10x GENOMICS

User Guide | CG000592 | Rev B

Chromium Next GEM Single Cell 5' Reagent Kits v2 (Dual Index)

with Feature Barcode technology for Barcode Enabled Antigen Mapping (BEAM) & Cell Surface Protein

For use with:

Chromium Next GEM Single Cell 5' Kit v2 *16 rxns PN-1000263 | 4 rxns PN-1000265*

Library Construction Kit *16 rxns PN-1000190*

Chromium Single Cell 5' BEAM Core Kit, PE, Set A *128 rxns PN-1000539*

Chromium Human MHC Class I
A0201 Monomer Kit 32 rxns PN- 1000542 | A1101 Monomer Kit 32 rxns PN- 1000543
B0702 Monomer Kit 32 rxns PN-1000544 | A2402 Monomer Kit 32 rxns PN-1000545

Chromium Mouse MHC Class I H2Kb Monomer Kit *32 rxns PN-1000546*

5' Feature Barcode Kit *16 rxns PN-1000541*

Chromium Single Cell V(D)J Amplification Kits
Human 16 rxns TCR PN-1000252 / BCR PN-1000253
Mouse 16 rxns TCR PN-1000254 / BCR PN-1000255

Chromium Next GEM Chip K Single Cell Kit, *48 rxns PN-1000286 | 16 rxns PN-1000287*

Dual Index Kit TT Set A *96 rxns PN-1000215* | TS Set A *96 rxns PN-1000251* | TN Set A *96 rxns PN-1000250*

Notices

Document Number

CG000592 | Rev B

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

CG000592 | Rev B

Title

Chromium Next GEM Single Cell 5' Reagent Kits v2 (Dual Index) with Feature Barcode technology for Barcode Enabled Antigen Mapping (BEAM) & Cell Surface Protein

Revision

Rev A to Rev B

Revision Date

March 15, 2023

Specific Changes

- Added guidance on the requirement of sequence data from 5' Gene Expression library for analysis in the Cell Ranger.

General Changes

Updated for general minor consistency of language and terms throughout.

Table of Contents

Introduction

Chromium Next GEM Single Cell 5' Reagent Kits v2 (Dual Index) 8
10x Genomics Accessories 15
Recommended Thermal Cyclers 16
Additional Kits, Reagents & Equipment 17
Recommended Pipette Tips 18
Protocol Steps & Timing 20
Stepwise Objectives 21
BEAM Reagent Assembly, Sample Labeling & Sorting Guidelines 30

Tips & Best Practices

Step 1: GEM Generation and Barcoding

1.0 Get Started 41
1.1 Prepare Master Mix 42
Assemble Chromium Next GEM Chip K 42
1.2 Load Chromium Next GEM Chip K 45
1.3 Run the Chromium Controller or X/iX 47
1.4 Transfer GEMs 49
1.5 GEM-RT Incubation 50

Step 2: Post GEM–RT Cleanup & cDNA Amplification

2.0 Get Started 52
2.1 Post GEM-RT Cleanup – Dynabeads 53
Step Overview (steps 2.2 & 2.3) 56
2.2 cDNA Amplification 57
2.3 cDNA Cleanup – SPRIselect 58
2.3A Pellet Cleanup for V(D)J & 5' Gene Expression Library 59
2.3B Transferred Supernatant Cleanup for Cell Surface Protein and BEAM Library 59
2.4 Post cDNA Amplification QC & Quantification 60

Step 3: V(D)J Amplification from cDNA

3.0 Get Started 64
3.1 V(D)J Amplification 1 66
3.2 Post V(D)J Amplification 1 Cleanup Double Sided – SPRIselect 67

Table of Contents

3.3 V(D)J Amplification 2 68
3.4 Post V(D)J Amplification 2 Cleanup Double Sided – SPRIselect 69
3.5 Post V(D)J Amplification QC & Quantification 70

Step 4: V(D)J Library Construction
4.0 Get Started 74
4.1 Fragmentation, End Repair & A-tailing 76
4.2 Adaptor Ligation 77
4.3 Post Ligation Cleanup – SPRIselect 77
4.4 Sample Index PCR 78
4.5 Post Sample Index PCR Cleanup – SPRIselect 78
4.6 Post Library Construction QC 79

Step 5: 5' Gene Expression Library Construction
5.0 Get Started 81
5.1 GEX Fragmentation, End Repair & A-tailing 82
5.2 GEX Post Fragmentation, End Repair & A-tailing Double Sided – SPRIselect 83
5.3 GEX Adaptor Ligation 84
5.4 GEX Post Ligation Cleanup – SPRIselect 84
5.5 GEX Sample Index PCR 85
5.6 Post Sample Index PCR Double Sided Size Selection – SPRIselect 86
5.7 Post Library Construction QC 87

Step 6: Barcode Enabled Antigen Mapping (BEAM) Library Construction
6.0 Get Started 89
6.1 Sample Index PCR 90
6.2 Post Sample Index PCR Size Selection – SPRIselect 91
6.3 Post Library Construction QC 92

Step 7: Cell Surface Protein Library Construction
7.0 Get Started 94
7.1 Sample Index PCR 95
7.2 Post Sample Index PCR Size Selection – SPRIselect 96

Table of Contents

7.3 Post Library Construction QC 97

Step 8: Sequencing

Sequencing Libraries 99
Illumina Sequencer Compatibility 100
Sample Indices 100
Library Sequencing Depth & Run Parameters 100
Library Loading 101
Library Pooling 101
Data Analysis and Visualization 102

Troubleshooting

GEM Generation & Barcoding 104
Chromium Controller Errors 106

Appendix

Post Library Construction Quantification 109
LabChip Traces 110
Agilent TapeStation Traces 111
Oligonucleotide Sequences 112

Introduction

Chromium Next GEM Single Cell 5' Reagent Kits v2 (Dual Index)	8
10x Genomics Accessories	15
Recommended Thermal Cyclers	16
Additional Kits, Reagents & Equipment	17
Recommended Pipette Tips	18
Protocol Steps & Timing	20
Stepwise Objectives	21
BEAM Reagent Assembly, Sample Labeling & Sorting Guidelines	30

Chromium Next GEM Single Cell 5' Reagent Kits v2 (Dual Index)

Refer to SDS for handling and disposal information

Chromium Next GEM Single Cell 5' Kit v2, 16 rxns PN-1000263

Chromium Next GEM
Single Cell 5' GEM Kit v2
16 rxns, PN-1000244
Store at -20°C

		#	PN
●	RT Reagent B	1	2000165
●	RT Enzyme C	1	2000085
○	Reducing Agent B	1	2000087
●	Poly-dT RT Primer	1	2000007
●	Cleanup Buffer	2	2000088
○	Amp Mix	1	2000047
●	cDNA Primers	1	2000089

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Chromium Next GEM
Library Construction Kit
16 rxns, PN-1000190
Store at -20°C

		#	PN
●	Fragmentation Buffer	1	2000091
●	Fragmentation Enzyme	1	2000090
●	Ligation Buffer	1	2000092
●	DNA Ligase	1	220110
●	Adaptor Oligos	1	2000094
○	Amp Mix	1	2000047

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Chromium Next GEM
Single Cell 5' Gel Bead Kit v2
16 rxns, PN-1000264
Store at -80°C

	#	PN
Single Cell VDJ 5' Gel Bead	2	1000264

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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' Kit v2, 4 rxns PN-1000265

Chromium Next GEM
Single Cell 5' GEM Kit v2
4 rxns, PN-1000266
Store at -20°C

		#	PN
●	RT Reagent B	1	2000165
●	RT Enzyme C	1	2000102
○	Reducing Agent B	1	2000087
●	Poly-dT RT Primer	1	2000007
●	Cleanup Buffer	1	2000088
○	Amp Mix	1	2000103
●	cDNA Primers	1	2000089

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Chromium Next GEM
Library Construction Kit
4 rxns, PN-1000196
Store at -20°C

		#	PN
●	Fragmentation Buffer	1	2000091
●	Fragmentation Enzyme	1	2000104
●	Ligation Buffer	1	2000092
●	DNA Ligase	1	220131
●	Adaptor Oligos	1	2000094

10x GENOMICS

Chromium Next GEM
Single Cell 5' Gel Bead Kit v2
4 rxns, PN-1000267
Store at -80°C

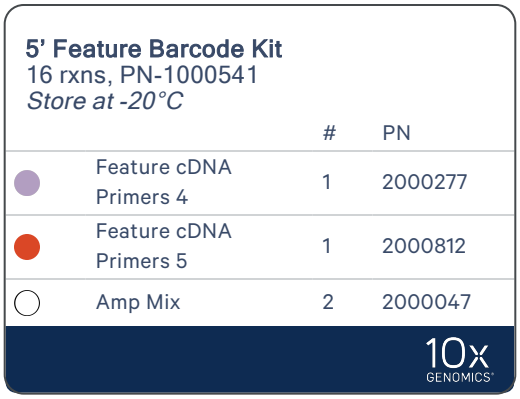
	#	PN
Single Cell VDJ 5' Gel Bead	1	1000267

