CG000207 Rev G

USER GUIDE

Chromium Next GEM Single Cell V(D)J Reagent Kits v1.1

FOR USE WITH

Chromium Next GEM Single Cell 5' Library & Gel Bead Kit v1.1, 16 rxns PN-1000165

Chromium Next GEM Single Cell 5' Library & Gel Bead Kit v1.1, 4 rxns PN-1000167

Chromium Single Cell 5' Library Construction Kit, 16 rxns PN-1000020

Chromium Single Cell V(D)J Enrichment Kit, Human T Cell, 96 rxns PN-1000005

Chromium Single Cell V(D)J Enrichment Kit, Human B Cell, 96 rxns PN-1000016

Chromium Single Cell V(D)J Enrichment Kit, Mouse T Cell, 96 rxns PN-1000071

Chromium Single Cell V(D)J Enrichment Kit, Mouse B Cell, 96 rxns PN-1000072

Chromium Next GEM Chip G Single Cell Kit, 48 rxns PN-1000120

Chromium Next GEM Chip G Single Cell Kit, 16 rxns PN-1000127

Single Index Kit T Set A, 96 rxns PN-1000213

Next GEM reagents are specific to Next GEM products and should not be used interchangeably with non-Next GEM reagents.

Notices

Document Number

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Legal Notices

Instrument & Licensed Software Updates Warranties

Updates to existing Instruments and Licensed Software may be required to enable customers to use new or existing products. In the event of an Instrument failure resulting from an update, such failed Instrument will be replaced or repaired in accordance with the 10x Limited Warranty, Assurance Plan or service agreement, only if such Instrument is covered by any of the foregoing at the time of such failure. Instruments not covered under a current 10x Limited Warranty, Assurance Plan or service agreement will not be replaced or repaired.

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Document Revision Summary

Document Number	CG000207
Title	Chromium Next GEM Single Cell V(D)J Reagent Kits v1.1 User Guide
Revision	Rev F to Rev G
Revision Date	October 2021

Specific Changes:

- Updated Step 1.3 — Run the Chromium Controller or X/iX.

General Changes:

- Updates for general minor consistency of language and terms throughout.

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Chromium Next GEM Single Cell V(D)J Reagent Kits v1.1

Chromium Next GEM Single Cell 5' Library and Gel Bead Kit v1.1, 16 rxns PN-1000165

Chromium Next GEM Single Cell 5' Library Kit v1.1, 16 rxns PN-1000166 (store at –20°C)

Chromium Next GEM Single Cell 5' Reagents Module 1 v1.1	#	PN
RT Reagent B	1	2000165
RT Enzyme Mix B	1	2000010
Additive A	1	220074
Poly-dT RT Primer	1	2000007
Buffer Sample Clean Up 1	2	220020
Amplification Master Mix	2	220125
cDNA Primer Mix	1	220106
cDNA Additive	1	220067

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Chromium Next GEM Single Cell 5' Reagents Module 2 v1.1	#	PN
Fragmentation Enzyme Blend	1	220107
Fragmentation Buffer	1	220108
Ligation Buffer	1	220109
DNA Ligase	1	220110
Adaptor Mix	1	220026
SI-PCR Primer	1	220111
Amplification Master Mix	1	220125

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Chromium Next GEM Single Cell 5' Gel Bead Kit v1.1, 16 rxns PN-1000169 (store at –80°C)

Chromium Single Cell 5' Gel Beads	#	PN
Single Cell 5' Gel Beads	2	2000209

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Dynabeads™ MyOne™ SILANE PN-2000048 (store at 4°C)

	#	PN
Dynabeads MyOne SILANE	1	2000048

Chromium Next GEM Single Cell 5' Library and Gel Bead Kit v1.1, 4 rxns PN-1000167

Chromium Next GEM Single Cell 5' Library Kit v1.1, 4 rxns PN-1000168 (store at –20°C)

Chromium Next GEM Single Cell 5' Reagents Module 1 v1.1

	#	PN
RT Reagent B	1	2000165
RT Enzyme Mix B	1	2000021
Additive A	1	220074
Poly-dT RT Primer	1	2000007
Buffer Sample Clean Up 1	1	220020
Amplification Master Mix	1	220125
cDNA Primer Mix	1	220106
cDNA Additive	1	220067

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Chromium Next GEM Single Cell 5' Reagents Module 2 v1.1

	#	PN
Fragmentation Enzyme Blend	1	220130
Fragmentation Buffer	1	220108
Ligation Buffer	1	220109
DNA Ligase	1	220131
Adaptor Mix	1	220026
SI-PCR Primer	1	220111

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Chromium Next GEM Single Cell 5' Gel Bead Kit v1.1, 4 rxns PN-1000170 (store at –80°C)

Chromium Single Cell 5' Gel Beads

	#	PN
Single Cell 5' Gel Beads	1	2000209

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Dynabeads™ MyOne™ SILANE PN-2000048 (store at 4°C)

	#	PN
Dynabeads MyOne SILANE	1	2000048

Chromium Single Cell 5' Library Construction Kit, 16 rxns PN-1000020 (store at -20°C)

Chromium
Single Cell 5'
Library Construction Kit

	#	PN
cDNA Additive	2	220067
Fragmentation Enzyme Blend	1	220107
Fragmentation Buffer	1	220108
Ligation Buffer	1	220109
DNA Ligase	1	220110
Amplification Master Mix	3	220125
Adapter Mix	1	220026
SI-PCR Primer	1	220111

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Chromium Single Cell V(D)J Enrichment Kits, Human (store at –20°C)

Human T Cell, 96 rxns
PN-1000005

Chromium
Single Cell V(D)J Enrichment Kit
Human T Cell

	#	PN
Human T Cell Mix 1	6	2000008
Human T Cell Mix 2	6	2000009

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Human B Cell, 96 rxns
PN-1000016

Chromium
Single Cell V(D)J Enrichment Kit
Human B Cell

	#	PN
Human B Cell Mix 1	6	2000035
Human B Cell Mix 2	6	2000036

10xGenomics.com

Chromium Single Cell V(D)J Enrichment Kits, Mouse (store at –20°C)

Mouse T Cell, 96 rxns
PN-1000071

Chromium
Single Cell V(D)J Enrichment Kit
Mouse T Cell

	#	PN
Mouse T Cell Mix 1	6	2000075
Mouse T Cell Mix 2	6	2000079

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Mouse B Cell, 96 rxns
PN-1000072

Chromium
Single Cell V(D)J Enrichment Kit
Mouse B Cell

	#	PN
Mouse B Cell Mix 1	6	2000080
Mouse B Cell Mix 2	6	2000081

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Chromium Next GEM Chip G Single Cell Kit, 48 rxns PN-1000120 (store at ambient temperature)

Chromium
Partitioning Oil

	#	PN
Partitioning Oil	6	2000190

Chromium
Recovery Agent

	#	PN
Recovery Agent	6	220016

Chromium
Next GEM
Chip G & Gaskets

	#	PN
Chromium Next GEM Chip G	6	2000177
Gasket, 6-pack	1	370017

10xGenomics.com

Chromium Next GEM Chip G Single Cell Kit, 16 rxns PN-1000127 (store at ambient temperature)

Chromium
Partitioning Oil

	#	PN
Partitioning Oil	2	2000190

Chromium
Recovery Agent

	#	PN
Recovery Agent	2	220016

Chromium
Next GEM
Chip G & Gaskets

	#	PN
Chromium Next GEM Chip G	2	2000177
Gasket, 2-pack	1	3000072

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

Single Index Kit T set A

	#	PN
Single Index Plate T Set A	1	2000240

Chromium Accessories

Product	PN (Kit)	PN (Item)
10x Vortex Adapter	120251	330002
Chromium Next GEM Secondary Holder	1000195	3000332
10x Magnetic Separator	120250	230003

Recommended Thermal Cyclers

Thermal cyclers used must support uniform heating of 100 µl emulsion volumes.

Supplier	Description	Part Number
BioRad	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

Additional Kits, Reagents & Equipment

The items in the table below have been validated by 10x Genomics and are highly recommended for the Single Cell V(D)J protocol. Substituting materials may adversely affect system performance. The list does not include standard laboratory equipment, such as water baths, centrifuges, vortex mixers, pH meters, freezers, etc.

Supplier	Description		Part Number (US)
Plastics			
Eppendorf	PCR Tubes 0.2 ml 8-tube strips DNA LoBind Tubes, 1.5 ml DNA LoBind Tubes, 2.0 ml	Choose either Eppendorf, USA Scientific or Thermo Fisher Scientific PCR 8-tube strips.	951010022 022431021 022431048
USA Scientific	TempAssure PCR 8-tube strip		1402-4700
Thermo Fisher Scientific	MicroAmp 8-Tube Strip, 0.2 ml MicroAmp 8-Cap Strip, clear		N8010580 N8010535
Rainin	Tips LTS 200UL Filter RT-L200FLR Tips LTS 1ML Filter RT-L1000FLR Tips LTS 20UL Filter RT-L10FLR		30389240 30389213 30389226
Kits & Reagents			
Thermo Fisher Scientific	Nuclease-free Water		AM9937
Millipore Sigma	Ethanol, Pure (200 Proof, anhydrous)		E7023-500ML
Beckman Coulter	SPRIselect Reagent Kit		B23318
Bio-Rad	10% Tween 20		1662404
Ricca Chemical Company	Glycerin (glycerol), 50% (v/v) Aqueous Solution		3290-32
Qiagen	Qiagen Buffer EB		19086
Equipment			
VWR	Vortex Mixer Divided Polystyrene Reservoirs		10153-838 41428-958
Thermo Fisher Scientific	MYFUGE 12 Mini Centrifuge (alternatively, use any equivalent mini centrifuge)		C1012
Eppendorf	Eppendorf ThermoMixer C Eppendorf SmartBlock 1.5 ml, Thermoblock for 24 reaction vessel (alternatively, use a temperature-controlled Heat Block)		5382000023 5360000038
Rainin	Pipet-Lite Multi Pipette L8-50XLS+ Pipet-Lite Multi Pipette L8-200XLS+ Pipet-Lite Multi Pipette L8-10XLS+ Pipet-Lite Multi Pipette L8-20XLS+ Pipet-Lite LTS Pipette L-2XLS+ Pipet-Lite LTS Pipette L-10XLS+ Pipet-Lite LTS Pipette L-20XLS+ Pipet-Lite LTS Pipette L-100XLS+ Pipet-Lite LTS Pipette L-200XLS+ Pipet-Lite LTS Pipette L-1000XLS+		17013804 17013805 17013802 17013803 17014393 17014388 17014392 17014384 17014391 17014382

Additional Kits, Reagents & Equipment

The items in the table below have been validated by 10x Genomics and are highly recommended for the Single Cell V(D)J protocol. Substituting materials may adversely affect system performance. The list does not include standard laboratory equipment, such as water baths, centrifuges, vortex mixers, pH meters, freezers, etc.

Supplier	Description		Part Number (US)
Quantification & Quality Control			
Agilent	2100 Bioanalyzer Laptop Bundle High Sensitivity DNA Kit 4200 TapeStation High Sensitivity D5000 ScreenTape High Sensitivity D5000 Reagents	Choose Bioanalyzer, TapeStation or Qubit based on availability & preference.	G2943CA 5067-4626 G2991AA 5067-5592 5067-5593
Thermo Fisher Scientific	Qubit 4.0 Fluorometer Qubit dsDNA HS Assay Kit		Q33238 Q32854
KAPA Biosystems	KAPA Library Quantification Kit for Illumina Platforms		KK4824

Protocol Steps & Timing

Day	Steps		Timing	Stop & Store
3 h	**Cell Preparation**			
	Dependent on Cell Type		~1-1.5 h	
	Step 1 – GEM Generation & Barcoding			
	1.1	Prepare Reaction Mix	20 min	
	1.2	Load Chromium Next GEM Chip G	10 min	
	1.3	Run the Chromium Controller or X/iX	18 min	
	1.4	Transfer GEMs	3 min	
	1.5	GEM-RT Incubation	55 min	STOP 4°C ≤72 h or –20°C ≤1 week
6 h	**Step 2 – Post GEM RT Cleanup**			
	2.1	Post GEM-RT Cleanup – Dynabead	45 min	
	Step 3 – cDNA Amplification & QC*			
	3.1	cDNA Amplification	50 min	STOP 4°C ≤72 h
	3.2	cDNA Cleanup	15 min	STOP 4°C ≤72 h or –20°C ≤4 weeks
	3.3	cDNA Quantification & QC	50 min	
	*After cDNA Amplification & QC, for Target Enrichment & Enriched Library Construction proceed to steps 4-5. For 5' Gene Expression Library Construction proceed directly to step 6.			
8 h plus* (*Time dependent on Stop options used and protocol steps executed (steps 4-5 only/ steps 4,5 & 6))	**Step 4 – Target Enrichment from cDNA**			
	4.1	Target Enrichment 1	40 min	STOP 4°C ≤72 h
	4.2	Post Target Enrichment 1 Cleanup – SPRIselect	20 min	STOP 4°C ≤72 h or –20°C ≤1 week
	4.3	Target Enrichment 2	40 min	STOP 4°C ≤72 h
	4.4	Post Target Enrichment 2 Double Sided Size Selection – SPRIselect	30 min	STOP 4°C ≤72 h or –20°C ≤1 week
	4.5	Post Target Enrichment QC & Quantification	50 min	
	Step 5 – Enriched Library Construction			
	5.1	Fragmentation, End Repair & A – tailing	45 min	
	5.2	Adaptor Ligation	25 min	
	5.3	Post Ligation Cleanup – SPRIselect	20 min	
	5.4	Sample Index PCR	40 min	STOP 4°C ≤72 h
	5.5	Post Sample Index PCR Cleanup – SPRIselect	20 min	STOP 4°C ≤72 h or –20°C long-term
	5.6	Post Library Construction QC	50 min	
	Step 6 – 5' Gene Expression (GEX) Library Construction			
	6.1	GEX Fragmentation, End Repair & A-tailing	45 min	
	6.2	GEX Post Fragmentation, End Repair & A-tailing Double Sided Size Selection – SPRIselect	30 min	
	6.3	GEX Adaptor Ligation	25 min	
	6.4	GEX Post Ligation Cleanup – SPRIselect	20 min	
	6.5	GEX Sample Index PCR	40 min	STOP 4°C ≤72 h
	6.6	GEX Post Sample Index PCR Double Sided Cleanup – SPRIselect	30 min	STOP 4°C ≤72 h or –20°C long-term
	6.7	GEX Post Library Construction QC	50 min	

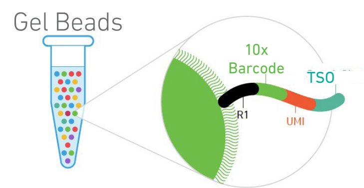
Stepwise Objectives

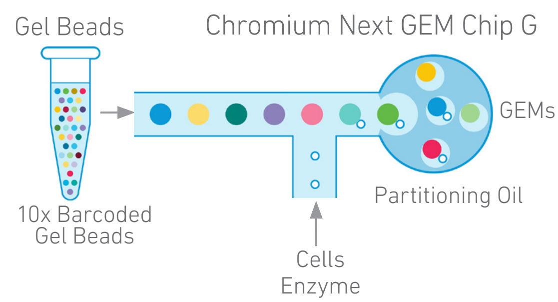
The Single Cell V(D)J protocols offer comprehensive, scalable solutions for measuring immune repertoire information and gene expression from the same cell. Profile full-length (5' UTR to constant region), paired T-cell receptor (TCR), or B-cell immunoglobulin (Ig) transcripts from 100-10,000 individual cells per sample. A pool of ~750,000 barcodes are sampled separately to index each cell's transcriptome. It is done by partitioning thousands of cells into nanoliter-scale Gel Beads-in-emulsion (GEMs), where all generated cDNA share a common 10x Barcode. Libraries are generated and sequenced and 10x Barcodes are used to associate individual reads back to the individual partitions. This document outlines the protocol to generate an enriched T-cell library and/or an enriched B-cell library, and/or a 5' Gene Expression library from amplified cDNA from the same cells.

