **300 rcf** for **1 min** at **22°C.** • Retrieve V(D)J Module 2 from **4°C** thaw. DO NOT vortex. Invert-mix and centrifuge at **300 rcf** for **1 min** at **22°C.**
Dual Index Plate TT Set A	• Vortex Dual Index Plate for 15 sec at maximum speed and centrifuge at **300 rcf** for **1 min** at **22°C.**
Primer Mix 1 & 2	• Vortex and centrifuge before loading.

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

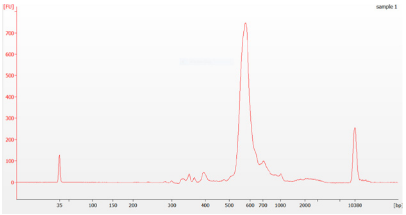
V(D)J Amplification QC & Quantification

a. Follow the instruction on the touchscreen for V(D)J Amplification QC & quantification.

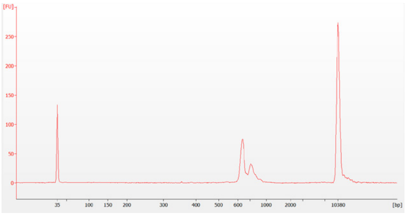
b. Run **1 µl** sample at **1:5 dilution** (Dilution Factor 5) on an Agilent Bioanalyzer High Sensitivity chip.

Samples of RNA-rich cells may require additional dilution in nuclease-free water. The number of distinct peaks may vary. Higher molecular weight product (2,000- 9,000 bp) may be present. This does not affect sequencing.

Representative Trace - PBMCs amplified for TCR



Representative Trace - PBMCs amplified for BCR



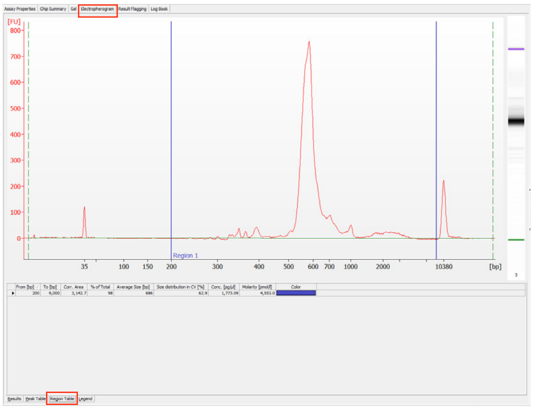
c. Determine yield for each sample using the example calculation below.

d. Enter the V(D)J amplified product concentration (pg/µl) and the calculated input volume (µl) on the touchscreen to proceed with V(D)J library construction.

EXAMPLE CALCULATION

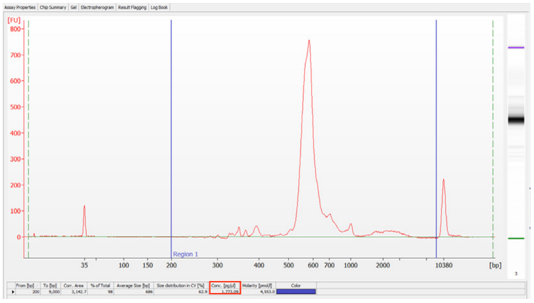
i. Select Region

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



From [bp]	To [bp]	Corr. Area	% of Total	Average Size [bp]	Size distribution in CV [%]	Conc. [pg/µl]	Molarity [pmol/l]	Color
200	9,000	3,142.7	98	686	62.9	1,773.09	4,553.0	🟦

ii. Note Concentration [pg/µl]



From [bp]	To [bp]	Corr. Area	% of Total	Average Size [bp]	Size distribution in CV [%]	Conc. [pg/µl]	Molarity [pmol/l]	Color
200	9,000	3,142.7	98	686	62.9	1,773.09	4,553.0	🟦

iii. Calculate

Concentration: 1773.07 pg/µl

Dilution Factor:

V(D)J Amplified Product Conc.

$$\frac{\text{Conc. (pg/µl) x Dilution Factor}}{\text{1000 (pg/ng)}} = \frac{1773.07 \text{ x } 5}{1000}$$

= **8.9 ng/µl**

Example Calculation for Carrying Forward 60 ng Sample for V(D)J Library Construction

$$\text{Volume for 60 ng} = \frac{\text{60 ng}}{\text{8.9 (ng/µl)}} = \mathbf{6.7\ µl}$$

- The sample input volume should be in the 5-22 µl range.