10x GENOMICS

Dynabeads™ MyOne™ SILANE
PN-2000048
Store at 4°C

	#	PN
Dynabeads MyOne SILANE	1	2000048

5' Feature Barcode Kit, 16 rxns PN-1000541

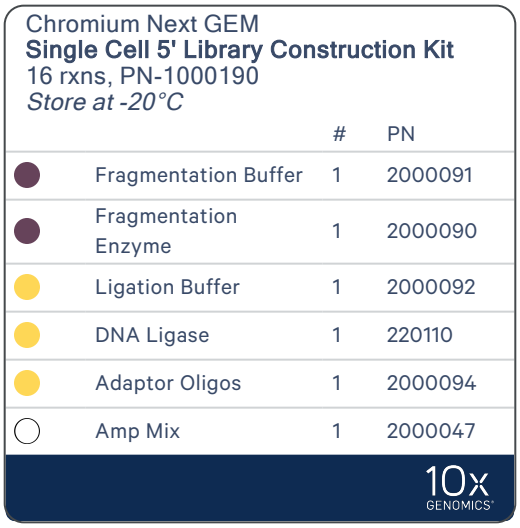


5' Feature Barcode Kit
16 rxns, PN-1000541
Store at -20°C

	#	PN
Feature cDNA Primers 4	1	2000277
Feature cDNA Primers 5	1	2000812
Amp Mix	2	2000047

10x GENOMICS

Library Construction Kit, 16 rxns PN-1000190



Chromium Next GEM
Single Cell 5' Library Construction Kit
16 rxns, PN-1000190
Store at -20°C

	#	PN
Fragmentation Buffer	1	2000091
Fragmentation Enzyme	1	2000090
Ligation Buffer	1	2000092
DNA Ligase	1	220110
Adaptor Oligos	1	2000094
Amp Mix	1	2000047

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Depending on the experimental goals, additional Library Construction Kits (PN-1000190) may be required. Refer to 10x Genomics support website for further guidance.

Chromium Single Cell V(D)J Amplification Kits, Human

Chromium
Single Cell Human TCR Amplification
16 rxns, PN-1000252
Store at -20°C

		#	PN
●	Human T Cell Mix 1 v2	1	2000242
●	Human T Cell Mix 2 v2	1	2000246
○	Amp Mix	2	2000047

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Chromium
Single Cell Human BCR Amplification
16 rxns, PN-1000253
Store at -20°C

		#	PN
●	Human B Cell Mix 1 v2	1	2000254
●	Human B Cell Mix 2 v2	1	2000255
○	Amp Mix	2	2000047

10x GENOMICS

Chromium Single Cell V(D)J Amplification Kits, Mouse

Chromium
Single Cell Mouse TCR Amplification
16 rxns, PN-1000254
Store at -20°C

		#	PN
●	Mouse T Cell Mix 1 v2	1	2000256
●	Mouse T Cell Mix 2 v2	1	2000257
○	Amp Mix	2	2000047

10x GENOMICS

Chromium
Single Cell Mouse BCR Amplification
16 rxns, PN-1000255
Store at -20°C

		#	PN
●	Mouse B Cell Mix 1 v2	1	2000258
●	Mouse B Cell Mix 2 v2	1	2000259
○	Amp Mix	2	2000047

10x GENOMICS

Chromium Single Cell 5' BEAM Core Kit, PE, Set A, 128 rxns PN-1000539

Chromium
Single Cell 5' BEAM Core Kit, PE, Set A
128 rxns, Module 1 PN-1000539
Store at -20°C

		#	PN
●	BEAM Conjugate 1, PE	1	2000774
●	BEAM Conjugate 2, PE	1	2000775
●	BEAM Conjugate 3, PE	1	2000776
●	BEAM Conjugate 4, PE	1	2000777
●	BEAM Conjugate 5, PE	1	2000778
●	BEAM Conjugate 6, PE	1	2000779
●	BEAM Conjugate 7, PE	1	2000780
●	BEAM Conjugate 8, PE	1	2000781
○	Quenching Reagent	1	2000790

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Chromium
Single Cell 5' BEAM Core Kit, PE, Set A
128 rxns, Module 2 PN-1000539
Store at -20°C

		#	PN
●	BEAM Conjugate 9, PE	1	2000782
●	BEAM Conjugate 10, PE	1	2000783
●	BEAM Conjugate 11, PE	1	2000784
●	BEAM Conjugate 12, PE	1	2000785
●	BEAM Conjugate 13, PE	1	2000786
●	BEAM Conjugate 14, PE	1	2000787
●	BEAM Conjugate 15, PE	1	2000788
●	BEAM Conjugate 16, PE	1	2000789
○	Quenching Reagent	1	2000790

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Cap colors for BEAM Conjugates 1-16 tubes may vary depending on the lot. Verify reagent using the part number.

Chromium Human MHC Class I A0201 Monomer Kit, 32 rxns PN-1000542

Chromium
Human MHC Class I A0201 Monomer Kit
32 rxns, PN-1000542
Store at -20°C

		#	PN
●	Monomer A0201	1	2000802
●	Negative Control Peptide A0201	1	2000803
○	Dilution Buffer	1	2000801

10x GENOMICS

Chromium, Human MHC Class I A1101 Monomer Kit, 32 rxns PN-1000543

Chromium
Human MHC Class I A1101 Monomer Kit
32 rxns, PN-1000543
Store at -20°C

		#	PN
●	Monomer A1101	1	2000804
●	Negative Control Peptide A1101	1	2000805
○	Dilution Buffer	1	2000801

10x GENOMICS

Chromium Human MHC Class I B0702 Monomer Kit, 32 rxns PN-1000544

Chromium
Human MHC Class I B0702 Monomer Kit
32 rxns, PN-1000544
Store at -20°C

		#	PN
●	Monomer B0702	1	2000806
●	Negative Control Peptide B0702	1	2000807
○	Dilution Buffer	1	2000801

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Chromium Human MHC Class I A2402 Monomer Kit, 32 rxns PN-1000545

Chromium
Human MHC Class I A2402 Monomer Kit
32 rxns, PN-1000545
Store at -20°C

		#	PN
●	Monomer A2402	1	2000808
●	Negative Control Peptide A2402	1	2000809
○	Dilution Buffer	1	2000801

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Chromium Mouse MHC Class I H2Kb Monomer Kit, 32 rxns PN-1000546

Chromium
Mouse MHC Class I H2Kb Monomer Kit
32 rxns, PN-1000546
Store at -20°C

		#	PN
●	Monomer H2Kb	1	2000810
●	Negative Control Peptide H2Kb	1	2000811
○	Dilution Buffer	1	2000801

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Chromium Next GEM Chip K Single Cell Kit, 48 rxns PN-1000286

Chromium
Partitioning Oil
Store at ambient temperature

	#	PN
Partitioning Oil	6	2000190

Chromium
Recovery Agent
Store at ambient temperature

		#	PN
○	Recovery Agent	6	220016

Chromium Next GEM
Chip K & Gaskets
Store at ambient temperature

	#	PN
Chromium Next GEM Chip K	6	2000182
Gasket, 6-pack	1	370017

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Chromium Next GEM Chip K Single Cell Kit, 16 rxns PN-1000287

Chromium
Partitioning Oil
Store at ambient temperature

	#	PN
Partitioning Oil	2	2000190

Chromium
Recovery Agent
Store at ambient temperature

		#	PN
○	Recovery Agent	2	220016

Chromium Next GEM
Chip K & Gaskets
Store at ambient temperature

	#	PN
Chromium Next GEM Chip K	2	2000182
Gasket, 2-pack	1	3000072

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Dual Index Kit TT Set A, 96 rxns PN-1000215

Dual Index Kit TT Set A
Store at -20°C

	#	PN
Dual Index Plate TT Set A	1	3000431

Dual Index Kit TN Set A, 96 rxns PN-1000250

Dual Index Kit TN Set A
Store at -20°C

	#	PN
Dual Index Plate TN Set A	1	3000510

Dual Index Kit TS Set A, 96 rxns PN-1000251

Dual Index Kit TS Set A
Store at -20°C

	#	PN
Dual Index Plate TS Set A	1	3000511

10x Genomics Accessories

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

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
Analytik Jena	Biometra TAdvanced 96 SG with 96 SG	846-x-070-241 *(x = 2 for 230 V; 4 for 115 V; 5 for 100 V, 50–60 Hz)*
Eppendorf	MasterCycler X50s	6311000010
	MasterCycler Pro *(discontinued)*	North America 950030010 International 6321 000.019
Thermo Fisher Scientific	Veriti 96-Well Thermal Cycler *(discontinued)*	4375786

For select instruments, ramp rates should be adjusted for all the steps as described below:

- Analytik Jena Biometra TAdvanced 96 SG: 2°C/sec for both heating and cooling
- Eppendorf MasterCycler X50s: 3°C/sec heating and 2°C/sec cooling

Additional Kits, Reagents & Equipment

The items in the table below have been tested by 10x Genomics and perform optimally with the assay. Substituting materials may adversely affect system performance. This list does not include standard laboratory equipment, such as water baths, pH meters, freezers, etc.