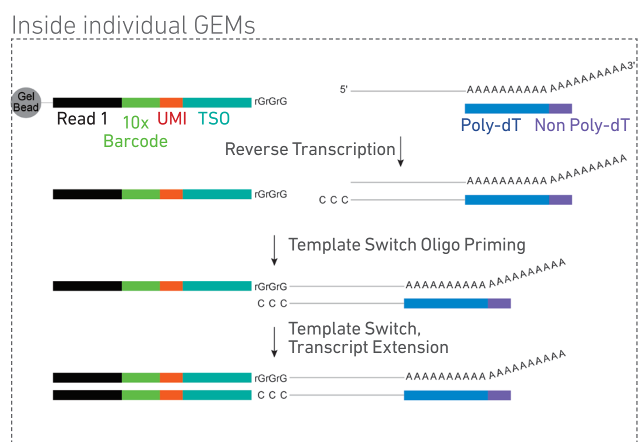
Step 1
GEM Generation & Barcoding

GEMs are generated by combining barcoded Single Cell 5' Gel Beads, a Master Mix with cells, and Partitioning Oil on Chromium Next GEM Chip G. To achieve single cell resolution, cells are delivered at a limiting dilution, such that the majority (~90 – 99%) of generated GEMs contains no cell, while the remainder largely contain a single cell.

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.







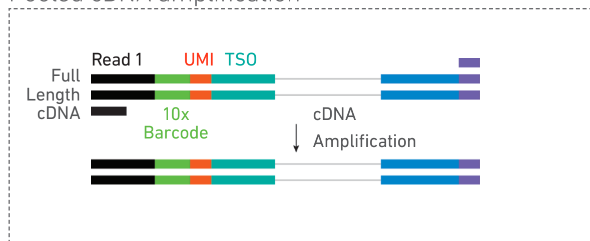
Step 2
Post GEM-RT Cleanup & QC

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. After cleanup a user may decide to pursue target enrichment directly from first-strand cDNA, in which case, consult Demonstrated Protocol - Chromium Single Cell V(D)J Reagent Kits-Direct Target Enrichment (Document CG000166). Otherwise, users should proceed to cDNA amplification in this protocol.

Step 3
cDNA Amplification & QC

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 enriched libraries (steps 4 and 5) and 5' Gene Expression libraries (step 6).

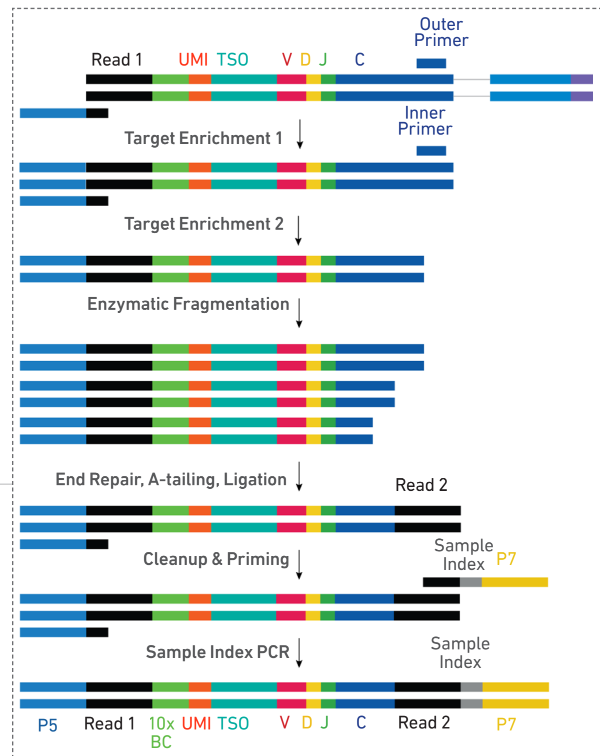
Pooled cDNA amplification



Step 4
Target Enrichment from cDNA

Full-length V(D)J segments (10x Barcoded) are enriched from amplified cDNA via PCR amplification with primers specific to either the TCR or Ig constant regions. If both T and B cells are expected to be present in the partitioned cell population, TCR and Ig transcripts can be enriched in separate reactions from the same amplified cDNA material. P5 is added during enrichment.

Pooled amplified cDNA processed in bulk



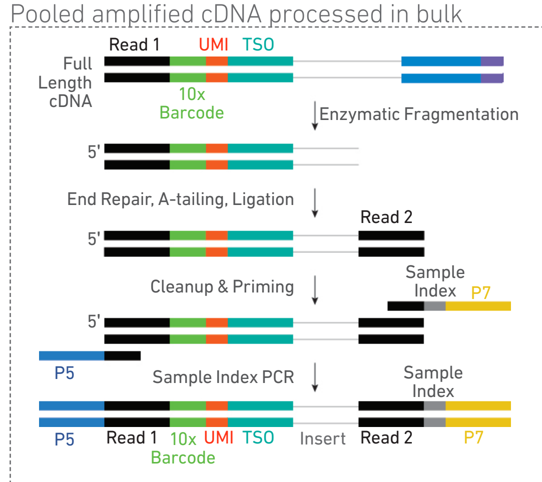
Step 5
Enriched Library Construction

Enzymatic fragmentation and size selection are used to generate variable length fragments that collectively span the V(D)J segments of the enriched TCR or Ig transcripts prior to library construction. P7, a sample index, 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.

Step 6
5' Gene Expression (GEX) Library Construction

Enzymatic fragmentation and size selection are used to optimize the cDNA amplicon size prior to 5' Gene Expression library construction. P5, P7, a sample index, 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.

Pooled amplified cDNA processed in bulk

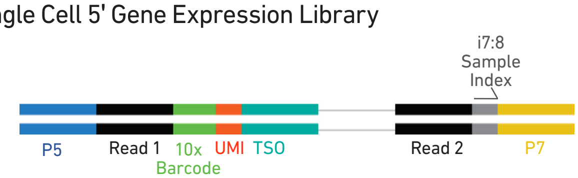


Step 7
Sequencing

Illumina-ready sequencing libraries can be sequenced at the recommended depth & run parameters. Illumina sequencer compatibility, sample indices, library loading and pooling for sequencing are summarized in step 7.

Chromium Single Cell V(D)J Enriched Library

Chromium Single Cell 5' Gene Expression Library



See Appendix for Oligonucleotide Sequences

Tips & Best Practices

TIPS

Icons

TIPS	!		Next GEM
Tips & Best Practices section includes additional guidance	Signifies critical step requiring accurate execution	Troubleshooting section includes additional guidance	Next GEM specific protocol step updates

Emulsion-safe Plastics

- Use validated emulsion-safe plastic consumables when handling GEMs as some plastics can destabilize GEMs.

Cell Concentration

- Recommended starting point is to load ~1700 cells per reaction, resulting in recovery of ~1000 cells, and a multiplet rate of ~0.8%. The optimal input cell concentration is 700-1,200 cells/µl.
- 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, for example, the Demonstrated Protocol for Enrichment of CD3+ T Cells from Dissociated Tissues for Single Cell RNA Sequencing and Immune Repertoire Profiling (Document CG000123).

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

General Reagent Handling

- Fully thaw and thoroughly mix reagents before use.
- Keep all enzymes and Master Mixes on ice during setup and use. Promptly move reagents back to the recommended storage after use.
- Calculate reagent volumes with 10% excess of 1 reaction values.
- Cover Partitioning Oil tubes and reservoirs to minimize evaporation.
- If using multiple chips, use separate reagent reservoirs for each chip during loading.
- Thoroughly mix samples with the beads during bead-based cleanup steps.

50% Glycerol Solution

- Purchase 50% glycerol solution from Ricca Chemical Company, Glycerin (glycerol), 50% (v/v) Aqueous Solution, PN-3290-32.
- Prepare 50% glycerol solution:

  i. Mix an equal volume of water and 99% Glycerol, Molecular Biology Grade.

  ii. Filter through a 0.2-µm filter.

  iii. Store at –20°C in 1-ml LoBind tubes. 50% glycerol solution should be equilibrated to room temperature before use.

Pipette Calibration

- Follow manufacturer's calibration and maintenance schedules.
- Pipette accuracy is particularly important when using SPRIselect reagents.

Chromium Next GEM Chip Handling

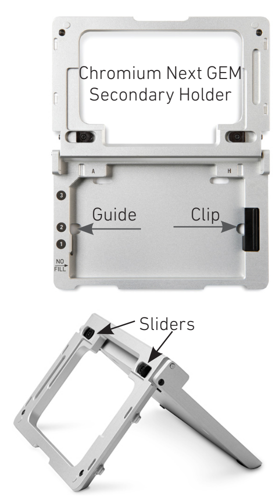
Next GEM

- Minimize exposure of reagents, chips, and gaskets to sources of particles and fibers, laboratory wipes, frequently opened flip-cap tubes, clothing that sheds fibers, and dusty surfaces.
- After removing the chip from the sealed bag, use in ≤ 24 h.
- Execute steps without pause or delay, unless indicated. When multiple chips are to be used, load, run, and collect the content from one chip before loading the next.
- Fill all unused input wells in rows labeled 1, 2, and 3 on a chip with an appropriate volume of 50% glycerol solution before loading the used wells. DO NOT add glycerol to the wells in the bottom NO FILL row.
- Avoid contacting the bottom surface of the chip with gloved hands and other surfaces. Frictional charging can lead to inadequate priming of the channels, potentially leading to either clogs or wetting failures.
- Minimize the distance that a loaded chip is moved to reach the Chromium Controller.
- Keep the chip horizontal to prevent wetting the gasket with oil, which depletes the input volume and may adversely affect the quality of the resulting emulsion.

Chromium Next GEM Secondary Holders

Next GEM

- Chromium Next GEM Secondary Holders encase Chromium Next GEM Chips.
- The holder lid flips over to become a stand, holding the chip at 45 degrees for optimal recovery well content removal.
- Squeeze the black sliders on the back side of the holder together to unlock the lid and return the holder to a flat position.



Chromium Next GEM Chip & Holder Assembly

Next GEM

- Align notch on the chip (upper left corner) and the holder.
- Insert the left-hand side of the chip under the guide. Depress the right-hand side of the chip until the spring-loaded clip engages.
- Close the lid before dispensing reagents into the wells.

Chromium Next GEM Chip

Chromium Next GEM Secondary Holder

Assembled Next GEM Chip

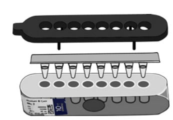
Chromium Next GEM Chip Loading

Next GEM

- Place the assembled chip and holder flat on the bench with the lid closed.
- Dispense at the bottom of the wells without introducing bubbles.
- When dispensing Gel Beads into the chip, wait for the remainder to drain into the bottom of the pipette tips and dispense again to ensure complete transfer.
- Refer to Load Chromium Next GEM Chip G for specific instructions.

Gel Bead Handling

Next GEM



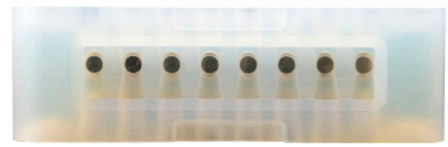
- Use one tube of Gel Beads per sample. DO NOT puncture the foil seals of tubes not used at the time.
- Equilibrate the Gel Beads strip to room temperature before use.
- Store unused Gel Beads at −80°C and avoid more than 10 freeze-thaw cycles. DO NOT store Gel Beads at −20°C.
- Snap the tube strip holder 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.
- If the required volume of beads cannot be recovered, place the pipette tips against the sidewalls and slowly dispense the Gel Beads back into the tubes. DO NOT introduce bubbles into the tubes and verify that the pipette tips contain no leftover Gel Beads. Withdraw the full volume of beads again by pipetting slowly.

10x Gasket Attachment

- After reagents are loaded, attach the gasket by holding the tongue (curved end, to the right) and hook it on the left-hand tabs of the holder. Gently pull the gasket toward the right and hook it on the two right-hand tabs.
- DO NOT touch the smooth side of the gasket. DO NOT press down on the top of the gasket after attachment.
- Keep the assembly horizontal to avoid wetting the gasket with Partitioning Oil.

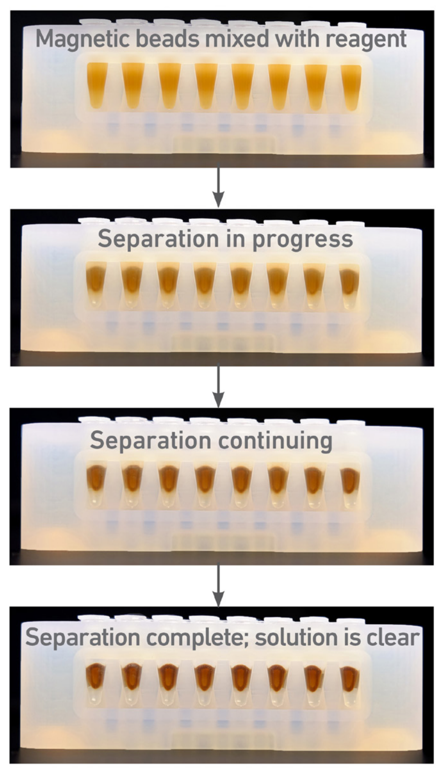
10x Magnetic Separator

- Offers two positions of the magnets (high and low) relative to a tube, depending on its orientation. Flip the magnetic separator over to switch between high (magnet•**High**) or low (magnet•**Low**) positions.
- If using MicroAmp 8-Tube Strips, use the high position (magnet•**High**) only throughout the protocol.