If <60* ng available, carry forward 22 µl sample (2-60 ng) into V(D)J Library Construction.
*Note that the intended sample amount differs from manual protocol to account for pipetting differences in automation.

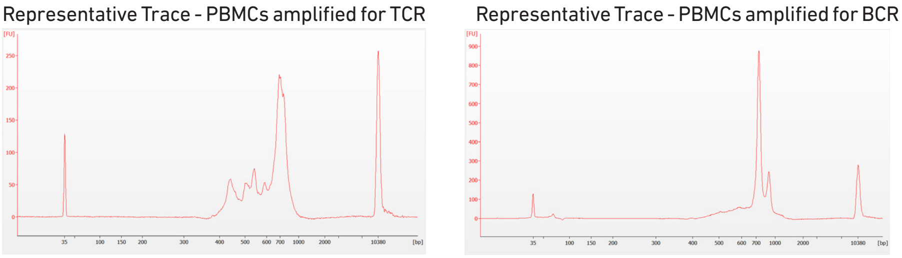
DO NOT exceed a mass of 60 ng in the 22 µl carry forward volume.

Alternate Quantification Methods

- PerkinElmer LabChip (See Appendix for representative traces)
- Agilent TapeStation

V(D)J Library Construction QC

Run sample on an Agilent Bioanalyzer High Sensitivity chip.