Item	Description	Supplier	Part Number (US)
Plastics			
1.5 ml tubes	DNA LoBind Tubes, 1.5 ml	Eppendorf	022431021
2.0 ml tubes	DNA LoBind Tubes, 2.0 ml	Eppendorf	022431048
0.2 ml PCR 8-tube strips *Choose either Eppendorf, USA Scientific, or Thermo Fisher Scientific PCR 8-tube strips*	PCR Tubes 0.2 ml 8-tube strips	Eppendorf	951010022
	TempAssure PCR 8-tube strip	USA Scientific	1402-4700
	MicroAmp 8-Tube Strip, 0.2 ml	Thermo Fisher Scientific	N8010580
	MicroAmp 8 -Cap Strip, clear	Thermo Fisher Scientific	N8010535
Kits & Reagents			
Nuclease-free water (not DEPC treated)	Nuclease-free Water	Thermo Fisher Scientific	AM9937
Ethanol	Ethyl alcohol, Pure (200 Proof, anhydrous)	Millipore Sigma	E7023-500ML
SPRIselect reagent	SPRIselect Reagent Kit	Beckman Coulter	B23318
Tween 20	10% Tween 20	Bio-Rad	1662404
50% glycerol	Glycerin (glycerol), 50% v/v Aqueous Solution	Ricca Chemical Company	3290-32
Buffer EB	Qiagen Buffer EB	Qiagen	19086
Equipment			
Vortex mixer	Vortex Mixer	VWR	10153-838
Reagent reservoirs	Divided Polystyrene Reservoirs	VWR	41428-958
Mini centrifuge	VWR Mini Centrifuge (or any equivalent mini centrifuge)	VWR	76269-064
Thermomixer	Eppendorf Thermomixer C	Eppendorf	5382000023
Heat block	Eppendorf SmartBlock 1.5 ml, Thermoblock for 24 reaction vessel or any equivalent temperature controlled heat block	Eppendorf	5360000038

Item	Description	Supplier	Part Number (US)
Quantification & Quality Control			
Choose Bioanalyzer, TapeStation, or LabChip based on availability & preference.			
Bioanalyzer & associated reagents	2100 Bioanalyzer Laptop Bundle (discontinued)	Agilent	G2943CA
	Replacement 2100 Bioanalyzer Instrument/2100 Expert Laptop Bundle	Agilent	G2939BA/G2953CA
	High Sensitivity DNA Kit	Agilent	5067-4626
TapeStation & associated reagents	4200 TapeStation	Agilent	G2991AA
	High Sensitivity D5000 ScreenTape/Reagents	Agilent	5067-5592/ 5067-5593
LabChip & associated reagents	LabChip GX Touch HT Nucleic Acid Analyzer	PerkinElmer	CLS137031
	DNA High Sensitivity Reagent Kit	PerkinElmer	CLS760672
Library quantification kit	KAPA Library Quantification Kit for Illumina Platforms	KAPA Biosystems	KK4824

Recommended Pipette Tips

10x Genomics recommends using only validated emulsion-safe pipette tips for all Single Cell protocols. Rainin pipette tips have been extensively validated by 10x Genomics and are highly recommended for all single cell assays. If Rainin tips are unavailable, any of the listed alternate pipette tips validated by 10x Genomics may be used.

Supplier	Description	Part Number (US)
Recommended Pipettes & Pipette tips		
Rainin	**Pipettes** Pipet-Lite Multi Pipette L8-50XLS+	17013804
	Pipet-Lite Multi Pipette L8-200XLS+	17013805
	Pipet-Lite Multi Pipette L8-10XLS+	17013802
	Pipet-Lite Multi Pipette L8-20XLS+	17013803
	Pipet-Lite LTS Pipette L-2XLS+	17014393
	Pipet-Lite LTS Pipette L-10XLS+	17014388
	Pipet-Lite LTS Pipette L-20XLS+	17014392
	Pipet-Lite LTS Pipette L-100XLS+	17014384

Supplier	Description	Part Number (US)
	Pipet-Lite LTS Pipette L-200XLS+	17014391
	Pipet-Lite LTS Pipette L-1000XLS+	17014382
	Pipette Tips Tips LTS 200UL Filter RT-L200FLR	30389240
	Tips LTS 1ML Filter RT-L1000FLR	30389213
	Tips LTS 20UL Filter RT-L10FLR	30389226
Alternate Recommendations *(If Rainin pipette tips are unavailable, any of the listed pipette tips may be used)*		
Eppendorf	**Pipettes** Eppendorf Research Plus, 8-channel, epT.I.P.S. Box, 0.5 – 10 µl	3125000010
	Eppendorf Research Plus, 8-channel, epT.I.P.S. Box, 10 – 100 µl	3125000036
	Eppendorf Research Plus, 8-channel, epT.I.P.S. Box, 30 – 300 µl	3125000052
	Eppendorf Research Plus, 1-channel, epT.I.P.S.® Box, 0.1 – 2.5 µl	3123000012
	Eppendorf Research Plus, 1-channel, epT.I.P.S.® Box, 0.5 – 10 µl	3123000020
	Eppendorf Research Plus, 1-channel, epT.I.P.S.® Box, 2 – 20 µl	3123000039
	Eppendorf Research Plus, 1-channel, epT.I.P.S.® Box, 2 – 200 µl	3123000055
	Eppendorf Research Plus, 1-channel, epT.I.P.S.® Box, 100 – 1000 µl	3123000063
	Pipette Tips (compatible with Eppendorf pipettes only) ep Dualfilter T.I.P.S., 2-20 µl	0030078535
	ep Dualfilter T.I.P.S., 2-200 µl	0030078551
	ep Dualfilter T.I.P.S., 2-1,000 µl	0030078578
Labcon*	ZAP SLIK 20 µL Low Retention Aerosol Filter Pipet Tips for Rainin LTS	4-1143-965-008
	ZAP SLIK 200 µL Low Retention Aerosol Filter Pipet Tips for Rainin LTS	4-1144-965-008
	ZAP SLIK 1200 µL Low Retention Aerosol Filter Pipet Tips for Rainin LTS	4-1145-965-008
Biotix*	xTIP4 Racked Pipette Tips, Rainin LTS Pipette Compatible, 0.1-20 µl	63300931
	xTIP4 Racked Pipette Tips, Rainin LTS Pipette Compatible, 200 µl	63300001
	xTIP4 Racked Pipette Tips, Rainin LTS Pipette Compatible, 1200 µl	63300004

*Compatible with Rainin pipettes

Protocol Steps & Timing

	Steps		Timing	Stop & Store
3 h	**Sample Preparation - Assembly, Labeling & Flow Sorting**		~1-2 day	
	Step 1 – GEM Generation & Barcoding			
	1.1	Prepare Reaction Mix	20 min	
	1.2	Load Chromium Next GEM Chip K	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 & cDNA Amplification**			
	2.1	Post GEM-RT Cleanup – Dynabead	45 min	
	2.2	cDNA Amplification	50 min STOP	4°C ≤72 h or –20°C ≤1 week
	2.3	cDNA Cleanup		
		2.3A Pellet Cleanup	15 min STOP	4°C ≤72 h or –20°C ≤4 weeks
		2.3 Supernatant Clenaup	20 min STOP	4°C ≤72 h or –20°C ≤4 weeks
	2.4	cDNA Quantification & QC	50 min	
	Step 3 – V(D)J Amplification from cDNA			
	3.1	V(D)J Amplification 1	40 min STOP	4°C ≤72 h
	3.2	Post V(D)J Amplification 1 Double Sided Size Selection – SPRIselect	20 min STOP	4°C ≤72 h or –20°C ≤1 week
	3.3	V(D)J Amplification 2	40 min STOP	4°C ≤72 h
	3.4	Post V(D)J Amplification 2 Double Sided Size Selection – SPRIselect	30 min STOP	4°C ≤72 h or –20°C ≤1 week
	3.5	Post V(D)J Amplification QC & Quantification	50 min	
8 h plus* *Time dependent on Stop options used and protocol steps executed	**Step 4 – V(D)J Library Construction**			
	4.1	Fragmentation, End Repair & A-tailing	45 min	
	4.2	Adaptor Ligation	25 min	
	4.3	Post Ligation Cleanup – SPRIselect	20 min	
	4.4	Sample Index PCR	40 min STOP	4°C ≤72 h
	4.5	Post Sample Index PCR Cleanup – SPRIselect	20 min STOP	4°C ≤72 h or –20°C long-term
	4.6	Post Library Construction QC	50 min	
	Step 5 – 5' Gene Expression (GEX) Library Construction**			
	5.1	GEX Fragmentation, End Repair & A-tailing	45 min	
	5.2	GEX Post Frag, End Repair & A-tailing Double Sided – SPRIselect	30 min	
	5.3	GEX Adaptor Ligation	25 min	
	5.4	GEX Post Ligation Cleanup – SPRIselect	20 min	
	5.5	GEX Sample Index PCR	40 min STOP	4°C ≤72 h
	5.6	GEX Post Sample Index PCR Double Sided Cleanup – SPRIselect	30 min STOP	4°C ≤72 h or –20°C long-term
	5.7	GEX Post Library Construction QC	50 min	
		** Sequence data from the 5' Gene Expression library is required for analysis in Cell Ranger, as it is used for cell calling.		
	Step 6 – BEAM Library Construction			
	6.1	Sample Index PCR	30 min	
	6.2	Post Sample Index PCR Size Selection – SPRIselect	20 min STOP	4°C ≤72 h or –20°C long-term
	6.3	Post Library Construction QC	50 min	
	Step 7 – 5' Cell Surface Protein Library Construction			
	7.1	Sample Index PCR	30 min	
	7.2	Post Sample Index PCR Size Selection – SPRIselect	20 min STOP	4°C ≤72 h or –20°C long-term
	7.3	Post Library Construction QC	50 min	

Stepwise Objectives

The Chromium Single Cell 5' v2 workflows with Feature Barcode technology for Barcode Enabled Antigen Mapping (BEAM) offer comprehensive, scalable solutions for measuring immune repertoire, gene expression, antigen specificity, and cell surface proteins from the same cell. Profile full-length (5' UTR to constant region), paired T-cell receptor (TCR), or B-cell receptor (BCR) transcripts, antigen specificity, and cell surface proteins from 500-10,000 individual cells per sample. This is accomplished by first assembling 10x Genomics BEAM Conjugates, each containing a streptavidin, a fluorophore molecule (Phycoerythrin, PE), and a Feature Barcode oligonucleotide, with appropriate antigens or peptides. The samples are then labeled with the assembled reagents (BEAM-Ab or BEAM-T Assembly) and flow sorted to collect antigen-specific B or T cells. The guidelines on reagent assembly, sample labeling, and cell sorting are provided in the Sample Prep User Guide (Document CG000595).