Magnetic Bead Cleanup Steps

- During magnetic bead based cleanup steps that specify waiting "until the solution clears", visually confirm clearing of solution before proceeding to the next step. See adjacent panel for an example.
- The time need for the solution to clear may vary based on specific step, reagents, volume of reagents used etc.



cDNA Amplification PCR Cycle Numbers

- Follow cycle number recommendations for high and low RNA content cells based on Targeted Cell Recovery and cell sample.
- Cycle numbers in the table below have been optimized assuming that the sample has >80% T and/or B cells. Samples with lower fraction of T and/or B cells may require additional cycle number optimization and/or may be enriched to increase the fraction of T or B cells. Refer to the Demonstrated Protocol for Enrichment of CD3+ T Cells from Dissociated Tissues for Single Cell RNA Sequencing and Immune Repertoire Profiling (Document CG000123).
- If the fraction of T and/or B cells in a cell sample is known, adjust PCR cycle number based on that fraction to ensure sufficient product generation.

Tutorial – Optimizing cDNA Amplification PCR Cycle Numbers

Examples

Sample A:
Primary cells with 15% T cell fraction.
Targeted Cell Recovery is 10,000 cells.
Only 1,500 (15%) cells are T cells.
Total PCR cycles – 16.

Sample B:
Cell line with high RNA content.
Targeted Cell Recovery is 10,000 cells.
Total PCR cycles – 11.

Sample C:
Cell mix with 90% low RNA content and 10% high RNA content B cells.
Targeted Cell Recovery is 10,000 cells.
90% B cells are low RNA content.
Total PCR cycles – 13.

Recommended starting point for cycle number optimization.

Targeted Cell Recovery	Low RNA Content Cells Total Cycles	High RNA Content Cells Total Cycles
100–500	18	16
501–2,000	16	14
2,001–6,000	14	12
6,001–10,000	13	11

Enzymatic Fragmentation

- Ensure enzymatic fragmentation reactions are prepared on ice and then loaded into a thermal cycler pre-cooled to 4°C prior to initiating the Fragmentation, End Repair, and A-tailing incubation steps.

SPRIselect Cleanup & Size Selection

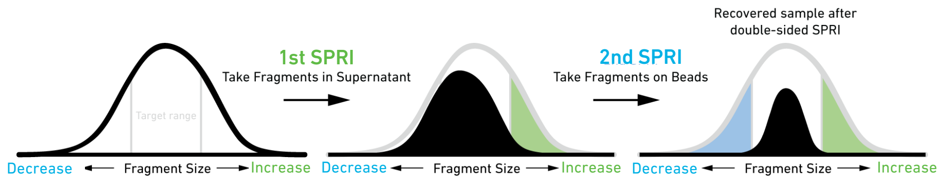
- After aspirating the desired volume of SPRIselect reagent, examine the pipette tips before dispensing to ensure the correct volume is transferred.
- Pipette mix thoroughly as insufficient mixing of sample and SPRIselect reagent will lead to inconsistent results.
- Use fresh preparations of 80% Ethanol.

Tutorial — SPRIselect Reagent:DNA Sample Ratios

SPRI beads selectively bind DNA according to the ratio of SPRIselect reagent (beads).

Example: $\text{Ratio} = \dfrac{\text{Volume of SPRIselect reagent added to the sample}}{\text{Volume of DNA sample}} = \dfrac{50\ \mu l}{100\ \mu l} = \mathbf{0.5X}$

Schematic of Double Sided Size Selection



After the first SPRI, supernatant is transferred for a second SPRI while larger fragments are discarded (green). After the second SPRI, fragments on beads are eluted and kept while smaller fragments are discarded (blue). Final sample has a tight fragment size distribution with reduced overall amount (black).

Tutorial — Double Sided Size Selection

Step a – First SPRIselect: Add **50 µl** SPRIselect reagent to **100 µl** sample (**0.5X**).

$\text{Ratio} = \dfrac{\text{Volume of SPRIselect reagent added to the sample}}{\text{Volume of DNA sample}} = \dfrac{50\ \mu l}{100\ \mu l} = \mathbf{0.5X}$

Step b – Second SPRIselect: Add **30 µl** SPRIselect reagent to supernatant from step a (**0.8X**).

$\text{Ratio} = \dfrac{\text{Total Volume of SPRIselect reagent added to the sample (step a + b)}}{\text{Original Volume of DNA sample}} = \dfrac{50\ \mu l + 30\ \mu l}{100\ \mu l} = \mathbf{0.8X}$

Sample Indices in Sample Index PCR

- Choose the appropriate sample index sets to ensure that no sample indices overlap in a multiplexed sequencing run.
- Each well in the Single Index plate T, Set A contains a unique mix of 4 oligos.
- The sample indices can therefore be used in any combination.
- Each sample index set is base-balanced to avoid monochromatic signal issues when it is the sole sample loaded on an Illumina sequencer.

Step 1

GEM Generation & Barcoding

1.0
GEM Generation & Barcoding

Next GEM

GET STARTED!

Action		Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature		**Chromium Single Cell 5' Gel Beads**	2000209	Equilibrate to room temperature 30 min before loading the chip.	−80°C
	●	**RT Reagent B**	2000165	Vortex, verify no precipitate, centrifuge briefly.	−20°C
	●	**Poly-dT RT Primer**	2000007	Vortex, verify no precipitate, centrifuge briefly.	−20°C
	●	**Additive A**	220074	Vortex, verify no precipitate, centrifuge briefly.	−20°C
Place on Ice	○	**RT Enzyme Mix B**	2000010/ 2000021	Centrifuge briefly before adding to the mix.	−20°C
Obtain		**Partitioning Oil**	2000190	-	Ambient
		Chromium Next GEM Chip G	2000177	-	Ambient
		10x Gasket	370017/ 3000072	See Tips & Best Practices.	Ambient
		Chromium Next GEM Secondary Holder	3000332	See Tips & Best Practices.	Ambient
		10x Vortex Adapter	330002	See Tips & Best Practices.	Ambient
		50% glycerol solution If using <8 reactions	-	See Tips & Best Practices.	-

!

Firmware Version 4.0 or higher is required in the Chromium Controller or the Chromium Single Cell Controller used for the Single Cell V(D)J v1.1 protocols.

1.1 Prepare Reaction Mix

Next GEM

a. Prepare Master Mix on ice. Pipette mix 15x and centrifuge briefly.

Master Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
RT Reagent B	2000165	18.8	82.7	165.4
Poly-dT RT Primer	2000007	6.4	28.2	56.3
Additive A	220074	2.0	8.8	17.6
RT Enzyme Mix B	2000010/ 2000021	10.0	44.0	88.0
Total	-	**37.2**	**163.7**	**327.3**

b. Add **37.2 µl** Master Mix into each tube of a PCR 8-tube strip on ice.

Assemble Chromium Next GEM Chip G

After removing the chip from the sealed bag, use the chip in ≤ 24 h.

TIPS

See Tips & Best Practices for chip handling instructions.

- Align notch on the chip (upper left corner) and the holder.
- Insert the left-hand side of the chip under the guide. Depress the right-hand side of the chip until the spring-loaded clip engages.
- Close the lid before dispensing reagents into the wells.
- The assembled chip is ready for loading the indicated reagents. Refer to step 1.2 for reagent volumes and loading order.

For GEM generation, load the indicated reagents only in the specified rows, starting from row labeled 1, followed by rows labeled 2 and 3. DO NOT load reagents in the bottom row labeled NO FILL. See step 1.2 for details.

Next GEM

Cell Suspension Volume Calculator Table

(for step 1.2 of Chromium Next GEM Single Cell V(D)J v1.1 protocol)

Volume of Cell Suspension Stock per reaction (µl) | Volume of Nuclease-free Water per reaction (µl)

Cell Stock Concentration (Cells/µl)	Targeted Cell Recovery										
	500	1000	2000	3000	4000	5000	6000	7000	8000	9000	10000
100	8.3 29.5	16.5 21.3	33.0 4.8	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
200	4.1 33.7	8.3 29.5	16.5 21.3	24.8 13.0	33.0 4.8	n/a	n/a	n/a	n/a	n/a	n/a
300	2.8 35.0	5.5 32.3	11.0 26.8	16.5 21.3	22.0 15.8	27.5 10.3	33.0 4.8	n/a	n/a	n/a	n/a
400	2.1 35.7	4.1 33.7	8.3 29.5	12.4 25.4	16.5 21.3	20.6 17.2	24.8 13.0	28.9 8.9	33.0 4.8	n/a	n/a
500	1.7 36.1	3.3 34.5	6.6 31.2	9.9 27.9	13.2 24.6	16.5 21.3	19.8 18.0	23.1 14.7	26.4 11.4	29.7 8.1	33.0 4.8
600	1.5 36.3	2.8 35.0	5.5 32.3	8.3 29.5	11.0 26.8	13.8 24.0	16.5 21.3	19.3 18.6	22.0 15.8	24.8 13.0	27.5 10.3
700	1.2 36.6	2.4 35.4	4.7 33.1	7.1 30.7	9.4 28.4	11.8 26.0	14.1 23.7	16.5 21.3	18.9 18.9	21.2 16.6	23.6 14.2
800	1.0 36.8	2.1 35.7	4.1 33.7	6.2 31.6	8.3 29.5	10.3 27.5	12.4 25.4	14.4 23.4	16.5 21.3	18.6 19.2	20.6 17.2
900	0.9 36.9	1.8 36.0	3.7 34.1	5.5 32.3	7.3 30.5	9.2 28.6	11.0 26.8	12.8 25.0	14.7 23.1	16.5 21.3	18.3 19.5
1000	0.8 37.0	1.7 36.1	3.3 34.5	5.0 32.8	6.6 31.2	8.3 29.5	9.9 27.9	11.6 26.3	13.2 24.6	14.9 23.0	16.5 21.3
1100	0.8 37.0	1.5 36.3	3.0 34.8	4.5 33.3	6.0 31.8	7.5 30.3	9.0 28.8	10.5 27.3	12.0 25.8	13.5 24.3	15.0 22.8
1200	0.7 37.1	1.4 36.4	2.8 35.1	4.1 33.7	5.5 32.3	6.9 30.9	8.3 29.5	9.6 28.2	11.0 26.8	12.4 25.4	13.8 24.0
1300	0.6 37.2	1.3 36.5	2.5 35.3	3.8 34.0	5.1 32.7	6.3 31.5	7.6 30.2	8.9 28.9	10.2 27.6	11.4 26.4	12.7 25.1
1400	0.6 37.2	1.2 36.6	2.4 35.4	3.5 34.3	4.7 33.1	5.9 31.9	7.1 30.7	8.3 29.5	9.4 28.4	10.6 27.2	11.8 26.0
1500	0.6 37.3	1.1 36.7	2.2 35.6	3.3 34.5	4.4 33.4	5.5 32.3	6.6 31.2	7.7 30.1	8.8 29.0	9.9 27.9	11.0 26.8
1600	0.5 37.3	1.0 36.8	2.1 35.7	3.1 34.7	4.1 33.7	5.2 32.6	6.2 31.6	7.2 30.6	8.3 29.5	9.3 28.5	10.3 27.5
1700	0.5 37.3	1.0 36.8	1.9 35.9	2.9 34.9	3.9 33.9	4.9 32.9	5.8 32.0	6.8 31.0	7.8 30.0	8.7 29.1	9.7 28.1
1800	0.5 37.3	0.9 36.9	1.8 36.0	2.8 35.0	3.7 34.1	4.6 33.2	5.5 32.3	6.4 31.4	7.3 30.5	8.3 29.5	9.2 28.6
1900	0.4 37.4	0.9 36.9	1.7 36.1	2.6 35.2	3.5 34.3	4.3 33.5	5.2 32.6	6.1 31.7	6.9 30.9	7.8 30.0	8.7 29.1
2000	0.4 37.4	0.8 37.0	1.7 36.1	2.5 35.3	3.3 34.5	4.1 33.7	5.0 32.8	5.8 32.0	6.6 31.2	7.4 30.4	8.3 29.5

Grey boxes: Volumes that would exceed the allowable water 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

1.2 Load Chromium Next GEM Chip G

Next GEM

After removing the chip from the sealed bag, use in ≤ 24 h. For all **chip loading steps**, raising and depressing the pipette plunger should each take **~5 sec**. When dispensing, raise the pipette tips at the same rate as the liquid is rising, keeping the tips slightly submerged.

a. Dispense 50% Glycerol into Unused Chip Wells (if < 8 samples per chip)

i. **70 µl** to unused wells in **row labeled 1**.
ii. **50 µl** to unused wells in **row labeled 2**.
iii. **45 µl** to unused wells in **row labeled 3**.

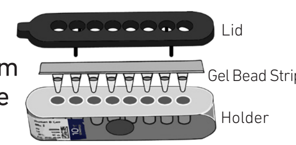
DO NOT add 50% glycerol solution to the bottom row of NO FILL wells. DO NOT use any substitute for 50% glycerol solution.

b. Prepare Master Mix + Cell Suspension
Refer to the Cell Suspension Volume Calculator Table. Add the appropriate volume of nuclease-free water first, followed by corresponding volume of single cell suspension to Master Mix for a total of **75 µl** in each tube. Gently pipette mix the cells suspension before adding to the Master Mix.

c. Load Row Labeled 1
Gently pipette mix the Master Mix + Cell Suspension and using the same pipette tip, dispense **70 µl** Master Mix + Cell Suspension into the bottom center of each well in **row labeled 1** without introducing bubbles.

The illustrated chip is being loaded for 8 samples.

d. Prepare Gel Beads
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.** Confirm there are no bubbles at the bottom of the tubes and the liquid levels are even. Place the Gel Bead strip back in the holder. Secure the holder lid.