Representative Trace

Representative Trace - PBMCs amplified for TCR

Representative Trace - PBMCs amplified for BCR

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

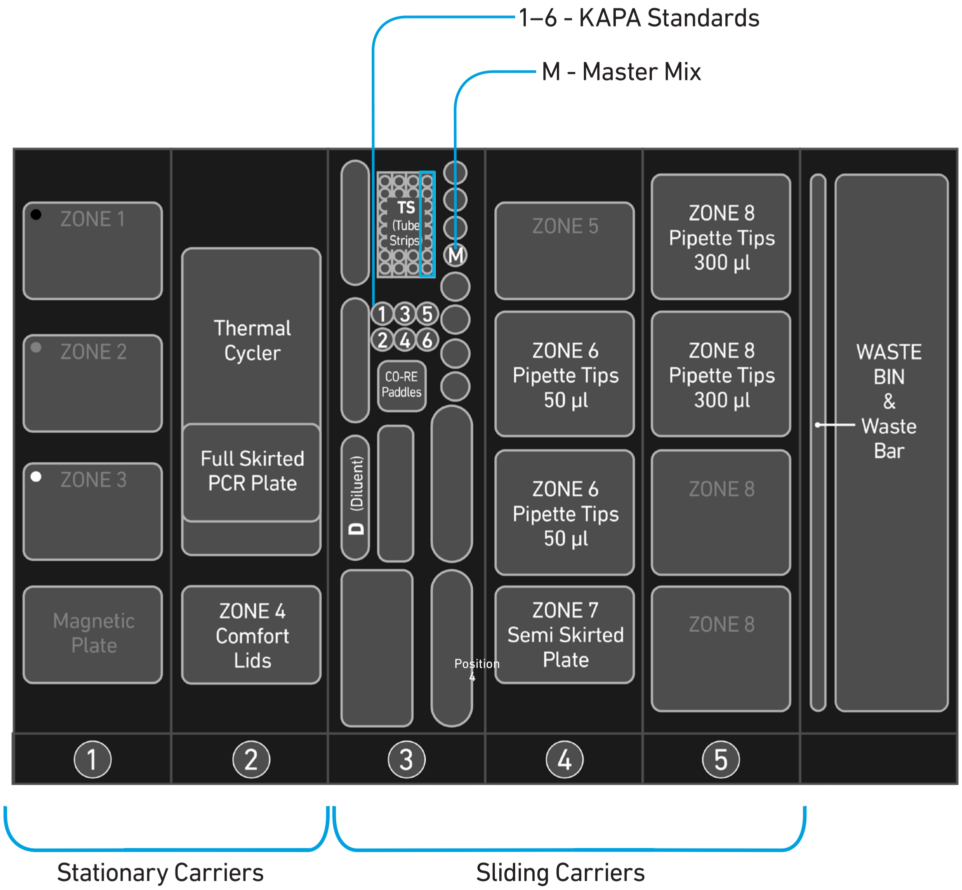
Alternate QC Methods

- LabChip (See Appendix for representative traces)
- Agilent TapeStation

Post Library Construction Quantification & 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-setup can be run directly on Chromium Connect after library generation and final library QC. Alternatively, the option can be selected from the instrument home screen, at the user's convenience. Up to 8 samples can be quantified on a 96 well reaction plate, including duplicates for each sample. The minimum sample volume required is **25 µl.** Only **6 µl** of the sample will be used for qPCR plate setup.



Gather Items & Reagents

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

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

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

Reagent	Qty
Qiagen Buffer EB	50 ml
Nuclease-free Water	1 ml
10% Tween-20	250 µl
Libraries (in an 8-tube strip)	1-8
KAPA Library Quantification Kit, thawed	
SYBR FAST Master Mix Primer Mix Standards	5 ml 1 ml 6

Post Library Construction Quantification

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

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

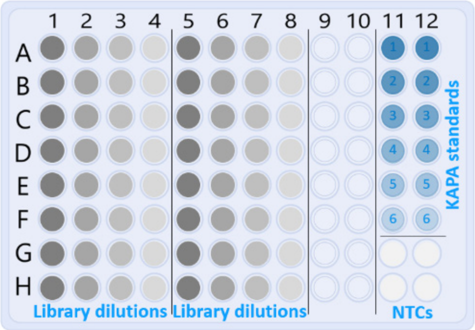
Volumes listed take into account volume for 6 standards

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

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

Dilutions:

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



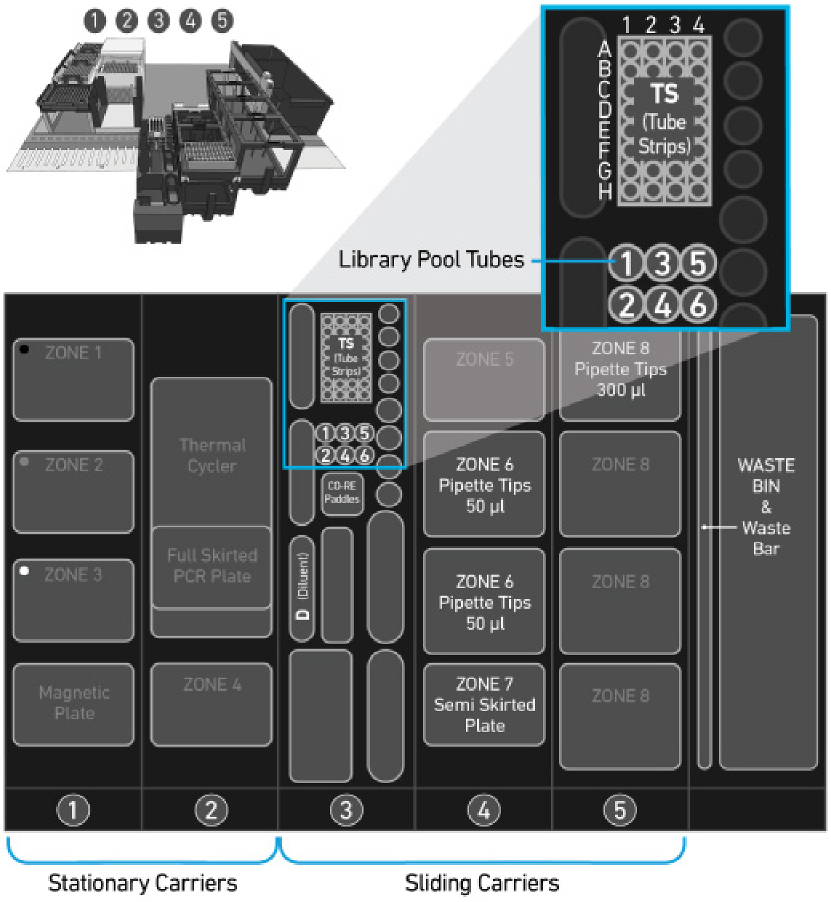
- After the run is completed, follow the unloading instructions on the touchscreen.
- Cap and store libraries at **4°C ≤72 h** or **-20°C ≤4 weeks**.
- Remove Full Skirted Plate. Seal plate and centrifuge at **300 rcf** for **1 min** at **22°C**.
- Follow the manufacturer's recommendations for qPCR-based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