A pool of ~750,000 barcodes are sampled separately to index each cell's transcriptome and antigen specificity. It is done by partitioning thousands of cells into nanoliter-scale Gel Beads-in-emulsion (GEMs), where all generated cDNA/DNA (from polyadenylated mRNA, BEAM-Ab or BEAM-T Assembly Feature Barcode, and cell surface protein Feature Barcode) 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 protocols to generate the following libraries:

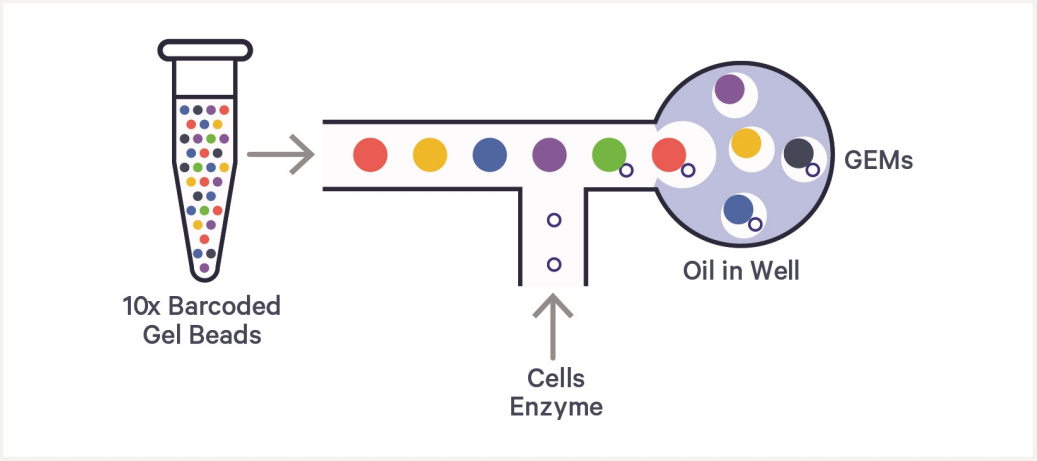
- Single Cell V(D)J libraries from V(D)J-amplified cDNA derived from poly-adenylated mRNA
- Single Cell 5' Gene Expression libraries from amplified cDNA derived from poly-adenylated mRNA
- Single Cell Barcode Enabled Antigen Mapping (BEAM) libraries
- Single Cell 5' Cell Surface Protein libraries

Sequence data from the 5' Gene Expression library is required for analysis in Cell Ranger, as it is used for cell calling.

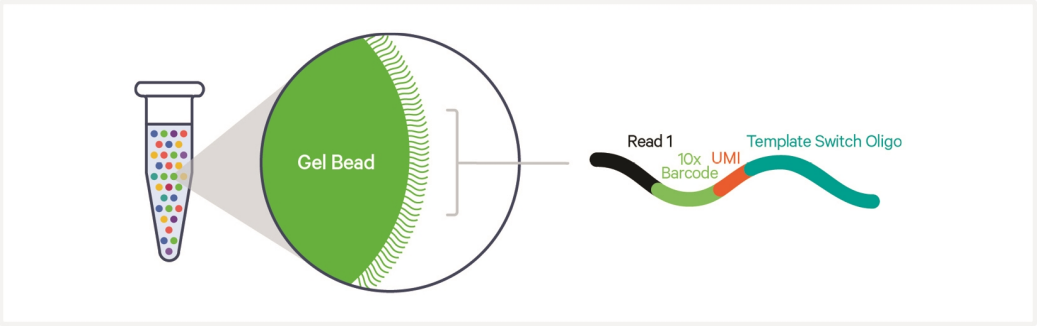
Step 1: GEM Generation & Barcoding

GEMs are generated by combining barcoded Single Cell VDJ 5' Gel Beads, a Master Mix with antigen-specific sorted cells (also labeled with cell surface protein), and Partitioning Oil onto Chromium Next GEM Chip K. To achieve single cell resolution, cells are delivered at a limiting dilution, such that the

majority (~90-99%) of generated GEMs contain no cell, while the remainders largely contain a single cell.



Immediately following GEM generation, the Gel Bead is dissolved and any co-partitioned cell is lysed. Gel Bead primers containing (i) an Illumina TruSeq Read 1 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) primers.



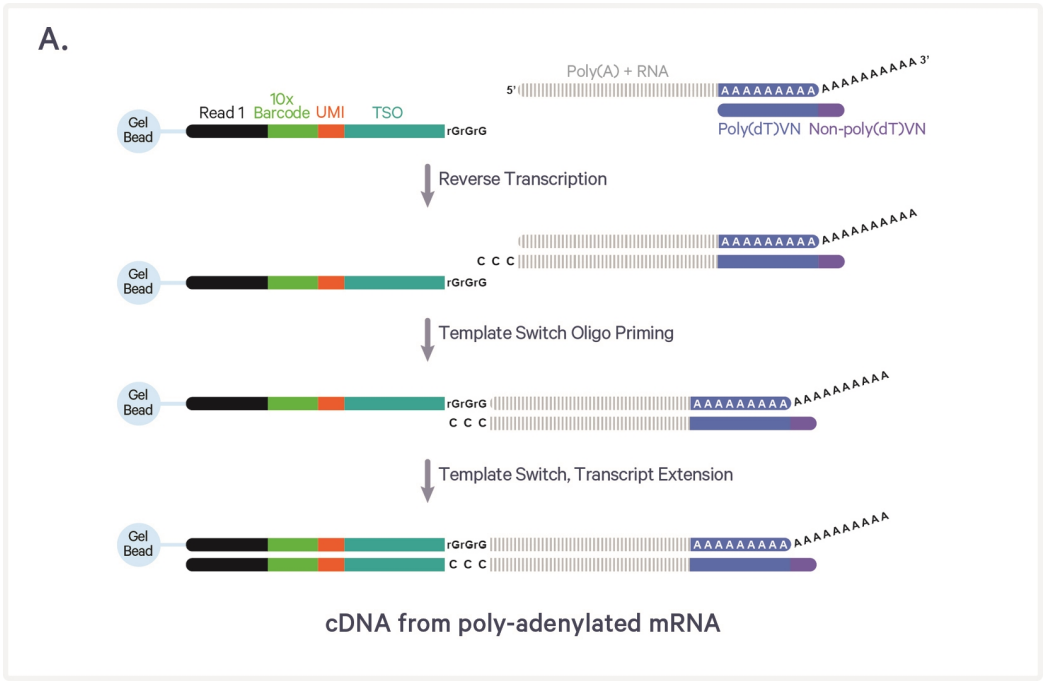
A. The cell lysate and the released Gel Bead primer incubated with the Master Mix containing RT reagents, produce 10x Barcoded, full-length cDNA from poly-adenylated mRNA.

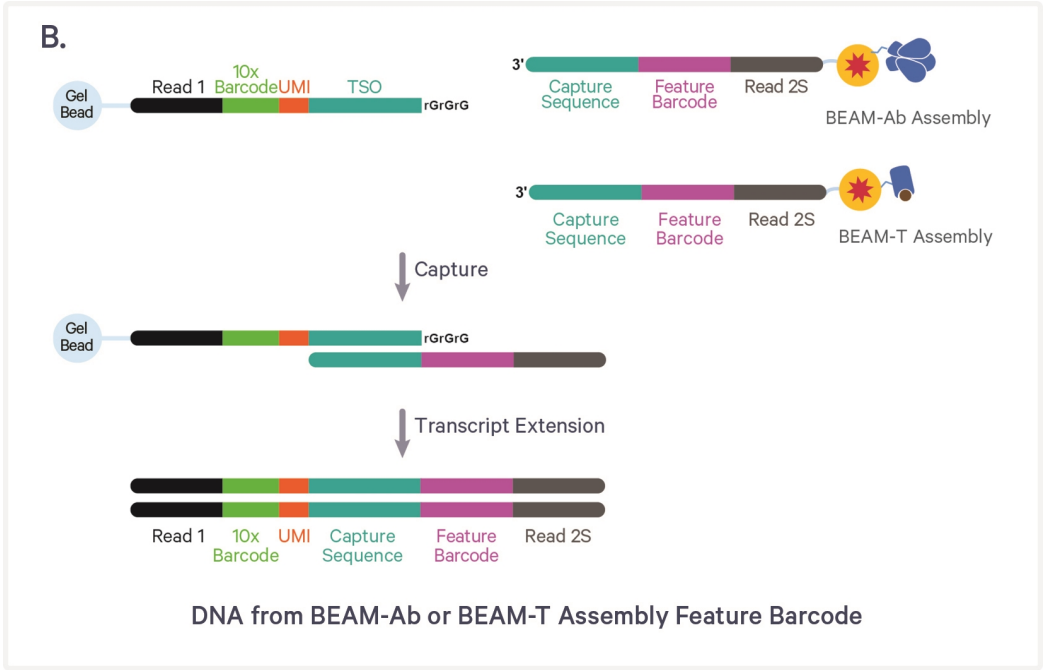
B. In the same partition, the Gel Bead primer captures the Feature Barcode conjugated to the BEAM-Ab or BEAM-T Assembly containing (i) a small RNA Read 2 (Read 2S), (ii) a 15 nt Feature Barcode, and (iii) Capture Sequence. Incubation of the GEMs with the Master Mix containing RT reagents, produces 10x Barcoded DNA from the BEAM assembly Feature Barcode.