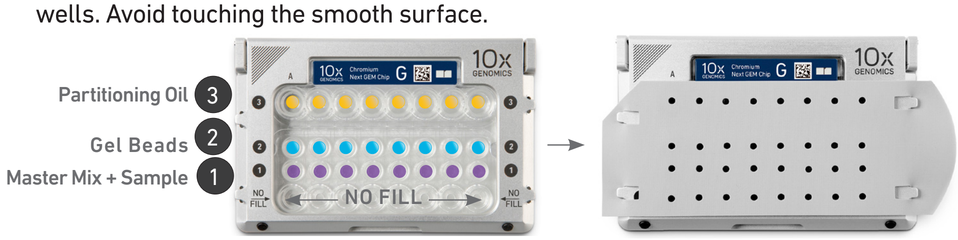


e. Load Row Labeled 2
Puncture the foil seal of the Gel Bead tubes. Slowly aspirate **50 µl** Gel Beads. Dispense into the wells in **row labeled 2** without introducing bubbles. Wait **30 sec**.

f. Load Row Labeled 3
Dispense **45 µl** Partitioning Oil into the wells in **row labeled 3** from a reagent reservoir. Failure to add Partitioning Oil to the top row labeled 3 will prevent GEM generation and can damage the Chromium Controller.

Attach the gasket and run the chip in the Chromium Controller **immediately** after loading the Partitioning Oil.

g. Attach 10x Gasket
Align the notch with the top left-hand corner. Ensure the gasket holes are aligned with the wells. Avoid touching the smooth surface.



Keep horizontal to avoid wetting the gasket. DO NOT press down on the gasket.

1.3 Run the Chromium Controller

If using Chromium Controller:

a. Press the eject button on the Controller to eject the tray.

b. Place the assembled chip with the gasket in the tray, ensuring that the chip stays horizontal. Press the button to retract the tray.

c. Confirm the Chromium Chip G program on screen. Press the play button.

d. At completion of the run **(~18 min),** the Controller will chime. **Immediately** proceed to the next step.

Firmware Version 4.0 or higher is required in the Chromium Controller or the Chromium Single Cell Controller used for the Single Cell V(D)J v1.1 protocol.

If using Chromium X/iX:

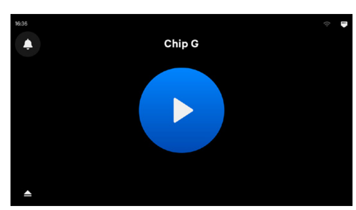
Consult the Chromium X Series (X/iX) User Guide (CG000396) for detailed instrument operation instructions and follow the instrument touchscreen prompts for execution.

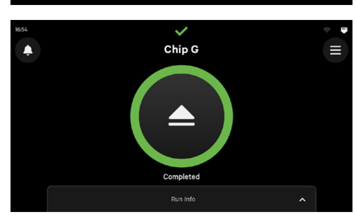
a. Press the eject button on Chromium X/iX to eject the tray.
If the eject button is not touched within 1 min, tray will close automatically. System requires a few seconds before the tray can be ejected again.

b. Place the assembled chip with the gasket in the tray, ensuring that the chip stays horizontal. Press the button to retract the tray.

c. Press the play button.

d. At completion of the run (~18 min), Chromium X/iX will chime. **Immediately** proceed to the next step.





1.4 Transfer GEMs

Next GEM

a. Place a tube strip on ice.

b. Press the eject button of the Controller and remove the chip.

c. Discard the gasket. Open the chip holder. Fold the lid back until it clicks to expose the wells at 45 degrees.

d. Check the volume in rows labeled 1-2. Abnormally high volume in any well indicates a clog.

e. Slowly aspirate **100 µl** GEMs from the lowest points of the recovery wells in the top row labeled 3 without creating a seal between the pipette tips and the bottom of the wells.

f. Withdraw pipette tips from the wells. GEMs should appear opaque and uniform across all channels. Excess Partitioning Oil (clear) in the pipette tips indicates a potential clog.

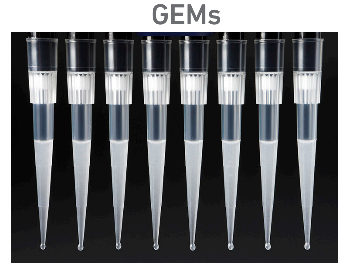
g. Over the course of **~20 sec**, dispense GEMs into the tube strip on ice with the pipette tips against the sidewalls of the tubes.

h. If multiple chips are run back-to-back, cap/cover the GEM-containing tube strip and place on ice for no more than **1 h**.

Expose Wells at 45 Degrees

Transfer GEMs

GEMs



1.5 GEM-RT Incubation

Use a thermal cycler that can accommodate at least 100 µl volume. A volume of 125 µl is the preferred setting on Bio-Rad C1000 Touch. In alternate thermal cyclers, use highest reaction volume setting.

a. Incubate in a thermal cycler with the following protocol.

Lid Temperature	Reaction Volume	Run Time
53°C	125 µl	~55 min
Step	**Temperature**	**Time**
1	53°C	00:45:00
2	85°C	00:05:00
3	4°C	Hold

STOP

b. Store at **4°C** for up to **72 h** or at **–20°C** for up to a **week**, or proceed to the next step.

Step 2

Post GEM-RT Cleanup

2

2.0
Post GEM-RT Cleanup

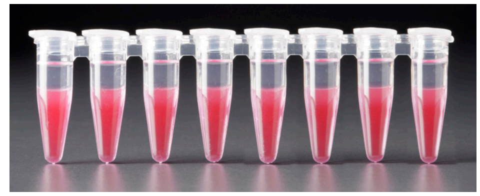
GET STARTED!

Action	Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature	**Additive A**	220074	Thaw, vortex, verify no precipitate, centrifuge briefly.	–20°C
	Dynabeads MyOne SILANE	2000048	Vortex thoroughly (≥30 sec) **immediately** before adding to the mix. If still clumpy, pipette mix to resuspend completely. DO NOT centrifuge before use.	4°C
Thaw at 65°C	**Buffer Sample Clean Up 1**	220020	Thaw for 10 min at 65°C at max speed on a thermomixer. Verify there are no visible crystals. Cool to room temperature.	–20°C
Obtain	**Recovery Agent**	220016	-	Ambient
	Qiagen Buffer EB	-	Manufacturer's recommendations.	-
	Bio-Rad 10% Tween 20	-	Manufacturer's recommendations.	-
	10x Magnetic Separator	230003	-	Ambient
	Prepare 80% Ethanol Prepare 15 ml for 8 reactions.	-	Prepare fresh.	-

2.1 Post GEM-RT Cleanup – Dynabeads

a. Add **125 µl** Recovery Agent to each sample (post GEM-RT incubation) at room temperature. DO NOT pipette mix or vortex the biphasic mixture. Wait **2 min**.

Biphasic Mixture

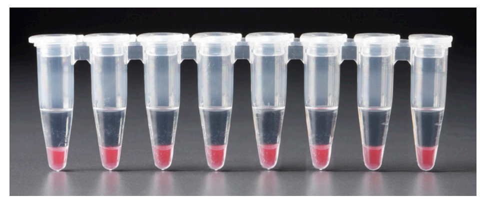


The resulting biphasic mixture contains Recovery Agent/Partitioning Oil (pink) and aqueous phase (clear), with no persisting emulsion (opaque).

If biphasic separation is incomplete:
Firmly secure the cap on the tube strip, ensuring that no liquid is trapped between the cap and the tube rim. Mix by inverting the capped tube strip 5x, centrifuge briefly, and proceed to step b. DO NOT invert without firmly securing the caps.

A smaller aqueous phase volume indicates a clog during GEM generation.

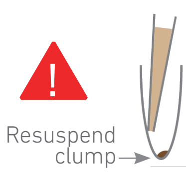
Remove Recovery Agent



b. Slowly remove and discard **125 µl** Recovery Agent/Partitioning Oil (pink) from the bottom of the tube. DO NOT aspirate any aqueous sample.

c. Prepare Dynabeads Cleanup Mix.

Dynabeads Cleanup Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Nuclease-free Water		5	22	44
Buffer Sample Clean Up 1	220020	182	801	1602
Dynabeads MyOne SILANE Vortex thoroughly (≥30 sec) **immediately** before adding to the mix. Aspirate the full liquid volume with a pipette tip to verify that the beads have not settled in the bottom of the tube. If clumps are present, pipette mix to resuspend completely. DO NOT centrifuge before use.	2000048	8	35	70
Additive A	220074	5	22	44
Total	-	**200**	**880**	**1760**



Add Dynabeads Cleanup Mix

d. Vortex and add **200 µl** to each sample. Pipette mix 5x (pipette set to 200 µl).

e. Incubate **10 min** at **room temperature**.

f. Prepare Elution Solution I. Vortex and centrifuge briefly.

Elution Solution I *Add reagents in the order listed*	PN	1X (µl)	10X (µl)
Buffer EB	-	98	980
10% Tween 20	-	1	10
Additive A	220074	1	10
Total	-	**100**	**1000**

g. At the end of **10 min** incubation, place on a 10x Magnetic Separator•**High position** (magnet•**High)** until the solution clears.

A white interface between the aqueous phase and Recovery Agent is normal.

h. Remove the supernatant.

i. Add **300 µl** 80% ethanol to the pellet while on the magnet. Wait **30 sec**.

j. Remove the ethanol.

k. Add **200 µl** 80% ethanol to pellet. Wait **30 sec**.

l. Remove the ethanol.

m. Centrifuge briefly. Place on the magnet•**Low**.

n. Remove remaining ethanol. Air dry for **2 min**.

o. Remove from the magnet. Immediately add **35.5 µl** Elution Solution I.

p. Pipette mix (pipette set to 30 µl) without introducing bubbles. Pipette mix 15x. If beads still appear clumpy, continue pipette mixing until fully resuspended.

q. Incubate **1 min** at **room temperature**.

r. Place on the magnet•**Low** until the solution clears.

s. Transfer **35 µl** sample to a new tube strip.

Step 3

cDNA Amplification & QC

3

3.0
cDNA Amplification & QC

GET STARTED!

Action	Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature	**cDNA Additive**	220067	Vortex, centrifuge briefly.	–20°C
	cDNA Primer Mix	220106	Vortex, centrifuge briefly.	–20°C
	Beckman Coulter SPRIselect Reagent	-	Manufacturer's recommendations.	-
	Agilent Bioanalyzer High Sensitivity Kit If used for QC and quantification	-	Manufacturer's recommendations.	-
	Agilent TapeStation ScreenTape and Reagents If used for QC and quantification	-	Manufacturer's recommendations.	-
	Qubit dsDNA HS Assay Kit If used for quantification	-	Manufacturer's recommendations.	-
Place on ice	**Amplification Master Mix**	220125	Vortex, centrifuge briefly.	–20°C
Obtain	**Qiagen Buffer EB**	-	Manufacturer's recommendations.	-
	10x Magnetic Separator	230003	-	Ambient
	Prepare 80% Ethanol Prepare 15 ml for 8 samples	-	Prepare fresh.	-

3.1 cDNA Amplification

a. Prepare cDNA Amplification Mix on ice. Vortex and centrifuge briefly.

cDNA Amplification Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Nuclease-free Water	-	8	35	70
Amplification Master Mix	220125	50	220	440
cDNA Additive	220067	5	22	44
cDNA Primer Mix	220106	2	9	18
Total	-	**65**	**286**	**572**

b. Add **65 µl** cDNA Amplification Mix to **35 µl** sample (Post GEM-RT Cleanup).

c. Pipette mix 5x (pipette set to 90 µl). Centrifuge briefly.

d. Incubate in a thermal cycler with the following protocol.

Lid Temperature	Reaction Volume	Run Time
105°C	100 µl	~25-50 min
Step	**Temperature**	**Time**
1	98°C	00:00:45
2	98°C	00:00:20
3	67°C	00:00:30
4	72°C	00:01:00
5	Go to Step 2, see table below for total # of cycles	
6	72°C	00:01:00
7	4°C	Hold

TIPS

The optimal number of cycles is a trade-off between generating sufficient final mass for library construction and minimizing PCR amplification artifacts. Cycle numbers were optimized assuming that sample includes >80% T or B cells. If testing cells types with a known fraction of T and/or B cells, adjust cycle number based on that fraction to generate sufficient product. See Tips and Best Practices for examples.

Recommended starting point for cycle number optimization.

Targeted Cell Recovery	Primary Cells Total Cycles	Cell Lines Total Cycles
100 – 500	18	16
501 – 2,000	16	14
2,001 – 6,000	14	12
6,001 – 10,000	13	11

STOP

e. Store at **4°C** for up to **72 h** or proceed to the next step.

3.2 cDNA Cleanup – SPRIselect

a. Vortex to resuspend the SPRIselect reagent. Add **60 µl** SPRIselect reagent **(0.6X)** to each sample and pipette mix 15x (pipette set to 150 µl).

b. Incubate **5 min** at **room temperature**.

c. Place on the magnet•**High** until the solution clears.

d. Remove the supernatant.

e. Add **200 µl** 80% ethanol to the pellet. Wait **30 sec**.

f. Remove the ethanol.

g. **Repeat** steps e and f for a total of 2 washes.

h. Centrifuge briefly and place on the magnet•**Low**.

i. Remove any remaining ethanol. Air dry for **2 min**.

j. Remove from the magnet. Add **45.5 µl** Buffer EB. Pipette mix 15x.

k. Incubate **2 min** at **room temperature**.

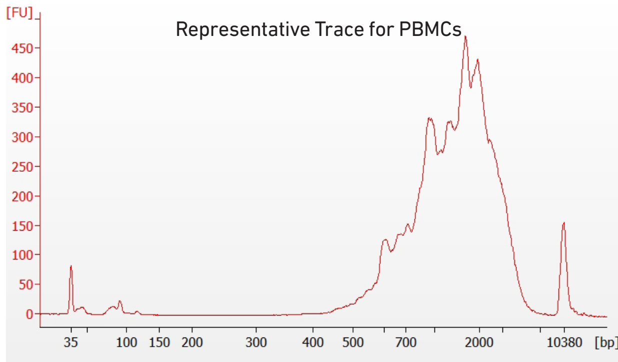
l. Place the tube strip on the magnet•**High** until the solution clears.

m. Transfer **45 µl** sample to a new tube strip.

STOP **n.** Store at **4°C** for up to **72 h** or at **–20°C** for up to **4 weeks,** or proceed to the next step.

3.3 cDNA QC & Quantification

a. Run **1 µl** undiluted sample (Dilution Factor 1) on an Agilent Bioanalyzer High Sensitivity chip.