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

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

Deck Orientation – Library Pooling

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



Gather Items & Reagents

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

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

Item	Qty
Hamilton	
50 µl CO-RE Pipette Tips, with filter (Black, Conductive)	1-2 rack
300 µl CO-RE Pipette Tips, with filter (Black, Conductive)	1-2 rack
Reagent Reservoir, 60 ml	1
Eppendorf	
96-well Semi Skirted Plate	1
Thermo Fisher Scientific	
0.5-ml Tube with Screw Cap	6
MicroAmp 8-Tube Strip, 0.2 ml	1-4
Reagent	**Qty**
Qiagen Buffer EB	50 ml
Libraries (in up to four 8-tube strips)	up to 32 libraries

Library Pooling

- Follow the touchscreen prompts for loading, scanning, and executing the run.
- Briefly vortex and centrifuge libraries in the 8-tube strip.
- Confirm that there are no bubbles at the bottoms of any library tubes.
- 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.

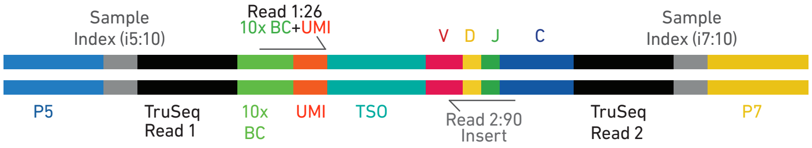
Sequencing

Sequencing Libraries

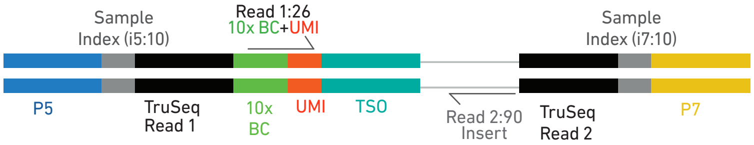
Chromium Single Cell V(D)J and 5' Gene Expression Dual Index libraries comprise standard Illumina paired-end constructs which begin with P5 and end with P7. These libraries include 16 bp 10x Barcodes encoded at the start of TruSeq Read 1. Sample index sequences are incorporated as the 10 bp i5 and i7 index reads.

TruSeq Read 1 and TruSeq Read 2 are standard Illumina sequencing primer sites used in paired-end sequencing of V(D)J and 5' Gene Expression libraries. Sequencing these libraries produce a standard Illumina BCL data output folder.