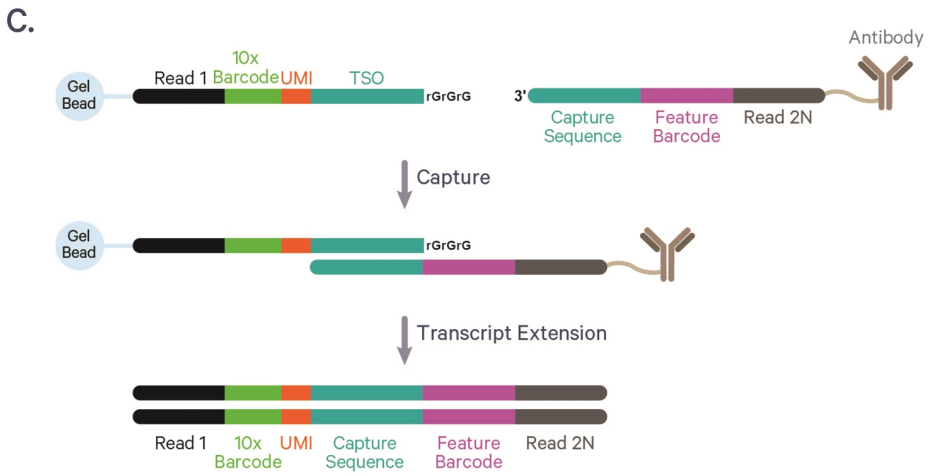
C. Simultaneously, in the same partition, the Gel Bead primer captures the cell surface protein Feature Barcode conjugated to the antibody containing (i) a Nextera Read 2 (Read 2N), (ii) a 15 nt Feature Barcode, and (iii) Capture Sequence. Incubation of the GEMs with the Master Mix containing RT

reagents, produces 10x Barcoded, DNA from the cell surface protein Feature Barcode.

Inside Individual GEMs





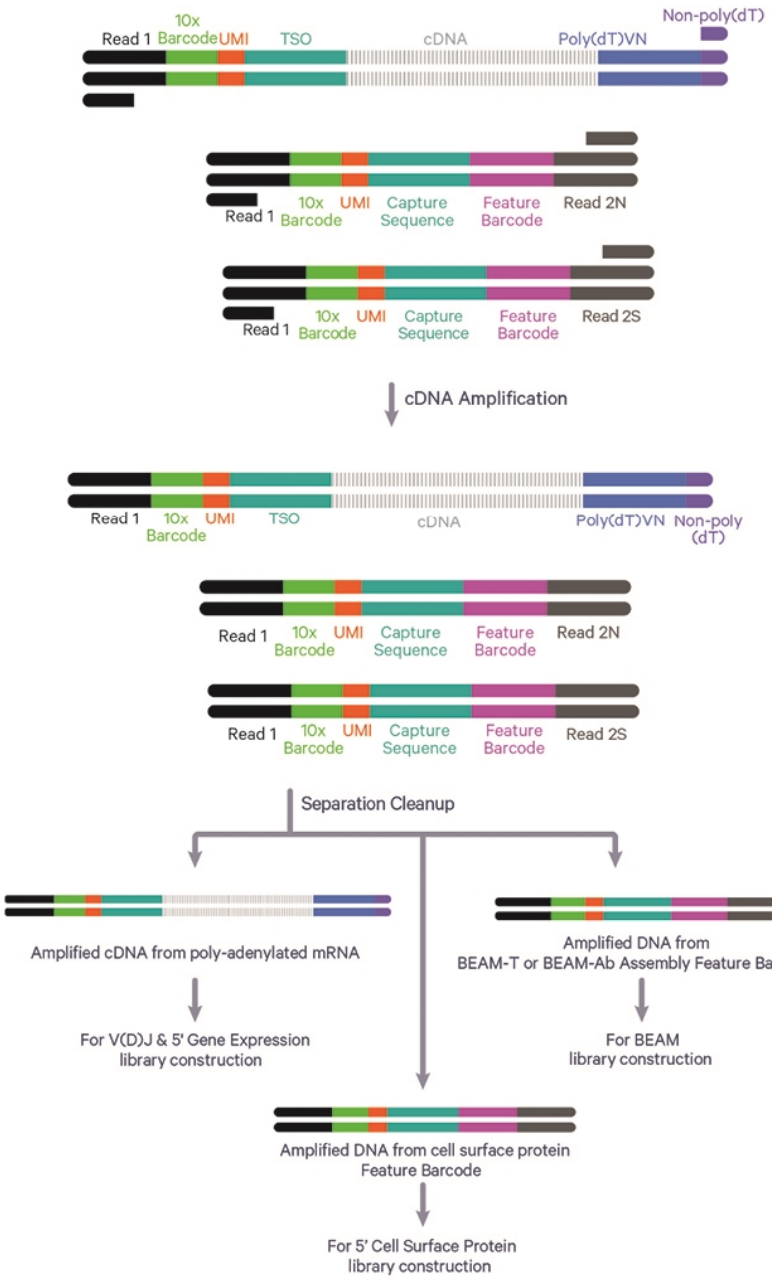


DNA from cell surface protein Feature Barcode

Step 2: Post GEM-RT Cleanup & cDNA Amplification

GEMs are broken and pooled after GEM-RT reaction mixtures are recovered. Silane magnetic beads are used to purify the 10x Barcoded first-strand cDNA from poly-adenylated mRNA, DNA from cell surface protein Feature Barcode, and DNA from BEAM-Ab or BEAM-T Assembly Feature Barcode from the post GEM-RT reaction mixture. 10x Barcoded, full-length cDNA from poly-adenylated mRNA and DNA from Feature Barcodes are amplified. Amplification generates sufficient material to construct multiple libraries from the same cells, e.g. T or B cell libraries, 5' Gene Expression libraries (step 5), Barcode Enabled Antigen Mapping libraries (step 6), and Cell Surface Protein libraries (step 7). The amplified cDNA from poly-adenylated mRNA and the amplified DNA from Feature Barcodes are separated by size selection. The amplified cDNA from mRNA is used for generating V(D)J and 5' Gene Expression libraries. The amplified DNA from BEAM assembly Feature Barcode is used for generating BEAM libraries and the amplified DNA from Cell Surface Protein Feature Barcode is used for generating Cell Surface Protein libraries.