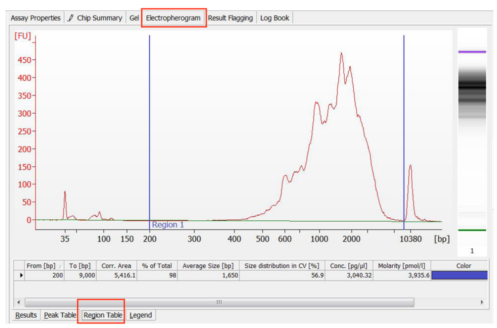
For 5' Gene Expression Library Construction proceed directly to step 6 after step 3.3.

b. If proceeding to 5' GEX Library Construction (step 6), determine cDNA yield for each sample. Example calculation below.

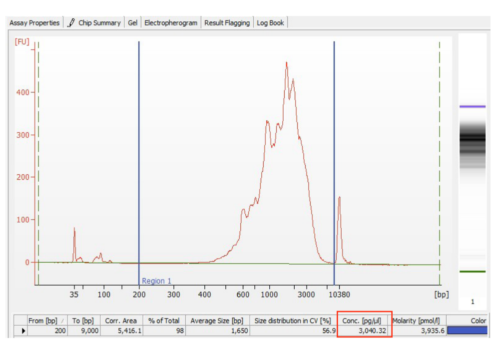
EXAMPLE CALCULATION

i. Select Region

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



ii. Note Concentration [pg/µl]



iii. Calculate

Concentration: 3040.32 pg/µl
Dilution Factor: 1

cDNA Conc. =

$$\frac{\text{Conc. (pg/µl) x Dilution Factor}}{\text{1000 (pg/ng)}} = \frac{3040.32 \times 1}{1000} = \mathbf{3\ ng/µl}$$

Example Calculation for Carrying Forward 50 ng Sample for 5' GEX Library Construction

$$\text{Volume for 50 ng} = \frac{50\ \text{ng}}{3\ \text{(ng/µl)}} = 16.7\ \text{µl}$$

5' GEX Library Construction Sample
=16.7 µl + 3.3 µl nuclease-free water
= 20 µl total

If <50 ng available, carry forward 20 µl sample (2-50 ng) into 5' GEX Library Construction.

DO NOT exceed a mass of 50 ng in the 20 µl carry forward volume.

Alternate Quantification Methods:

- Agilent TapeStation. See Appendix for representative traces
- Qubit Fluorometer and Qubit dsDNA HS Assay Kit.

Step 4

Target Enrichment from cDNA

4.1 Target Enrichment 1

4.2 Post Target Enrichment 1 Cleanup – SPRIselect

4.3 Target Enrichment 2

4.4 Post Target Enrichment 2 Double Sided Size Selection – SPRIselect

4.5 Post Target Enrichment QC & Quantification

4.0 Target Enrichment from cDNA

GET STARTED!

Action		Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature		**For Human Samples** (Choose B or T-cell primers based on desired enrichment products)			
	● (red)	**Human T Cell Mix 1**	2000008	Vortex, centrifuge briefly.	−20°C
	● (green)	**Human T Cell Mix 2**	2000009	Vortex, centrifuge briefly.	−20°C
	● (dark red)	**Human B Cell Mix 1**	2000035	Vortex, centrifuge briefly.	−20°C
	● (blue)	**Human B Cell Mix 2**	2000036	Vortex, centrifuge briefly.	−20°C
		For Mouse Samples (Choose B or T-cell primers based on desired enrichment products)			
	● (red)	**Mouse T Cell Mix 1**	2000075	Vortex, centrifuge briefly.	−20°C
	● (green)	**Mouse T Cell Mix 2**	2000079	Vortex, centrifuge briefly.	−20°C
	● (dark red)	**Mouse B Cell Mix 1**	2000080	Vortex, centrifuge briefly.	−20°C
	● (blue)	**Mouse B Cell Mix 2**	2000081	Vortex, centrifuge briefly.	−20°C
		For all Samples			
	● (lavender)	**cDNA Additive**	220067	Vortex, centrifuge briefly.	−20°C
		Beckman Coulter SPRIselect Reagent	-	Manufacturer's recommendations.	-
		Agilent Bioanalyzer High Sensitivity Kit If used for QC and quantification	-	Manufacturer's recommendations.	-
		Agilent TapeStation ScreenTape and Reagents If used for QC and quantification	-	Manufacturer's recommendations.	-
		Qubit dsDNA HS Assay Kit If used for quantification	-	Manufacturer's recommendations.	-
Place on Ice	● (light blue)	**Amplification Master Mix**	220125	Vortex, centrifuge briefly.	−20°C
Obtain		**Qiagen Buffer EB**	-	-	Ambient
		10x Magnetic Separator	230003	See Tips & Best Practices.	Ambient
		Prepare 80% Ethanol Prepare 15 ml for 8 reactions	-	See Tips & Best Practices.	Ambient

4.1 Target Enrichment 1

a. Add **33 µl** nuclease-free water into a tube strip on ice and then transfer **2 µl** sample (post cDNA Amplification & QC, step 3.3) to the same tube for a total of **35 µl.**

b. Prepare Target Enrichment 1 Reaction Mix on ice. Vortex and centrifuge briefly.

Target Enrichment 1 Reaction Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Nuclease-free Water	-	5	22	44
Amplification Master Mix	220125	50	220	440
cDNA Additive	220067	5	22	44
T Cell Mix 1 or **B Cell Mix 1**	Human 2000008/ Mouse 2000075 or Human 2000035/ Mouse 2000080	5	22	44
Total	-	**65**	**286**	**572**

c. Add **65 µl** Target Enrichment 1 Reaction Mix to each tube containing **35 µl** sample.

d. Pipette mix 5x (pipette set to 90 µl). Centrifuge briefly.

e. Incubate in a thermal cycler with the following protocol.

Lid Temperature	Reaction Volume	Run Time
105°C	100 µl	~20-30 min
Step	**Temperature**	**Time**
1	98°C	00:00:45
2	98°C	00:00:20
3	67°C	00:00:30
4	72°C	00:01:00
5 Different cycle numbers for T & B cells	T Cell: Go to Step 2, 9x (total 10 cycles) B Cell: Go to Step 2, 5x (total 6 cycles)	
6	72°C	00:01:00
7	4°C	Hold

STOP

f. Store at **4°C** for up to **72 h** or proceed to the next step.

4.2 Post Target Enrichment 1 Cleanup – SPRIselect

a. Vortex to resuspend the SPRIselect reagent. Add **80 µl** SPRIselect reagent **(0.8X)** to each sample. Pipette mix 15x (pipette set to 150 µl).

b. Incubate **5 min** at **room temperature**.

c. Place tube strip on the magnet•**High** until the solution clears.

d. Remove the supernatant.

e. Add **200 µl** 80% ethanol to the pellet. Wait **30 sec**.

f. Remove the ethanol.

g. **Repeat** steps e and f for a total of 2 washes.

h. Centrifuge briefly. Place on the magnet•**Low**.

i. Remove remaining ethanol. Air dry for **2 min**.

j. Remove from the magnet. Add **35.5 µl** Buffer EB. Pipette mix 15x.

k. Incubate **2 min** at **room temperature**.

l. Place on the magnet•**Low** until the solution clears.

m. Transfer **35 µl** sample to a new tube strip.

STOP **n.** Store at **4°C** in for up to **72 h** or at **–20°C** for up to **a week**, or proceed to the next step.

4.3 Target Enrichment 2

a. Prepare Target Enrichment 2 Reaction Mix on ice. Vortex and centrifuge briefly.

Target Enrichment 2 Reaction Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Nuclease-free Water	-	5	22	44
Amplification Master Mix	220125	50	220	440
cDNA Additive	220067	5	22	44
T Cell Mix 2 **or** **B Cell Mix 2**	Human 2000009/ Mouse 2000079 or Human 2000036/ Mouse 2000081	5	22	44
Total	-	**65**	**286**	**572**

c. Add **65 µl** Target Enrichment 2 Reaction Mix to each tube containing **35 µl** sample.

d. Pipette mix 5x (pipette set to 90 µl). Centrifuge briefly.

e. Incubate in a thermal cycler with the following protocol.

Lid Temperature	Reaction Volume	Run Time
105°C	100 µl	~25-30 min
Step	**Temperature**	**Time**
1	98°C	00:00:45
2	98°C	00:00:20
3	67°C	00:00:30
4	72°C	00:01:00
5 Different cycle numbers for T & B cells	T Cell: Go to Step 2, 9x (total 10 cycles) B Cell: Go to Step 2, 7x (total 8 cycles)	
6	72°C	00:01:00
7	4°C	Hold

STOP

f. Store at **4°C** for up to **72 h** or proceed to the next step.

4.4 Post Target Enrichment 2 Double Sided Size Selection – SPRIselect

a. Vortex to resuspend SPRIselect reagent. Add **50 µl** SPRIselect reagent **(0.5X)** to each sample. Pipette mix 15x (pipette set to 145 µl).

b. Incubate **5 min** at **room temperature**.

c. Place on the magnet•**High** until the solution clears. DO NOT discard supernatant.

d. Transfer **145 µl** supernatant to a new tube strip.

e. Vortex to resuspend SPRIselect reagent. Add **30 µl** SPRIselect reagent **(0.8X)** to each sample. Pipette mix 15x (pipette set to 150 µl).

f. Incubate **5 min** at **room temperature**.

g. Place on the magnet•**High** until the solution clears.

h. Remove **170 µl** supernatant. DO NOT discard any beads.

i. Add **200 µl** 80% ethanol. Wait **30 sec**.

j. Remove the ethanol.

k. **Repeat** steps i and j for a total of 2 washes.

l. Centrifuge briefly. Place on the magnet•**Low**.

m. Remove remaining ethanol wash. DO NOT over-dry beads to ensure maximum elution efficiency.

n. Remove from the magnet. Add **45.5 µl** Buffer EB. Pipette mix 15x.

o. Incubate **2 min** at **room temperature**.

p. Place on the magnet•**Low** until the solution clears.

q. Transfer **45 µl** sample to a new tube strip.

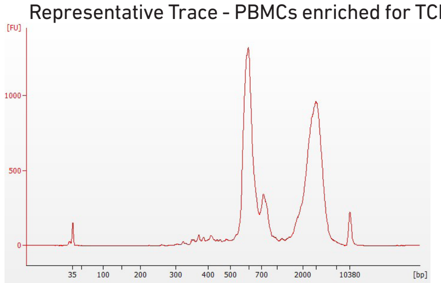
STOP **r.** Store at **4°C** for up to **72 h** or at **–20°C** for up to **1 week**, or proceed to the next step.

4.5 Post Target Enrichment QC & Quantification

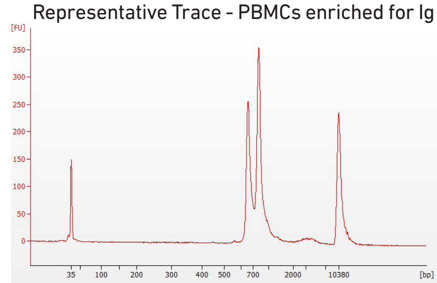
a. Run **1 µl** sample at **1:5 dilution** (Dilution Factor 5) on an Agilent Bioanalyzer High Sensitivity chip.

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 enriched for TCR



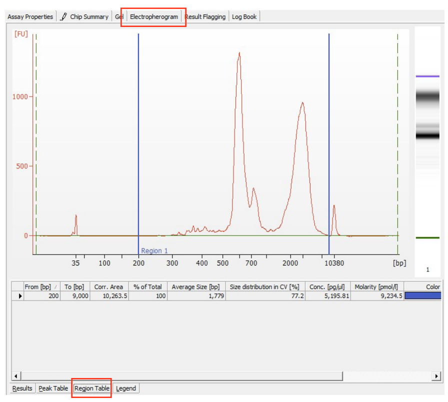
Representative Trace - PBMCs enriched for Ig



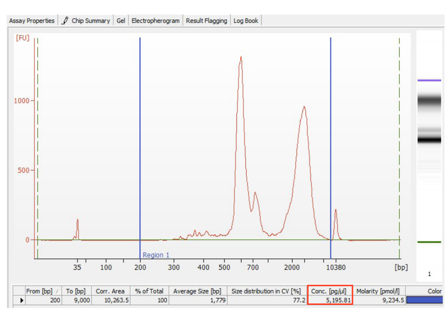
b. Determine yield for each sample. Example calculation below.

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]



iii. Calculate
Concentration: 5195.81 pg/µl
Dilution Factor: 5

Enriched Product Conc.

$$\frac{\text{Conc. (pg/µl) x Dilution Factor}}{\text{1000 (pg/ng)}} = \frac{5195.81 \times 5}{1000} = \mathbf{26\ ng/µl}$$

Example Calculation for Carrying Forward 50 ng Sample for Enriched Library Construction

$$\text{Volume for 50 ng} = \frac{\text{50 ng}}{\text{26 (ng/µl)}} = \mathbf{1.9\ µl}$$

Enriched Library Construction Sample
=1.9 µl + 18.1 µl nuclease-free water
=20 µl total

If <50 ng available, carry forward 20 µl sample (2-50 ng) into Enriched Library Construction.

! DO NOT exceed a mass of 50 ng in the 20 µl carry forward volume.

Alternate Quantification Methods:

- Qubit Fluorometer and Qubit dsDNA HS Assay Kit.

Step 5

Enriched Library Construction

5

5.0 Enriched Library Construction

GET STARTED!

Action	Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature	**Fragmentation Buffer**	220108	Thaw, vortex, verify no precipitate, centrifuge briefly.	−20°C
	Adaptor Mix	220026	Vortex, centrifuge briefly.	−20°C
	Ligation Buffer	220109	Thaw, vortex, verify no precipitate, centrifuge briefly.	−20°C
	SI-PCR Primer	220111	Vortex, centrifuge briefly.	−20°C
	Single Index Plate T Set A	2000240	-	−20°C
	Beckman Coulter SPRIselect Reagent	-	Manufacturer's recommendations.	-
	Agilent Bioanalyzer High Sensitivity Kit If used for QC	-	Manufacturer's recommendations.	-
	Agilent TapeStation ScreenTape and Reagents If used for QC	-	Manufacturer's recommendations.	-
Place on Ice	**Fragmentation Enzyme Blend**	220107/ 220130	Centrifuge briefly.	−20°C
	DNA Ligase	220110/ 220131	Centrifuge briefly.	−20°C
	Amplification Master Mix	220125	Vortex, centrifuge briefly.	−20°C
Obtain	**Qiagen Buffer EB**	-	-	Ambient
	10x Magnetic Separator	230003	See Tips & Best Practices.	Ambient
	Prepare 80% Ethanol Prepare 15 ml for 8 reactions	-	Prepare fresh.	Ambient

5.1 Fragmentation, End Repair & A-tailing

a. Determine the volume for **50 ng** mass of sample (see example calculation at step 4.5). Dispense the sample volume in a tube strip **on ice**. If the volume required for **50 ng** is less than **20 µl**, adjust the total volume of each sample to **20 µl** with nuclease-free water. If the volume for **50 ng** exceeds **20 µl**, carry only **20 µl** sample into library construction.

b. Prepare a thermal cycler with the following incubation protocol.