Chromium Single Cell V(D)J Dual Index Library



Chromium Single Cell 5' Gene Expression Dual Index 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 500/550/2000
- HiSeq 2500 (Rapid Run)
- HiSeq 3000/4000
- NovaSeq

Sample Indices

Each well of the Dual Index Kit TT Set A (PN-1000215) contains a mix of one 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 plate well ID) is needed in the sample sheet used for generating FASTQs with “cellranger mkfastq”.

If multiple libraries are pooled in a sequence lane, a separate sample index is needed with each library (see Tips & Best Practices).

Library Sequencing Depth & Run Parameters

Sequencing Depth	Minimum 5,000 read pairs per cell for V(D)J library Minimum 20,000 read pairs per cell for 5' Gene Expression library
Sequencing Type	Paired-end, Dual indexing
Sequencing Read	Read 1: 26 cycles i7 Index: 10 cycles i5 Index: 10 cycles Read 2: 90 cycles

Library Loading

Once quantified and normalized, V(D)J and 5' 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	10	1
NextSeq 500	1.5	1
NovaSeq	150*/300	1
NextSeq 2000	650	1

* Use 150 pM loading concentration for Illumina XP workflow.

Library Pooling

V(D)J and 5' Gene Expression libraries may be pooled for sequencing, taking into account the differences in depth requirements between the pooled libraries. 5' Gene Expression libraries may be sequenced using enriched library parameters, however the cost of sequencing using enriched library parameters is higher.

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

Library Pooling Examples:

Libraries	Sequencing Depth (read pairs per cell)	Library Pooling Ratio
Example 1		
V(D)J library	5,000	1
5' Gene Expression library	20,000	4
Example 2		
V(D)J library	5,000	1
5' Gene Expression library	50,000	10

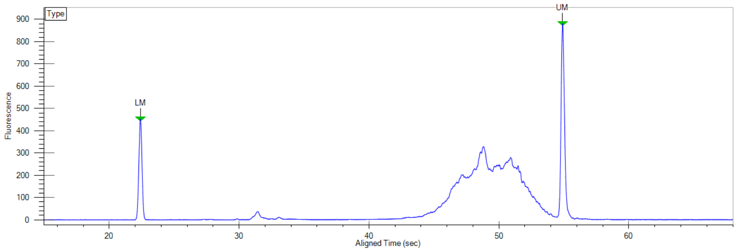
Appendix

LabChip Traces
Oligonucleotide Sequences

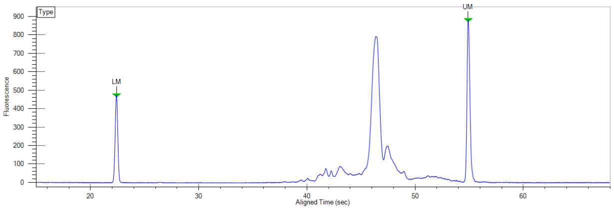
LabChip Traces

LabChip Traces DNA High Sensitivity Reagent Kit was used.