Pooled cDNA Amplification



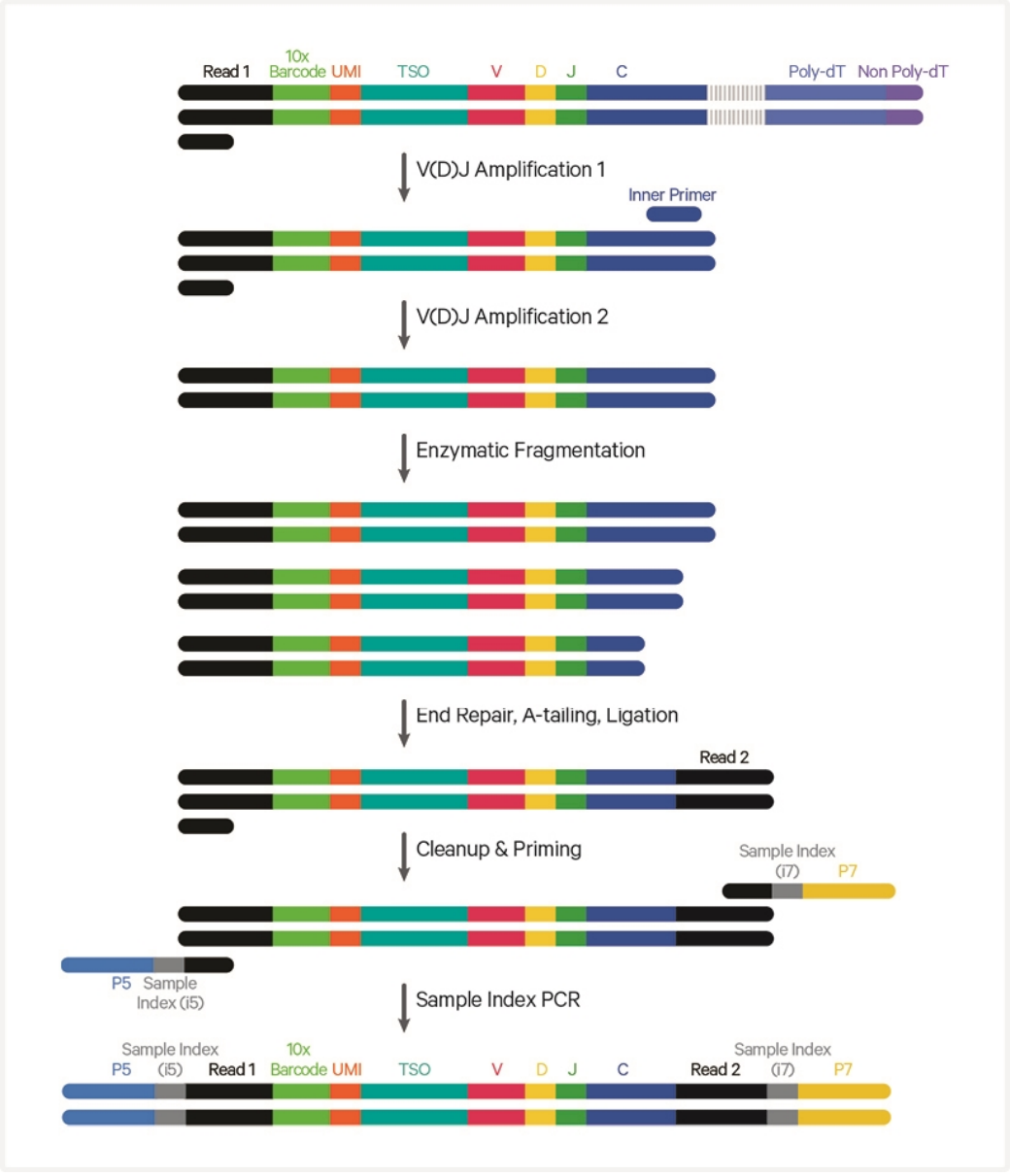
Step 3: V(D)J Amplification from cDNA

Amplified full-length cDNA from poly-adenylated mRNA is used to enrich full-length V(D)J segments (10x Barcoded) via PCR amplification with primers specific to either the TCR or BCR constant regions.

Step 4: V(D)J Library Construction

Enzymatic fragmentation and size selection are used to generate variable length fragments that collectively span the V(D)J segments of the amplified TCR or BCR transcripts prior to library construction. P5, P7, i5 and i7 sample indexes, and an Illumina R2 sequence (read 2 primer sequence) are added via End Repair, A-tailing, Adaptor Ligation, and Sample Index PCR. The final libraries contain the P5 and P7 priming sites used in Illumina sequencing.

Pooled Amplified cDNA Processed in Bulk



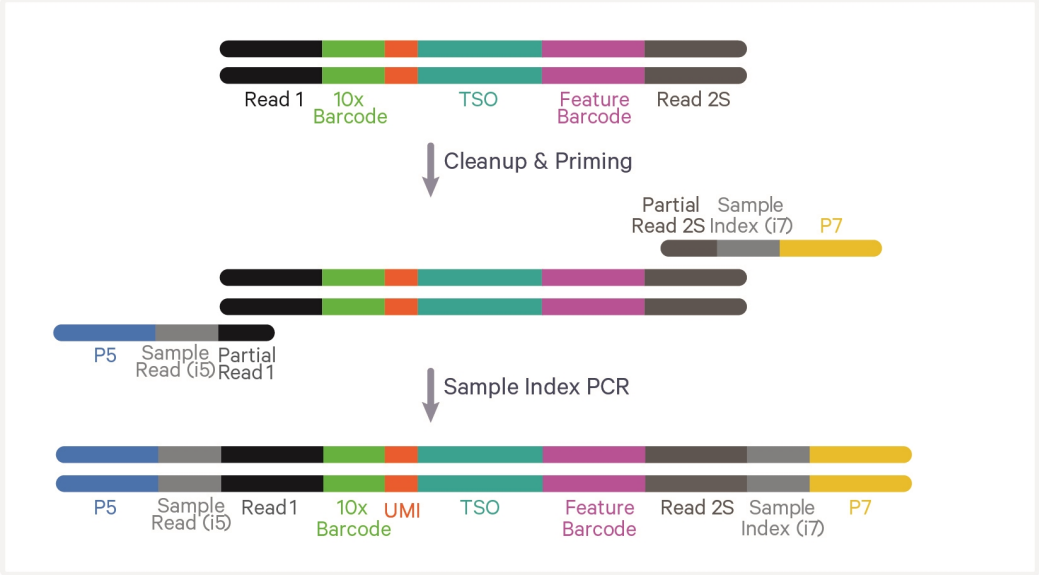
Step 5: 5' Gene Expression (GEX) Library Construction

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

Pooled Amplified cDNA Processed in Bulk

Step 6: BEAM Library Construction

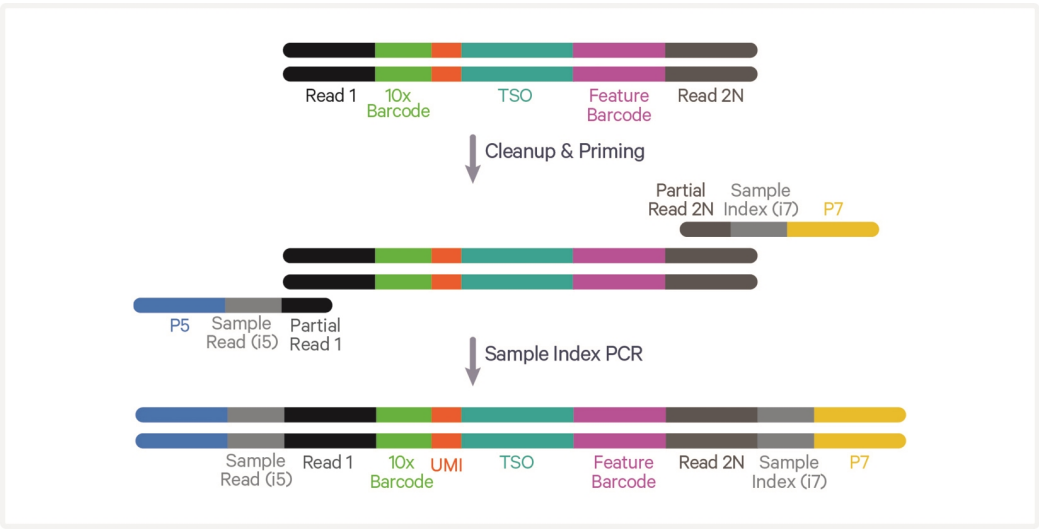
Amplified DNA from the BEAM assembly Feature Barcodes is used to construct the Barcode Enabled Antigen Mapping (BEAM) library. P5, P7, i5 and i7 sample indexes, and Small RNA Read 2 sequence (Read 2S primer sequence) are added via Sample Index PCR. The final libraries contain the P5 and P7 priming sites used in Illumina sequencers.



Step 7: Cell Surface Protein Library Construction

Amplified DNA from the cell surface protein Feature Barcodes derived from the antibody is used to construct the Cell Surface Protein library. P5, P7, i5 and i7 sample indexes, and Nextera Read 2 (Read 2N primer sequence) are added via Sample Index PCR. The final libraries contain the P5 and P7 priming sites used in Illumina sequencers.

Pooled Amplified DNA Processed in Bulk



Step 8: Sequencing

Illumina-ready dual index libraries can be sequenced at the recommended depth & run parameters. Illumina sequencer compatibility, sample indices,

library loading and pooling for sequencing are summarized in step 8.

Chromium Single Cell V(D)J Dual Index Library

Chromium Single Cell 5' Gene Expression Dual Index Library

Sequence data from the 5' Gene Expression library is required for analysis in Cell Ranger, as it is used for cell calling.

Chromium Single Cell Barcode Enabled Antigen Mappig (BEAM) Library

Chromium Single Cell 5' Cell Surface Protein Library

BEAM Reagent Assembly, Sample Labeling & Sorting Guidelines

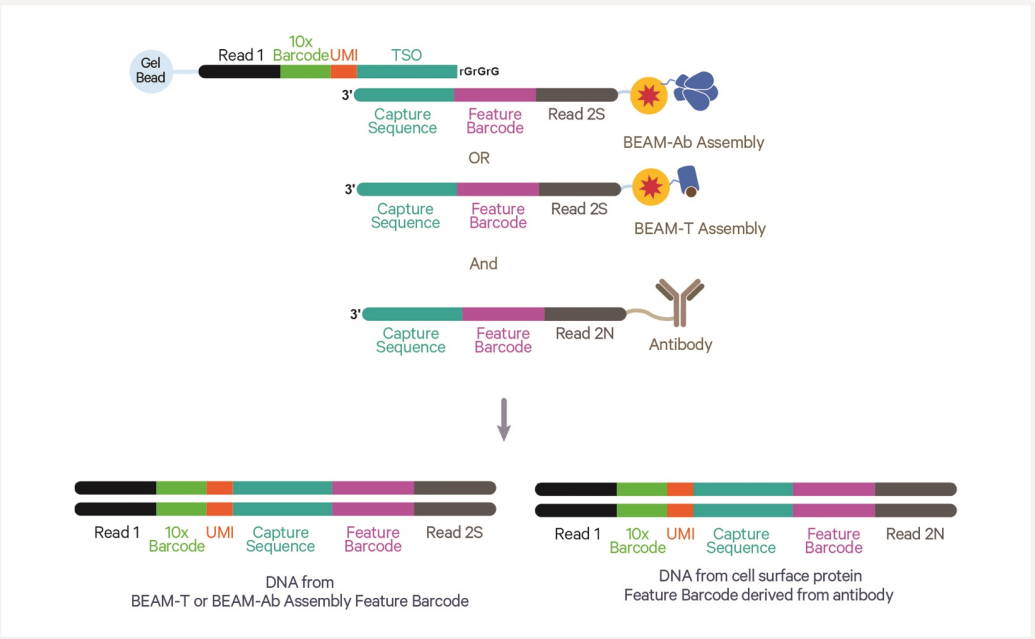
Overview

To generate BEAM and Cell Surface Protein libraries, the following sample preparation steps should be followed:

- Prepare BEAM-Ab or BEAM-T Assemblies.
- Prepare antibody-oligoncleotide conjugates
- Label samples with appropriate assembled reagents and antibody-oligonucelotide conjugates
- Sort labeled cells

Consult the Sample Prep User Guide Reagent Assembly, Sample Labeling & Flow Sorting for Barcode Enabled Antigen Mapping (BEAM) (Document CG000595) for details.