Lid Temperature	Reaction Volume	Run Time
65°C	50 µl	~35 min
Step	**Temperature**	**Time**
Pre-cool block Pre-cool block prior to preparing the Fragmentation Mix	4°C	Hold
Fragmentation	32°C	00:02:00
End Repair & A-tailing	65°C	00:30:00
Hold	4°C	Hold

c. Vortex Fragmentation Buffer. Verify there is no precipitate.

d. Prepare Fragmentation Mix on ice. Pipette mix and centrifuge briefly.

Fragmentation Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Nuclease-free Water	-	15	66	132
Fragmentation Buffer	220108	5	22	44
Fragmentation Enzyme Blend	220107/ 220130	10	44	88
Total	-	**30**	**132**	**264**

e. Add **30 µl** Fragmentation Mix into each tube containing **20 µl** sample.

f. Pipette mix 15x (pipette set to 30 µl) on ice. Centrifuge briefly.

g. Transfer into the pre-cooled thermal cycler **(4°C)** and press "SKIP" to initiate the protocol.

5.2 Adaptor Ligation

a. Prepare Adaptor Ligation Mix. Pipette mix and centrifuge briefly.

Adaptor Ligation Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Nuclease-free Water	-	17.5	77	154
Ligation Buffer	220109	20	88	176
DNA Ligase	220110/ 220131	10	44	88
Adaptor Mix	220026	2.5	11	22
Total	-	**50**	**220**	**440**

b. Remove the sample from the thermal cycler.

c. Add **50 µl** Adaptor Ligation Mix to **50 µl** sample. Pipette mix 15x (pipette set to 90 µl). Centrifuge briefly.

d. Incubate in a thermal cycler with the following protocol.

Lid Temperature	Reaction Volume	Run Time
30°C	100 µl	15 min
Step	**Temperature**	**Time**
1	20°C	00:15:00
2	4°C	Hold

5.3 Post Ligation Cleanup – SPRIselect

a. Vortex to resuspend SPRIselect Reagent. Add **80 µl** SPRIselect Reagent **(0.8X**) to each sample. Pipette mix 15x (pipette set to 150 µl).

b. Incubate **5 min** at **room temperature**.

c. Place on the magnet•**High** until the solution clears.

d. Remove the supernatant.

e. Add **200 µl** 80% ethanol to the pellet. Wait **30 sec**.

f. Remove the ethanol.

g. **Repeat** steps e and f for a total of 2 washes.

h. Centrifuge briefly. Place on the magnet•**Low**.

i. Remove any remaining ethanol. Air dry for **2 min**.

j. Remove from the magnet. Add **30.5 µl** Buffer EB. Pipette mix 15x. If beads still appear clumpy, continue pipette mixing until fully resuspended.

k. Incubate **2 min** at **room temperature**.

l. Place on the magnet•**Low** until the solution clears.

m. Transfer **30 µl** sample to a new tube strip.

5.4 Sample Index PCR

a. Choose the appropriate sample index sets to ensure that no sample indices overlap in a multiplexed sequencing run.
Record the 10x sample index name (PN-2000240 Single Index Plate T Set A well ID) used.

b. Prepare Sample Index PCR Mix.

Sample Index PCR Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Nuclease-free Water	-	8	35	70
Amplification Master Mix	220125	50	220	440
SI-PCR Primer	220111	2	9	18
Total	-	**60**	**264**	**528**

c. Add **60 µl** Sample Index PCR Mix to **30 µl** sample.

d. Add **10 µl** of an individual sample index (Single Index Plate T Set A) to each well and record the well ID. Pipette mix 5x (pipette set to 90 µl). Centrifuge briefly.

e. Incubate in a thermal cycler with the following protocol.

Lid Temperature	Reaction Volume	Run Time
105°C	100 µl	~30 min
Step	**Temperature**	**Time**
1	98°C	00:00:45
2	98°C	00:00:20
3	54°C	00:00:30
4	72°C	00:00:20
5	Go to step 2, 8x (total 9 cycles)	
6	72°C	00:01:00
7	4°C	Hold

STOP

f. Store at **4°C** for up to **72 h** or proceed to the next step.

5.5 Post Sample Index PCR Cleanup – SPRIselect

a. Vortex to resuspend the SPRIselect reagent. Add **80 µl** SPRIselect Reagent **(0.8X)** to each sample. Pipette mix 15x (pipette set to 150 µl).

b. Incubate **5 min** at **room temperature**.

c. Place the magnet•**High** until the solution clears.

d. Remove the supernatant.

e. Add **200 µl** 80% ethanol to the pellet. Wait **30 sec**.

f. Remove the ethanol.

g. **Repeat** steps e and f for a total of 2 washes.

h. Centrifuge briefly. Place on the magnet•**Low**.

i. Remove remaining ethanol. Air dry for **2 min**.

j. Remove from the magnet. Add **35.5 µl** Buffer EB. Pipette mix 15x.

k. Incubate **2 min** at **room temperature**.

l. Place on the magnet•**Low** until the solution clears.

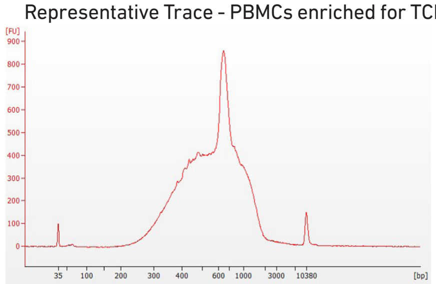
m. Transfer **35 µl** to a new tube strip.

STOP **n.** Store at **4°C** for up to **72 h** or at **–20°C** for long-term storage.

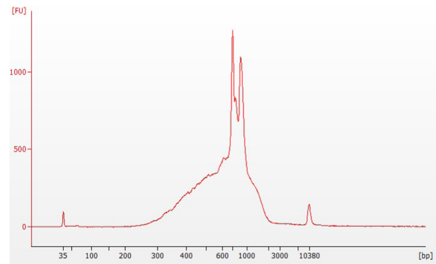
5.6 Post Library Construction QC

a. Run **1 µl** sample at **1:10 dilution** on an Agilent Bioanalyzer High Sensitivity chip.

Representative Trace - PBMCs enriched for TCR



Representative Trace - PBMCs enriched for Ig



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

Alternate QC Method:

- Agilent TapeStation. See Appendix for representative traces

See Appendix for Post Library Construction Quantification

Step 6

5' Gene Expression (GEX) Library Construction

6.0
5' Gene Expression (GEX) Library Construction

GET STARTED!

Action	Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature	**Fragmentation Buffer**	220108	Thaw, vortex, verify no precipitate, centrifuge briefly.	–20°C
	Adaptor Mix	220026	Vortex, centrifuge briefly.	–20°C
	Ligation Buffer	220109	Thaw, vortex, verify no precipitate, centrifuge briefly.	–20°C
	SI-PCR Primer	220111	Vortex, centrifuge briefly.	–20°C
	Single Index Plate T Set A	2000240	-	–20°C
	Beckman Coulter SPRIselect Reagent	-	Manufacturer's recommendations.	-
	Agilent Bioanalyzer DNA 1000 kit If used for QC	-	Manufacturer's recommendations.	-
	Agilent TapeStation ScreenTape and Reagents If used for QC	-	Manufacturer's recommendations.	-
Place on Ice	**Fragmentation Enzyme Blend**	220107/ 220130	Centrifuge briefly.	–20°C
	DNA Ligase	220110/ 220131	Centrifuge briefly.	–20°C
	Amplification Master Mix	220125	Vortex, centrifuge briefly.	–20°C
Obtain	**Qiagen Buffer EB**	-	-	Ambient
	10x Magnetic Separator	230003	See Tips & Best Practices.	Ambient
	Prepare 80% Ethanol Prepare 15 ml for 8 reactions	-	Prepare fresh.	Ambient

6.1 GEX Fragmentation, End Repair & A-tailing

a. Determine the volume for **50 ng** mass of sample (see example calculation at step 3.3). Dispense the sample volume in a tube strip **on ice**. If the volume required for **50 ng** is less than **20 µl**, adjust the total volume of each sample to **20 µl** with nuclease-free water. If the volume for **50 ng** exceeds **20 µl**, carry only **20 µl** sample into library construction.

b. Prepare a thermal cycler with the following incubation protocol.

Lid Temperature	Reaction Volume	Run Time
65°C	50 µl	~35 min
Step	**Temperature**	**Time**
Pre-cool block Pre-cool block prior to preparing the Fragmentation Mix	4°C	Hold
Fragmentation	32°C	00:05:00
End Repair & A-tailing	65°C	00:30:00
Hold	4°C	Hold

c. Vortex Fragmentation Buffer. Verify there is no precipitate.

d. Prepare Fragmentation Mix on ice. Pipette mix and centrifuge briefly.

Fragmentation Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Nuclease-free Water	-	15	66	132
Fragmentation Buffer	220108	5	22	44
Fragmentation Enzyme Blend	220107/ 220130	10	44	88
Total	**-**	**30**	**132**	**264**

e. Add **30 µl** Fragmentation Mix into each tube containing **20 µl** sample.

f. Pipette mix 15x (pipette set to 30 µl) on ice. Centrifuge briefly.

g. Transfer into the pre-cooled thermal cycler **(4°C)** and press "SKIP" to initiate the protocol.

6.2 GEX Post Fragmentation, End Repair & A-tailing Double Sided Size Selection – SPRIselect

a. Vortex to resuspend SPRIselect Reagent. Add 3**0 µl** SPRIselect Reagent **(0.6X)** to each sample. Pipette mix 15x (pipette set to 75 µl).

b. Incubate **5 min** at **room temperature**.

c. Place on the magnet•**High** until the solution clears. DO NOT discard supernatent.

d. Transfer **75 µl** supernatant to a new tube strip.

e. Add **10 µl** SPRIselect reagent **(0.8X)** to each sample. Pipette mix 15x (pipette set to 75 µl).

f. Incubate **5 min** at **room temperature**.

g. Place on the magnet•**High** until the solution clears.

h. Remove **80 µl** supernatant. DO NOT discard any beads.

i. With the tube strip still on the magnet, add **125 µl** 80% ethanol to the pellet. Wait **30 sec**.

j. Remove the ethanol.

k. **Repeat** steps i and j for a total of 2 washes.

l. Centrifuge briefly. Place on the magnet •**Low**.

m. Remove the ethanol. DO NOT over-dry beads to ensure maximum elution efficiency.

n. Remove from the magnet. Add **50.5 µl** Buffer EB. Pipette mix 15x.

o. Incubate **2 min** at **room temperature**.

p. Place on the magnet•**High** until the solution clears.

q. Transfer **50 µl** sample to a new tube strip.

6.3 GEX Adaptor Ligation

a. Prepare Adaptor Ligation Mix. Pipette mix and centrifuge briefly.

Adaptor Ligation Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Nuclease-free Water	-	17.5	77	154
Ligation Buffer	220109	20	88	176
DNA Ligase	220110/ 220131	10	44	88
Adaptor Mix	220026	2.5	11	22
Total	-	**50**	**220**	**440**

b. Add **50 µl** Adaptor Ligation Mix to **50 µl** sample. Pipette mix 15x (pipette set to 90 µl). Centrifuge briefly.

c. Incubate in a thermal cycler with the following protocol.

Lid Temperature	Reaction Volume	Run Time
30°C	100 µl	15 min
Step	**Temperature**	**Time**
1	20°C	00:15:00
2	4°C	Hold

6.4 GEX Post Ligation Cleanup – SPRIselect

a. Vortex to resuspend SPRIselect Reagent. Add **80 µl** SPRIselect Reagent **(0.8X)** to each sample. Pipette mix 15x (pipette set to 150 µl).

b. Incubate **5 min** at **room temperature**.

c. Place on the magnet•**High** until the solution clears.

d. Remove the supernatant.

e. Add **200 µl** 80% ethanol to the pellet. Wait **30 sec**.

f. Remove the ethanol.

g. **Repeat** steps e and f for a total of 2 washes.

h. Centrifuge briefly. Place on the magnet•**Low**.

i. Remove any remaining ethanol. Air dry for **2 min**.

j. Remove from the magnet. Add **30.5 µl** Buffer EB. Pipette mix 15x.

k. Incubate **2 min** at **room temperature**.

l. Place on the magnet•**Low** until the solution clears.

m. Transfer **30 µl** sample to a new tube strip.

6.5 GEX Sample Index PCR

a. Choose the appropriate sample index sets to ensure that no sample indices overlap in a multiplexed sequencing run.
Record the 10x sample index name (PN-2000240 Single Index Plate T Set A, well ID) used, especially if running more than one sample.

b. Prepare Sample Index PCR Mix. Pipette mix and centrifuge briefly.

Sample Index PCR Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Nuclease-free Water	-	8	35	70
Amplification Master Mix	220125	50	220	440
SI-PCR Primer	220111	2	9	18
Total	-	**60**	**264**	**528**

c. Add **60 µl** Sample Index PCR Mix to **30 µl** sample.

d. Add **10 µl** of an individual sample index (Single Index Plate T Set A) to each well and record their assignment. Pipette mix 5x (pipette set to 90 µl). Centrifuge briefly.

e. Incubate in a thermal cycler with the following protocol.

Lid Temperature	Reaction Volume	Run Time
105°C	100 µl	~40 min
Step	**Temperature**	**Time**
1	98°C	00:00:45
2	98°C	00:00:20
3	54°C	00:00:30
4	72°C	00:00:20
5	Go to step 2, see table below for # cycles	
6	72°C	00:01:00
7	4°C	Hold

The table recommends starting point for optimization. If less than 50 ng was carried into 5' Gene Expression Library Construction, refer to the product yield calculation example in step 3.3 to determine the mass input into Library Construction.

Input into Library Construction	Total Sample Index Cycles
1 - 25 ng	16
26 - 50 ng	14

STOP

f. Store at **4°C** for up to **72 h** or proceed to the next step.