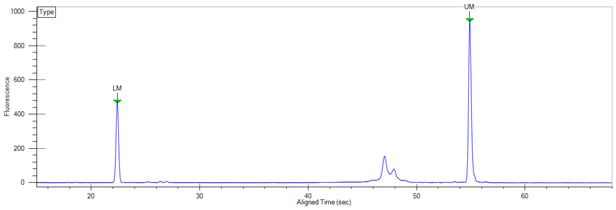
cDNA QC & Quantification



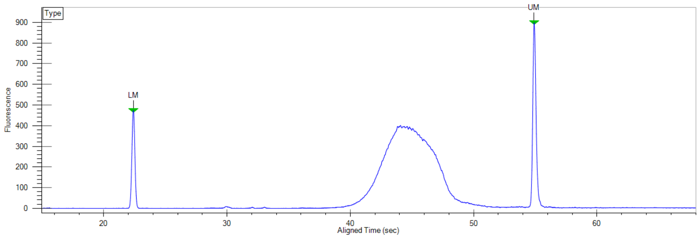
Post TCR Amplification QC



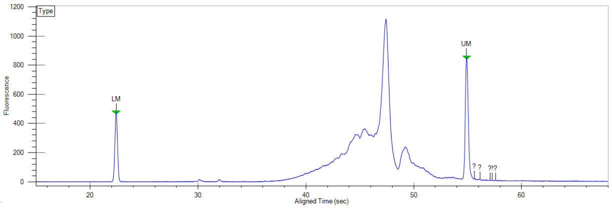
Post BCR Amplification QC



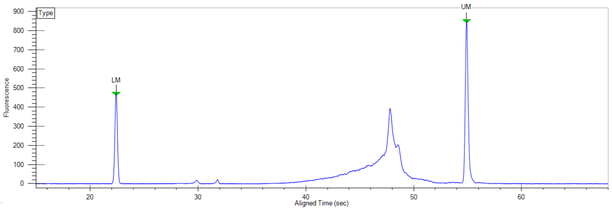
GEX Post Library Construction QC



Post V(D)J Library Construction QC (PBMCs amplified for TCR)



Post V(D)J Library Construction QC (PBMCs amplified for BCR)



Alternate QC Method:

Qubit Fluorometer and Qubit dsDNA HS Assay Kit

Oligonucleotide Sequences

Protocol steps correspond to the Chromium Next GEM Automated Single Cell 5' v2 protocol.

GEM-RT Incubation

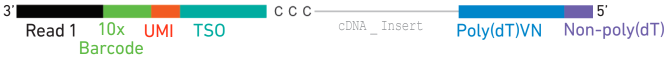
Gel Bead Primer

5'-CTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNNN-TTTCTTATATrGrGrG-3'

Poly-dT RT Primer
PN-2000007

5'-AAGCAGTGGTATCAACGCAGAGTACTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTVN-3'

GEM-RT Products



3'-GATGTGCTGCGAGAAGGCTAGA-N16-N10-AAAGAATATACCC-cDNA_Insert-NVTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-CATGAGACGCAACTATGGTGACGAA-5'

Automated Protocol Step – cDNA Amplification

cDNA Primers

Forward Primer:

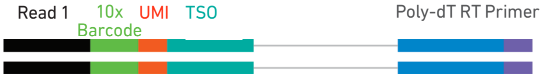
5'-CTACACGACGCTCTTCCGATCT-3'

Reverse Primer:

5'-AAGCAGTGGTATCAACGCAGAG-3'

Amplified Products

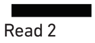
Amplified cDNA from poly-adenylated mRNA



5'–CTACACGACGCTCTTCCGATCT-N16-N10-TTTCTTATATGGG-cDNA_Insert-GTACTCTGCGTTGATACCACTGCTT–3'
3'–GATGTGCTGCGAGAAGGCTAGA-N16-N10-AAAGAATATACCC-cDNA_Insert-CATGAGACGCAACTATGGTGACGAA–5'

Automated Protocol Step –GEX Adaptor Ligation (for 5' Gene Expression (GEX) Library Construction)

Adaptor Read 2



5′ –GATCGGAAGAGCACACGTCTGAACTCCAGTCAC–3′
3′ –TCTAGCCTTCTCG–5′

Ligation Product

5'–GATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-N16-N10-TTTCTTATATGGG-cDNA_Insert-AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC–3'
3'–CTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-N16-N10-AAAGAATATACCC-cDNA_Insert-TCTAGCCTTCTCG–5'

Automated Protocol Step –Sample Index PCR (for 5' Gene Expression (GEX) Library Construction)

Dual Indexing
Dual Index TT Set A
PN-1000215

Forward Primer:

5'-AATGATACGGCGACCACCGAGATCT-N10-ACACTCTTTCCCTACACGACGCTC-3'

Reverse Primer:

5'-CAAGCAGAAGACGGCATACGAGAT-N10-GTGACTGGAGTTCAGACGTGT-3'