The Feature Barcode oligonucleotide conjugated to the BEAM-Ab or BEAM-T Assembly and bound to the BCR or TCR can be directly captured by the Gel Bead inside a GEM during GEM generation and amplified. The amplified DNA from the Feature Barcode can be used for BEAM Library Construction. Similarly, the Feature Barcode oligonucleotide conjugated to a specific protein binding molecule, such as an antibody for detecting cell surface protein expression can be directly captured by the Gel Bead inside a GEM during GEM generation and amplified. The amplified DNA generated from the Feature Barcode can be used for Cell Surface Protein Library Construction.



Tips & Best Practices

Icons

Tips & Best Practices section includes additional guidance

Signifies critical step requiring accurate execution

Troubleshooting section includes additional guidance

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 ~1,700 cells per reaction, resulting in recovery of ~1,000 cells, and a multiplet rate of ~0.8%. The optimal input cell concentration is 700-1,200 cells/µl.
- The presence of dead cells and debris in the suspension may also reduce the recovery rate. Consult the 10x Genomics Single Cell Protocols Cell Preparation Guide and Guidelines for Optimal Sample Preparation flowchart (Documents CG00053 and CG000126 respectively) for more information on preparing cells.

Multiplet Rate (%)	# of Cells Loaded	# of Cells Recovered
~0.4%	~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.
- 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.

  OR

- Prepare 50% glycerol solution:
  - Mix an equal volume of water and ≥99% Glycerol, Molecular Biology Grade.
  - Filter through a 0.2 µm filter.
  - 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 Secondary Holders

- 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 of the holder together to unlock the

lid and return the holder to a flat position.

Chromium Next GEM Chip Handling

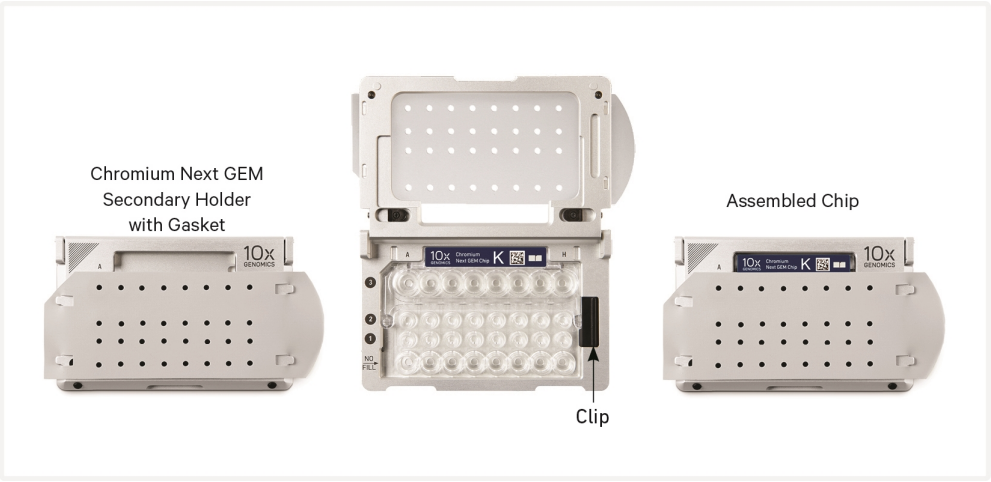
- Minimize exposure of reagents, chips, and gaskets to sources of particles and fibers, laboratory wipes, frequently opened flip-cap tubes, clothing that sheds fibers, and dusty surfaces.
- After removing the chip from the sealed bag, use in **≤24 h**.
- Execute steps without pause or delay, unless indicated. When using multiple chips, 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 or Chromium X.
- 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 Chip & Holder Assembly with Gasket

- Close the holder lid. Attach the gasket by holding the tongue (curved end, to the right) and hook the gasket 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.
- Open the chip holder.
- Align notch on the chip (upper left corner) and the open holder with the gasket attached.
- Slide the chip to the left until the chip is inserted under the guide on the holder.
- Depress the right hand side of the chip until the spring-loaded clip engages.
- Keep the assembled unit with the attached gasket until ready for dispensing reagents.



Chromium Next GEM Chip Loading

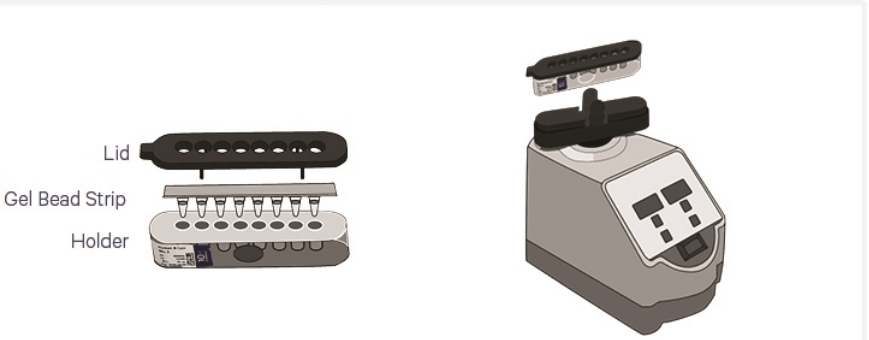
- Place the assembled chip and holder flat (gasket attached) on the bench with the lid open.
- Dispense at the bottom of the wells without introducing bubbles.
- When dispensing Gel Beads into the chip, wait for the remainder to drain into the bottom of the pipette tips and dispense again to ensure complete transfer.
- Refer to 1.2 Load Chromium Next GEM Chip K on page 45 for specific instructions.

Gel Bead Handling

- Use one tube of Gel Beads per sample. DO NOT puncture the foil seals of tubes not used at the time.
- After removing the Gel Bead strip from the packaging, equilibrate the Gel Bead strip to **room temperature** before use.
- Store unused Gel Beads at **−80°C** and avoid more than 12 freeze-thaw cycles.

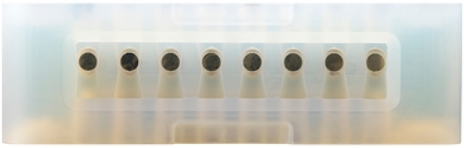
DO NOT store Gel Beads at **−20°C**.

- 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 tubes and the liquid levels look even. Place 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 Magnetic Separator

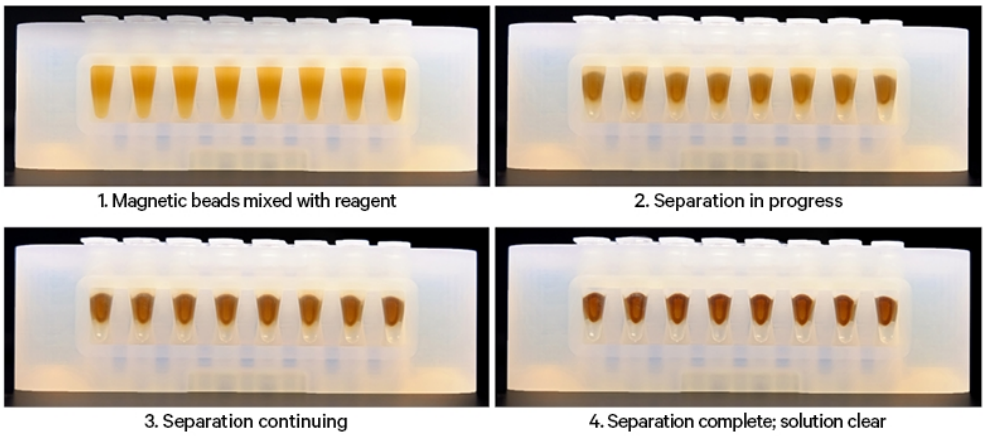
- Offers two positions of the magnets (high and low) relative to a tube, depending on its orientation. Flip the magnetic separator over to switch between high (magnet•**High**) or low (magnet•**Low**) positions.
- If using MicroAmp 8-Tube Strips, use the high position (magnet•**High**) only throughout 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 panel below for an example.
- The time needed for the solution to clear may vary based on specific step, reagents, volume of reagents etc.

Visually Confirm Clearing of Magnetic Bead Solution



SPRIselect Cleanup & Size Selection

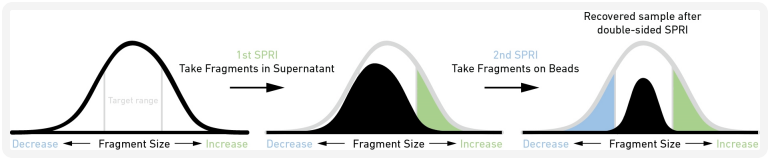
- After aspirating the desired volume of SPRIselect reagent, examine the pipette tips before dispensing to ensure the correct volume is transferred.
- Pipette mix thoroughly as insufficient mixing of sample and SPRIselect reagent will lead to inconsistent results.
- Use fresh preparations of 80% Ethanol.