6.6 GEX Post Sample Index PCR Double Sided Size Selection – SPRIselect

a. Vortex to resuspend SPRIselect reagent. Add **60 µl** SPRIselect reagent **(0.6X)** to each sample. Pipette mix 15x (pipette set to 150 µl).

b. Incubate **5 min** at **room temperature**.

c. Place on the magnet•**High** until the solution clears. DO NOT discard supernatant.

d. Transfer **150 µl** supernatant to a new tube strip.

e. Vortex to resuspend SPRIselect reagent. Add **20 µl** SPRIselect reagent **(0.8X)** to each sample. Pipette mix 15x (pipette set to 150 µl).

f. Incubate **5 min** at **room temperature.**

g. Place on the magnet•**High** until the solution clears.

h. Remove **165 µl** supernatant. DO NOT discard any beads.

i. With the tube strip still on the magnet, add **200 µl** 80% ethanol to the pellet. Wait **30 sec**.

j. Remove the ethanol.

k. **Repeat** steps i and j for a total of 2 washes.

l. Centrifuge briefly. Place on the magnet•**Low**.

m. Remove the remaining ethanol. DO NOT over-dry beads to ensure maximum elution efficiency.

n. Remove the tube strip from the magnet. Add **35.5 µl** Buffer EB. Pipette mix 15x.

o. Incubate **2 min** at **room temperature**.

p. Place on the magnet•**Low** until the solution clears.

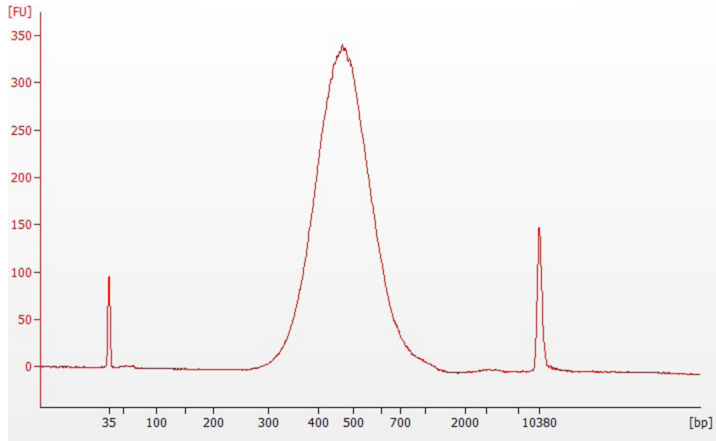
q. Transfer **35 µl** sample to a new tube strip.

STOP **r.** Store at **4°C** for up to **72 h** or at **–20°C** for long-term storage.

6.7 GEX Post Library Construction QC

a. Run **1 µl** sample at **1:10 dilution** on an Agilent Bioanalyzer High Sensitivity chip.

Representative Trace



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

Alternate QC Method:

- Agilent TapeStation. See Appendix for representative traces

See Appendix for GEX Post Library Construction Quantification

Sequencing

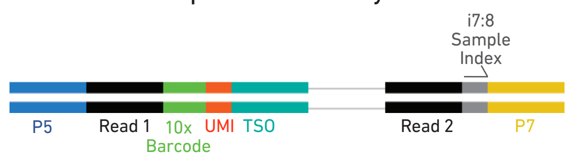
7

Sequencing Libraries

Chromium Single Cell V(D)J enriched libraries and 5' 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 Read 1, while sample index sequences are incorporated as the i7 index read. Read 1 and Read 2 are standard Illumina sequencing primer sites used in paired-end sequencing. Read 1 is used to sequence 16 bp 10x Barcodes and 10 bp UMI. Sequencing these libraries produce a standard Illumina BCL data output folder.

Chromium Single Cell V(D)J Enriched Library

Chromium Single Cell 5' 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
- NexSeq 500/550*
- HiSeq 2500 (Rapid Run)
- HiSeq 3000/4000
- NovaSeq

*Sequencing Chromium Single Cell libraries on the NextSeq 500/550 platform may yield reduced sequence quality and sensitivity relative to the MiSeq, HiSeq, and NovaSeq platforms. Refer to the 10x Genomics Support website for more information.

Sample Indices

Each sample index in the Single Index Kit T Set A (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 Single Index Plate T Set A well ID) is needed in the sample sheet used for generating FASTQs with “cellranger mkfastq”.

Library Sequencing Depth & Run Parameters

Sequencing Depth	Minimum 5,000 read pairs per cell for V(D)J Enriched library Minimum 20,000 read pairs per cell for 5' Gene Expression library
Sequencing Type	Paired-end, single indexing

Sequencing Read	**Recommended Cycles***			
	Read 1	**i7 index**	**i5 index**	**Read 2**
V(D)J Enriched library	26	8	0	91
5' Gene Expression library	26	8	0	91
V(D)J Enriched + 5' Gene Expression libraries	26	8	0	91

*Alternatively, all library types (alone or in combination) may be sequenced using previously recommended 150 x 150 bp cycles.

Library Loading

Once quantified and normalized, V(D)J Enriched libraries 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
HiSeq 2500 (RR)	10	1
HiSeq 4000	180	1
NovaSeq	200	1

Library Pooling

V(D)J Enriched libraries and the 5' Gene Expression libraries maybe 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.

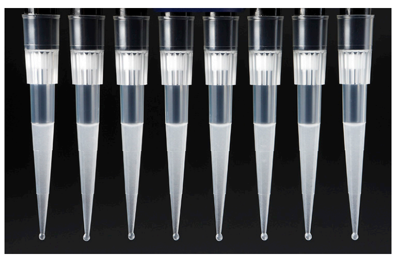
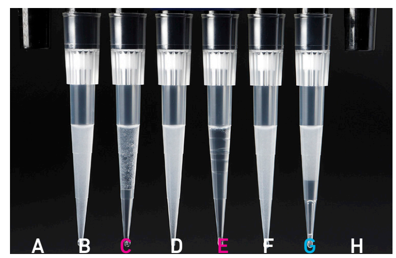
Library Pooling Examples:

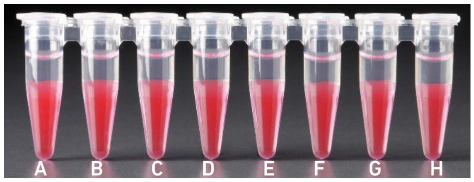
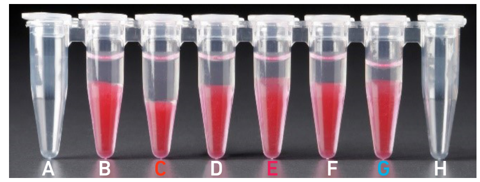
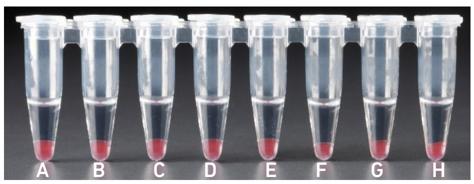
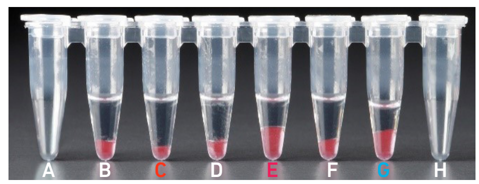
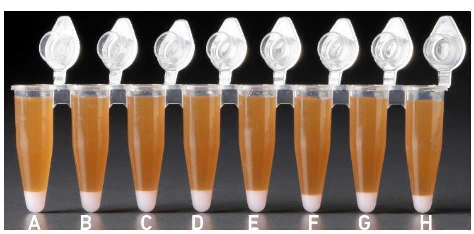
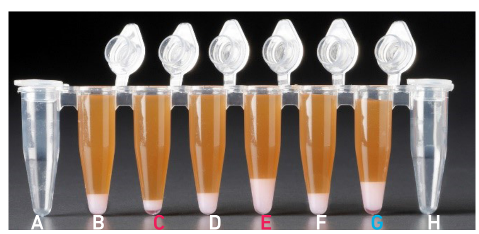
Libraries	Sequencing Depth (read pairs per cell)	Library Pooling Ratio
Example 1		
V(D)J Enriched library 5' Gene Expression library	5,000 20,000	1 4
Example 2		
V(D)J Enriched library 5' Gene Expression library	5,000 50,000	1 10

Troubleshooting

8

GEMs

STEP	NORMAL	REAGENT CLOGS & WETTING FAILURES
1.4 d After Chip G is removed from the Controller and the wells are exposed	 All 8 recovery wells are similar in volume and opacity.	 Recovery well G indicates a reagent clog. Recovery well C and E indicate a wetting failure. Recovery wells B, D, and F are normal. Wells A and H contain 50% Glycerol Solution.
1.4 e Transfer GEMs from Chip G Recovery Wells	 All liquid levels are similar in volume and opacity without air trapped in the pipette tips.	 Pipette tips C and E indicate a wetting failure. Pipette tip C contains partially emulsified GEMs. Emulsion is absent in pipette tip E. Pipette tip G indicates a reagent clog.

STEP	NORMAL	REAGENT CLOGS & WETTING FAILURES
2.1 a After transfer of the GEMs + Recovery Agent	 All liquid levels are similar in the aqueous sample volume (clear) and Recovery Agent/Partitioning Oil (pink).	 Tube G indicates a reagent clog has occurred. There is a decreased volume of aqueous layer (clear). Tube C and E indicate a wetting failure has occurred. There is an abnormal volume of Recovery Agent/Partitioning Oil (pink).
2.1 b After aspiration of Recovery Agent/ Partitioning Oil	 All liquid volumes are similar in the aqueous sample volume (clear) and residual Recovery Agent/Partitioning Oil (pink).	 Tube G indicates a reagent clog has occurred. There is a decreased volume of aqueous layer (clear). There is also a greater residual volume of Recovery Agent/Partitioning Oil (pink). Tube C and E indicate a wetting failure has occurred. There is an abnormal residual volume of Recovery Agent/Partitioning Oil (pink).
2.1 c After addition of Dynabeads Cleanup Mix	 All liquid volumes are similar after addition of the Dynabeads Cleanup Mix.	 Tube G indicates a reagent clog has occurred. There is an abnormal ratio of Dynabeads Cleanup Mix (brown) to Recovery Agent/Partitioning Oil (appears white). Tube C and E indicate a wetting failure has occurred. There is an abnormal ratio of Dynabeads Cleanup Mix (brown) to Recovery Agent/Partitioning Oil (appears white).

If a channel clogs or wetting failure occurs during GEM generation, it is recommended that the sample be remade. If any of the listed issues occur, take a picture and send it to support@10xgenomics.com for further assistance.

Chromium Controller Errors

If the Chromium Controller or the Chromium Single Cell Controller fails to start, an error tone will sound and one of the following error messages will be displayed:

a. **Chip not read – Try again:** Eject the tray, remove and/or reposition the Chromium Next GEM Secondary Holder assembly and try again. If the error message is still received after trying this more than twice, contact support@10xgenomics.com for further assistance.

b. **Check gasket:** Eject the tray by pressing the eject button to check that the 10x Gasket is correctly installed on the Chromium Next GEM Chip. If the error message persists, contact support@10xgenomics.com for further assistance.

c. **Error Detected: Row _ Pressure:**

   i. If this message is received within a few seconds of starting a run, eject the tray by pressing the eject button and check for dirt or deposits on the 10x Gasket. If dirt is observed, replace with a new 10x Gasket and try again. If the error message is still received after trying this more than twice, contact support@10xgenomics.com for further assistance.

   ii. If this message is received after a few minutes into the run, the Chromium Next GEM Chip must be discarded. **Do not try running this Chromium Next GEM Chip again as this may damage the Chromium Controller.**

d. **Invalid Chip CRC Value:** This indicates that a Chromium Next GEM Chip has been used with an older firmware version. The chip must be discarded. Contact support@10xgenomics.com for further assistance.

e. **Chip Holder Not Present:** Open the controller drawer and check if chip holder is present. Insert chip properly into chip holder and retry.

f. **Unauthorized Chip:** This indicates that an incompatible non-Next GEM chip has been used with an instrument that only can run Next GEM assays. Use only Chromium Controller (PN-120223;120246) or Chromium Single Cell Controller (PN-120263;120212) to run that chip or chip must be discarded. Contact support@10xgenomics.com for further assistance.

g. **Endpoint Reached Early:** If this message is received, contact support@10xgenomics.com for further assistance.

Chromium X Series Errors

The Chromium X touchscreen will guide the user through recoverable errors. If the error continues, or if the instrument has seen critical or intermediate errors, email support@10xgenomics.com with the displayed error code. Support will request a troubleshooting package. Upload pertinent logs to 10x Genomics by navigating to the Logs menu option on screen.

There are two types of errors:

Critical Errors — When the instrument has seen a critical error, the run will immediately abort. Do not proceed with any further runs. Contact support@10xgenomics.com with the error code.

a. System Error

b. Pressure Error

c. Chip Error

d. Run Error

e. Temperature Error

f. Software Error

User Recoverable Errors — Follow error handling instructions through the touchscreen and continue the run.

a. Gasket Error

b. Tray Error

c. Chip Error

d. Unsupported Chip Error

e. Update Error

Appendix

9

Post Library Construction Quantification

a. Thaw KAPA Library Quantification Kit for Illumina Platforms.

b. Dilute **1 µl** sample with deionized water to appropriate dilutions that fall within the linear detection range of the KAPA Library Quantification Kit for Illumina Platforms. (For more accurate quantification, make the dilution(s) in duplicate).

c. Make enough Quantification Master Mix for the DNA dilutions per sample and the DNA Standards (plus 10% excess) using the guidance for 1 reaction volume below.

Quantification Master Mix	1X (µl)
SYBR Fast Master Mix + Primer	12
Water	4
Total	**16**

d. Dispense **16 µl** Quantification Master Mix for sample dilutions and DNA Standards into a 96 well PCR plate.

e. Add **4 µl** sample dilutions and **4 µl** DNA Standards to appropriate wells. Centrifuge briefly.

f. Incubate in a thermal cycler with the following protocol.

Step	Temperature	Run Time
1	95°C	00:03:00
2	95°C	00:00:05
3	67°C	00:00:30
4	Go to Step 2, 29X (Total 30 cycles)	

g. Follow the manufacturer's recommendations for qPCR-based quantification. For library quantification for sequencer clustering, determine the concentration based on insert size derived from the Bioanalyzer/TapeStation trace.