Sample Index PCR Product

5'-AATGATACGGCGACCACCGAGATCTACAC-N10-ACACTCTTTCCCTACACGACGCTCTTCCGATCT-N16-N10-TTTCTTATATGGG-cDNA _ Insert-AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC-N10-ATCTCGTATGCCGTCTTCTGCTTG-3'

3'-TTACTATGCCGCTGGTGGCTCTAGATGTG-N10-TGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-N16-N10-AAAGAATATACCC-cDNA _ Insert-TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG-N10-TAGAGCATACGGCAGAAGACGAAC-5'

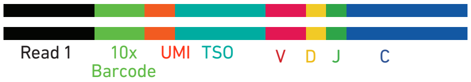
Automated Protocol Step – V(D)J Amplification 1

Human T Cell Mix 1 v2 PN-2000242	Forward Primer: ▬ PCR Primer 5’-GATCTACACTCTTTCCCTACACGACGC-3’	Reverse Outer Primers: 5’-TGAAGGCGTTTGCACATGCA-3’ 5’-TCAGGCAGTATCTGGAGTCATTGAG-3	▬ Outer Primer
Human B Cell Mix 1 v2 PN-2000254	Forward Primer: ▬ PCR Primer 5’-GATCTACACTCTTTCCCTACACGACGC-3’	Reverse Outer Primers: 5’-CAGGGCACAGTCACATCCT-3’ 5’-TGCTGGACCACGCATTTGTA-3’ 5’-GGTTTTGTTGTCGACCCAGTCT-3’ 5’-TTGTCCACCTTGGTGTTGCT-3’ 5’-CATGACGTCCTTGGAAGGCA-3’ 5’-TGTGGGACTTCCACTG-3’ 5’-TTCTCGTAGTCTGCTTTGCTCAG-3’	▬ Outer Primer
Mouse T Cell Mix 1 v2 PN-2000256	Forward Primer: ▬ PCR Primer 5’-GATCTACACTCTTTCCCTACACGACGC-3’	Reverse Outer Primers: 5’-CTGGTTGCTCCAGGCAATGG-3’ 5’-TGTAGGCCTGAGGGTCCGT-3’	▬ Outer Primer
Mouse B Cell Mix 1 v2 PN-2000258	Forward Primer: ▬ PCR Primer 5’-GATCTACACTCTTTCCCTACACGACGC-3’	Reverse Outer Primers: 5’-TCAGCACGGGACAAACTCTTCT-3’ 5’-GCAGGAGACAGACTCTTCTCCA-3’ 5’-AACTGGCTGCTCATGGTGT-3’ 5’-TGGTGCAAGTGTGGTTGAGGT-3’ 5’-TGGTCACTTGGCTGGTGGTG-3’ 5’-CACTTGGCAGGTGAACTGTTTTCT-3’ 5’-AACCTTCAAGGATGCTCTTGGGA-3’ 5’-GGACAGGGATCCAGAGTTCCA-3’ 5’-AGGTGACGGTCTGACTTGGC-3’ 5’-GCTGGACAGGGCTCCATAGTT-3’ 5’-GGCACCTTGTCCAATCATGTTCC-3’ 5’-ATGTCGTTCATACTCGTCCTTGGT-3’	▬ Outer Primer

Automated Protocol Step – V(D)J Amplification 2

Human T Cell Mix 2 v2 PN-2000246	Forward Primer: ▬ PCR Primer 5’-GATCTACACTCTTTCCCTACACGACGC-3’	Reverse Inner Primers: 5’-AGTCTCTCAGCTGGTACACG-3’ 5’-TCTGATGGCTCAAACACAGC-3’	▬ Inner Primer
Human B Cell Mix 2 v2 PN-2000255	Forward Primer: ▬ PCR Primer 5’-GATCTACACTCTTTCCCTACACGACGC-3’	Reverse Inner Primers: 5’-GGGAAGTTTCTGGCGGTCA-3’ 5’-GGTGGTACCCAGTTATCAAGCAT-3’ 5’-GTGTCCCAGGTCACCATCAC-3’ 5’-TCCTGAGGACTGTAGGACAGC-3’ 5’-CACGCTGCTCGTATCCGA-3’ 5’-TAGCTGCTGGCCGC-3’ 5’-GCGTTATCCACCTTCCACTGT-3’	▬ Inner Primer
Mouse T Cell Mix 2 v2 PN-2000257	Forward Primer: ▬ PCR Primer 5’-GATCTACACTCTTTCCCTACACGACGC-3’	Reverse Inner Primers: 5’-AGTCAAAGTCGGTGAACAGGCA-3’ 5’-GGCCAAGCACACGAGGGTA-3’	▬ Inner Primer
Mouse B Cell Mix 2 v2 PN-2000259	Forward Primer: ▬ PCR Primer 5’-GATCTACACTCTTTCCCTACACGACGC-3’	Reverse Inner Primers: 5’-TACACACCAGTGTGGCCTT-3’ 5’-CAGGCCACTGTCACACCACT-3’ 5’-CAGGTCACATTCATCGTGCCG-3’ 5’-GAGGCCAGCACAGTGACCT-3’ 5’-GCAGGGAAGTTCACAGTGCT-3’ 5’-CTGTTTGAGATCAGTTTGCCATCCT-3’ 5’-TGCGAGGTGGCTAGGTACTTG-3’ 5’-CCCTTGACCAGGCATCC-3’ 5’-AGGTCACGGAGGAACCAGTTG-3’ 5’-GGCATCCCAGTGTCACCGA-3’ 5’-AGAAGATCCACTTCACCTTGAAC-3’ 5’-GAAGCACACGACTGAGGCAC-3’	▬ Inner Primer

V(D)J Amplified Product



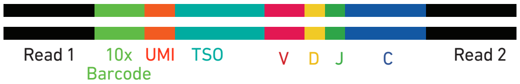
5'–GATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-N16-N10-TTTCTTATATGGG-cDNA_Insert-Inner_Primer–3'
3'–CTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-N16-N10-AAAGAATATACCC-cDNA_Insert-Inner_Primer–5'

Automated Protocol Step – Adaptor Ligation (for V(D)J Library Construction)

Adaptor (Read 2)

5'–GATCGGAAGAGCACACGTCTGAACTCCAGTCAC–3'
3'–TCTAGCCTTCTCG–5'

Ligation Product



5'–GATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-N16-N10-TTTCTTATATGGG-cDNA_Insert-AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC–3'
3'–CTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-N16-N10-AAAGAATATACCC-cDNA_Insert-TCTAGCCTTCTCG–5'

Automated Protocol Step – Sample Index PCR (for V(D)J Library Construction)

Dual Indexing

Dual Index Kit
TT Set A
PN-1000215

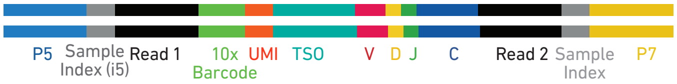
Forward Primer:

5'-AATGATACGGCGACCACCGAGATCTACAC-N10-ACACTCTTTCCCTACACGACGCTC-3'

Reverse Primer:

5'-CAAGCAGAAGACGGCATACGAGAT-N10-GTGACTGGAGTTCAGACGTGT-3'

Sample Index PCR Product



5'-AATGATACGGCGACCACCGAGATCTACAC-N10 -ACACTCTTTCCCTACACGACGCTCTTCCGATCT-N16-N10-TTTCTTATATGGG-Insert-AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC-N10-ATCTCGTATGCCGTCTTCTGCTTG-3'

3'-TTACTATGCCGCTGGTGGCTCTAGATGTG-N10-TGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-N16-N10-AAAGAATATACCC-Insert-TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG-N10-TAGAGCATACGGCAGAAGACGAAC-5'