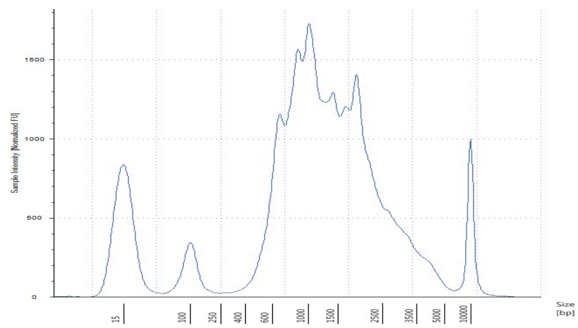
Agilent TapeStation Traces

Agilent TapeStation Traces

Agilent TapeStation High Sensitivity D5000 ScreenTape was used. Protocol steps correspond to the Chromium Next GEM Single Cell V(D)J v1.1 Reagent Kits User Guide (CG000207).

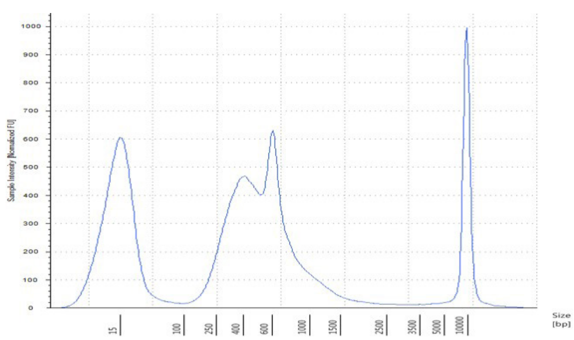
Protocol Step 3.3 – cDNA QC & Quantification

Representative Trace

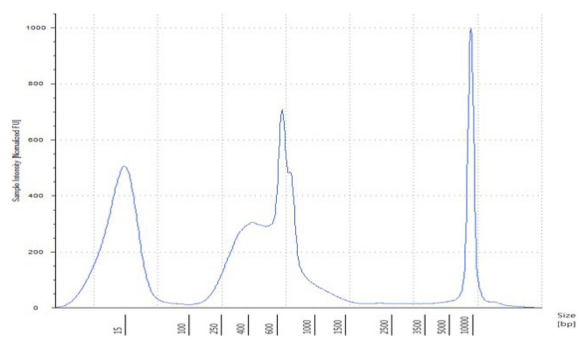


Protocol Step 5.6 – Post Library Construction QC

Representative Trace - PBMCs enriched for TCR

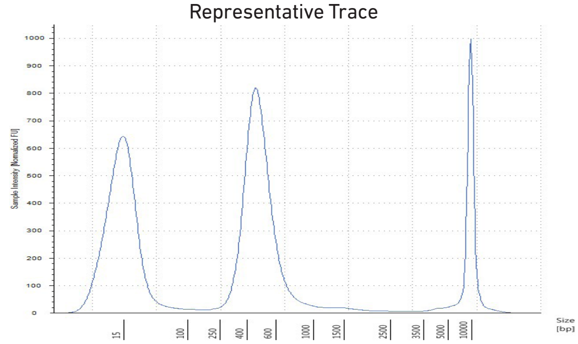


Representative Trace - PBMCs enriched for Ig



Protocol Step 6.7 – GEX Post Library Construction QC

Representative Trace



Oligonucleotide Sequences

Protocol steps correspond to the Chromium Next GEM Single Cell V(D)J v1.1 Reagent Kits User Guide (CG000207).

Protocol Step 1.5 – GEM-RT Incubation

Gel Bead Oligo Primer (TSO)

Gel Bead — Read 1 | 10x Barcode | UMI | TSO rGrGrG

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

Poly-dT RT Primer

Non-poly(dT) | Poly(dT)VN

5'-AAGCAGTGGTATCAACGCAGAGTAC-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTVN-3'

Reverse Transcript Product

3' Read 1 | 10x Barcode | UMI | TSO C C C ——— Poly(dT)VN | Non-poly(dT) 5'

3'-GATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNNNNNN-NNNNNNNNNN-AAAGAATATACCC-cDNA_Insert-NVTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-CATGAGACGCAACTATGGTGACGAA-5'

Protocol Step 3.1 – cDNA Amplification

cDNA Primer Mix PN-220106

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

Reverse Primer: Non-poly(dT)
5'-AAGCAGTGGTATCAACGCAGAG-3'

cDNA Amplified Product

Read 1 | 10x Barcode | UMI | TSO ——— Poly-dT RT Primer

5'-CTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNNN-TTTCTTATATGGG-cDNA_Insert-GTACTCTGCGTTGATACCACTGCTT-3'
3'-GATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNNNNNN-NNNNNNNNNN-AAAGAATATACCC-cDNA_Insert-CATGAGACGCAACTATGGTGACGAA-5'

Protocol Step 4.1 – Target Enrichment 1

Human T Cell Mix 1 PN-2000008

Forward Primer: (final conc. 2 µM) PCR Primer
5'-AATGATACGGCGACCACCGAGA-TCTACACTCTTTCCCTACACGACGCTC-3'

Reverse Outer Primers: (final conc. 1 µM each) Enrichment Outer Primer
5'-TGAAGGCGTTTGCACATGCA-3'
5'-TCAGGCAGTATCTGGAGTCATTGAG-3'

Human B Cell Mix 1 PN-2000035

Forward Primer: (final conc. 1 µM) PCR Primer
5'-AATGATACGGCGACCACCGAGA-TCTACACTCTTTCCCTACACGACGCTC-3'

Reverse Outer Primers: (final conc. 0.5 µM each) Enrichment Outer Primer
5'-CAGGGCACAGTCACATCCT-3'
5'-TGCTGGACCACGCATTTGTA-3'
5'-GGTTTTGTTGTCGACCCAGTCT-3'
5'-TTGTCCACCTTGGTGTTGCT-3'
5'-CATGACGTCCTTGGAAGGCA-3'
5'-TGTGGGACTTCCACTG-3'
5'-TTCTCGTAGTCTGCTTTGCTCAG-3'

Mouse T Cell Mix 1 PN-2000075

Forward Primer: (final conc. 2 µM) PCR Primer
5'-AATGATACGGCGACCACCGAGA-TCTACACTCTTTCCCTACACGACGCTC-3'

Reverse Outer Primers: (final conc. 0.5 µM each) Enrichment Outer Primer
5'-CTGGTTGCTCCAGGCAATGG-3'
5'-TGTAGGCCTGAGGGTCCGT-3'

Mouse B Cell Mix 1 PN-2000080

Forward Primer: (final conc. 1 µM) PCR Primer
5'-AATGATACGGCGACCACCGAGA-TCTACACTCTTTCCCTACACGACGCTC-3'

Reverse Outer Primers: Enrichment Outer Primer
5'-TCAGCACGGGACAAACTCTTCT-3' (final conc. 0.375 µM)
5'-GCAGGAGACAGACTCTTCTCCA-3' (final conc. 0.175 µM)
5'-AACTGGCTGCTCATGGTGT-3' (final conc. 0.1 µM)
5'-TGGTGCAAGTGTGGTTGAGGT-3' (final conc. 0.3 µM)
5'-TGGTCACTTGGCTGGTGGTG-3' (final conc. 0.25 µM)
5'-CACTTGGCAGGTGAACTGTTTTCT-3' (final conc. 0.25 µM)
5'-AACCTTCAAGGATGCTCTTGGGA-3' (final conc. 0.3 µM)
5'-GGACAGGGATCCAGAGTTCCA-3' (final conc. 0.5 µM)
5'-AGGTGACGGTCTGACTTGGC-3' (final conc. 0.125 µM)
5'-GCTGGACAGGGCTCCATAGTT-3' (final conc. 0.125 µM)
5'-GGCACCTTGTCCAATCATGTTCC-3' (final conc. 0.250 µM)
5'-ATGTCGTTCATACTCGTCCTTGGT-3' (final conc. 0.1 µM)

Protocol Step 4.3 – Target Enrichment 2

Human T Cell Mix 2
PN-2000009

Forward Primer: (final conc. 2 µM) PCR Primer

5'-AATGATACGGCGACCACCGAGA-TCT-3'

Reverse Inner Primers: (final conc. 1 µM each) Enrichment Inner Primer

5'-AGTCTCTCAGCTGGTACACG-3'
5'-TCTGATGGCTCAAACACAGC-3'

Human B Cell Mix 2
PN-2000036

Forward Primer: (final conc. 1 µM) PCR Primer

5'-AATGATACGGCGACCACCGAGA-TCT-3'

Reverse Inner Primers: (final conc. 0.5 µM each) Enrichment Inner Primer

5'-GGGAAGTTTCTGGCGGTCA-3'
5'-GGTGGTACCCAGTTATCAAGCAT-3'
5'-GTGTCCCAGGTCACCATCAC-3'
5'-TCCTGAGGACTGTAGGACAGC-3'
5'-CACGCTGCTCGTATCCGA-3'
5'-TAGCTGCTGGCCGC-3'
5'-GCGTTATCCACCTTCCACTGT-3'

Mouse T Cell Mix 2
PN-2000079

Forward Primer: (final conc. 0.5 µM) PCR Primer

5'-AATGATACGGCGACCACCGAGA-TCT-3'

Reverse Inner Primers: (final conc. 0.5 µM each) Enrichment Inner Primer

5'-AGTCAAAGTCGGTGAACAGGCA-3'
5'-GGCCAAGCACACGAGGGTA-3'

Mouse B Cell Mix 2
PN-2000081

Forward Primer: (final conc. 1 µM) PCR Primer

5'-AATGATACGGCGACCACCGAGA-TCT-3'

Reverse Inner Primers: Enrichment Inner Primer

5'-TACACACCAGTGTGGCCTT-3' (final conc. 0.375 µM)
5'-CAGGCCACTGTCACACCACT-3' (final conc. 0.175 µM)
5'-CAGGTCACATTCATCGTGCCG-3' (final conc. 0.1 µM)
5'-GAGGCCAGCACAGTGACCT-3' (final conc. 0.3 µM)
5'-GCAGGGAAGTTCACAGTGCT-3' (final conc. 0.25 µM)
5'-CTGTTTGAGATCAGTTTGCCATCCT-3' (final conc. 0.25 µM)
5'-TGCGAGGTGGCTAGGTACTTG-3' (final conc. 0.3 µM)
5'-CCCTTGACCAGGCATCC-3' (final conc. 0.5 µM)
5'-AGGTCACGGAGGAACCAGTTG-3' (final conc. 0.125 µM)
5'-GGCATCCCAGTGTCACCGA-3' (final conc. 0.125 µM)
5'-AGAAGATCCACTTCACCTTGAAC-3' (final conc. 0.250 µM)
5'-GAAGCACACGACTGAGGCAC-3' (final conc. 0.1 µM)

Target Enrichment Product

5'-AATGATACGGCGACCACCGAGA-TCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNNN-TTTCTTATATGGG-cDNA_Insert-Inner_Primer-3'

3'-TTACTATGCCGCTGGTGGCTCT-AGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNNNNNN-NNNNNNNNNN-AAAGAATATACCC-cDNA_Insert-Inner_Primer-5'

Protocol Step 5.2 – Adaptor Ligation

Adaptor (Read 2)
PN-220026

Read 2

5'-GATCGGAAGAGCACACGTCTGAACTCCAGTCAC-3'
3'-TCTAGCCTTCTCG-5'

Ligation Product

5'-AATGATACGGCGACCACCGAGA-TCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNNN-TTTCTTATATGGG-cDNA _ Insert-AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC-3'

3'-TTACTATGCCGCTGGTGGCTCT-AGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNNNNNN-NNNNNNNNNN-AAAGAATATACCC-cDNA _ Insert-TCTAGCCTTCTCG-5'

Protocol Step 5.4 – Sample Index PCR

Sample Index PCR Primer
PN-220111

Forward Primer:
SI-PCR Primer
P5 Partial Read 1
5'-AATGATACGGCGACCACCGAGA-TCTACACTCTTTCCCTACACGACGCTC-3'

Reverse Primer:
P7 Sample Index Partial Read 2
5'-CAAGCAGAAGACGGCATACGAGAT-NNNNNNNN-GTGACTGGAGTTCAGACGTGT-3'

Single Index Plate T Set A
PN-2000240

Sample Index PCR Product

5'-AATGATACGGCGACCACCGAGA-TCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNNN-TTTCTTATATGGG-cDNA _ Insert-AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC-NNNNNNNN-ATCTCGTATGCCGTCTTCTGCTTG-3'
3'-TTACTATGCCGCTGGTGGCTCT-AGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNNNNNN-NNNNNNNNNN-AAAGAATATACCC-cDNA _ Insert-TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG-NNNNNNNN-TAGAGCATACGGCAGAAGACGAAC-5'

Protocol Step 6.3 –GEX Adaptor Ligation

Adaptor (Read 2)
PN-220026

Read 2
5'-GATCGGAAGAGCACACGTCTGAACTCCAGTCAC-3'
3'-TCTAGCCTTCTCG-5'

Ligation Product

5'-CTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNNN-TTTCTTATATGGG-cDNA _ Insert-AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC-3'
3'-GATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNNNNNN-NNNNNNNNNN-AAAGAATATACCC-cDNA _ Insert-TCTAGCCTTCTCG-5'

Protocol Step 6.5 – Sample Index PCR

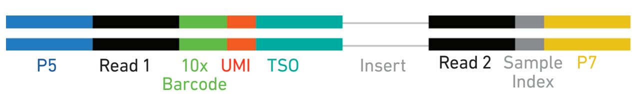
Sample Index PCR Primer
PN-220111

Forward Primer:
SI-PCR Primer
P5 Partial Read 1
5'-AATGATACGGCGACCACCGAGA-TCTACACTCTTTCCCTACACGACGCTC-3'

Reverse Primer:
P7 Sample Index Partial Read 2
5'-CAAGCAGAAGACGGCATACGAGAT-NNNNNNNN-GTGACTGGAGTTCAGACGTGT-3'

Single Index Plate T Set A
PN-2000240

Sample Index PCR Product



5'-AATGATACGGCGACCACCGAGA-TCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNNN-TTTCTTATATGGG-cDNA _ Insert-AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC-NNNNNNNN-ATCTCGTATGCCGTCTTCTGCTTG-3'
3'-TTACTATGCCGCTGGTGGCTCT-AGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNNNNNN-NNNNNNNNNN-AAAGAATATACCC-cDNA _ Insert-TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG-NNNNNNNN-TAGAGCATACGGCAGAAGACGAAC-5'