Tutorial — SPRIselect Reagent:DNA Sample Ratios

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

$$\text{Example Ratio: } \frac{\text{= Volume of SPRIselect reagent added to the sample}}{\text{Volume of DNA sample}} \frac{\text{= 50 µl}}{\text{100 µl}} = \textbf{0.5X}$$

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

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

$$\text{Ratio: } \frac{\text{= Volume of SPRIselect reagent added to the sample}}{\text{Volume of DNA sample}} \frac{\text{= 50 µl}}{\text{100 µl}} = 0.5\text{X}$$

2. Second SPRIselect: Add **30 µl** SPRIselect reagent to supernatant from step 1 (**0.8X**).

$$\text{Ratio: } \frac{\text{= Total Volume of reagent added to the sample (step a + b)}}{\text{Original Volume of DNA sample}} \frac{\text{= 50 µl + 30 µl}}{\text{100 µl}} = \textbf{0.8X}$$

cDNA Amplification PCR Cycle Numbers

Follow cycle number recommendations for high and low RNA content cells based on Targeted Cell Recovery and cell sample.

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

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 Dual Index Plate contains a unique i7 and a unique i5 oligonucleotide.
- Use ONLY Dual Index Plate TT, Set A for V(D)J and 5' Gene Expression libraries.

  Use ONLY Dual Index Plate TS, Set A for BEAM library. Use ONLY Dual Index Plate TN, Set A for Cell Surface Protein library.
- Consider sample index compatibility when pooling different libraries; a unique sample index for each of the pooled libraries is required.
- The sample indices of Dual Index Plate TT, Set A are unique from those of Dual Index Plate TS, Set A, and Dual Index Plate TN, Set A. Therefore, respective libraries from these plates may be pooled.

Index Hopping Mitigation

Index hopping can impact pooled samples sequenced on Illumina sequencing platforms that utilize patterned flow cells and exclusion amplification chemistry. To minimize index hopping, follow the guidelines listed below.

- Remove adapters during cleanup steps.
- Ensure no leftover primers and/or adapters are present when performing post-Library Construction QC.
- Store each library individually at **4°C** for up to **72 h** or at **−20°C** for **long-term** storage. DO NOT pool libraries during storage.
- Pool libraries prior to sequencing. An additional 1.0X SPRI may be performed for the pooled libraries to remove any free adapters before sequencing.
- Hopped indices can be computationally removed from the data generated from single cell dual index libraries.

Step 1:

GEM Generation and Barcoding

1.0 Get Started 41

1.1 Prepare Master Mix 42

Assemble Chromium Next GEM Chip K 42

1.2 Load Chromium Next GEM Chip K 45

1.3 Run the Chromium Controller or X/iX 47

1.4 Transfer GEMs 49

1.5 GEM-RT Incubation 50

1.0 Get Started

Firmware Version 4.0 or higher is required in the Chromium Controller or the Chromium Single Cell Controller used for this protocol.

Action	Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature				
☐	**Single Cell VDJ 5' Gel Beads**	1000264/1000267	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
☐ ○	**Reducing Agent B**	2000087	Vortex, verify no precipitate, centrifuge briefly.	−20°C
Place on Ice				
☐ ●	**RT Enzyme C**	2000085/2000102	Centrifuge briefly before adding to the mix.	−20°C
☐ ⚠	**Labeled & Sorted Cell Suspension**	—	Refer to Sample Prep User Guide (CG000595)	—
Obtain				
☐	**Partitioning Oil**	2000190	—	Ambient
☐ ⚠	**Chromium Next GEM Chip K** *Verify name and PN*	2000182	See Tips & Best Practices.	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.	—

1.1 Prepare Master Mix

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	7.3	32.1	64.2
○	**Reducing Agent B**	2000087	1.9	8.4	16.7
●	**RT Enzyme C**	2000085/ 2000102	8.3	36.5	73.0
	Total	–	**36.3**	**159.7**	**319.3**

b. Add **36.3 µl** Master Mix into each tube of a PCR 8-tube strip on ice.

Assemble Chromium Next GEM Chip K

TIPS See Tips & Best Practices on page 31 for chip handling instructions.

a. Close the holder lid.

b. Attach the gasket by holding the tongue (curved end, to the right) and hook the gasket on the left-hand tabs of the holder. Gently pull the gasket toward the right and hook it on the two right-hand tabs.

c. DO NOT touch the smooth side of the gasket.

d. Open the chip holder.

e. Remove the chip from the sealed bag. Use the chip within ≤ **24 h**.

f. Align notch on the chip (upper left corner) and the open holder with the gasket attached.

g. Slide the chip to the left until the guide on the holder is inserted into the chip. Depress the right hand side of the chip until the spring-loaded clip engages.

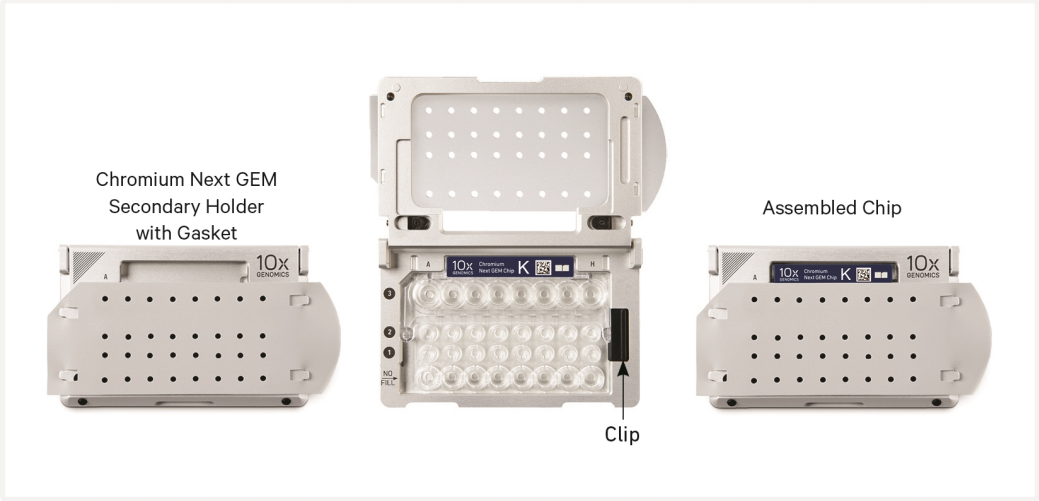
h. Keep the assembled unit with the attached gasket open until ready for and while dispensing reagents into the wells.

i. DO NOT touch the smooth side of the gasket.

j. After loading reagents, close the chip holder. DO NOT press down on the top of the gasket.

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.



Cell Suspension Volume Calculator Table

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

DO NOT add nuclease-free water directly to single cell suspension. Add nuclease-free water to the Master Mix.

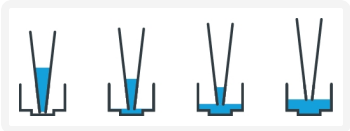
Cell Stock Concentration (Cells/µl)	Targeted Cell Recovery										
	500	1000	2000	3000	4000	5000	6000	7000	8000	9000	10000
100	8.3 30.4	16.5 22.2	33.0 5.7	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
200	4.1 34.6	8.3 30.4	16.5 22.2	24.8 13.9	33.0 5.7	n/a	n/a	n/a	n/a	n/a	n/a
300	2.8 35.9	5.5 33.2	11.0 27.7	16.5 22.2	22.0 16.7	27.5 11.2	33.0 5.7	n/a	n/a	n/a	n/a
400	2.1 36.6	4.1 34.6	8.3 30.5	12.4 26.3	16.5 22.2	20.6 18.1	24.8 13.9	28.9 9.8	33.0 5.7	n/a	n/a
500	1.7 37.0	3.3 35.4	6.6 32.1	9.9 28.8	13.2 25.5	16.5 22.2	19.8 18.9	23.1 15.6	26.4 12.3	29.7 9.0	33.0 5.7
600	1.4 37.3	2.8 35.9	5.5 33.2	8.3 30.5	11.0 27.7	13.8 24.9	16.5 22.2	19.3 19.4	22.0 16.7	24.8 13.9	27.5 11.2
700	1.2 37.5	2.4 36.3	4.7 34.0	7.1 31.6	9.4 29.3	11.8 26.9	14.1 24.6	16.5 22.2	18.9 19.8	21.2 17.5	23.6 15.1
800	1.0 37.7	2.1 36.6	4.1 34.6	6.2 32.5	8.3 30.4	10.3 28.4	12.4 26.3	14.4 24.3	16.5 22.2	18.6 20.1	20.6 18.1
900	0.9 37.8	1.8 36.9	3.7 35.0	5.5 33.2	7.3 31.4	9.2 29.5	11.0 27.7	12.8 25.9	14.7 24.0	16.5 22.2	18.3 20.4
1000	0.8 37.9	1.7 37.0	3.3 35.4	5.0 33.7	6.6 32.1	8.3 30.4	9.9 28.8	11.6 27.1	13.2 25.5	14.9 23.8	16.5 22.2
1100	0.8 37.9	1.5 37.2	3.0 35.7	4.5 34.2	6.0 32.7	7.5 31.2	9.0 29.7	10.5 28.2	12.0 26.7	13.5 25.2	15.0 23.7
1200	0.7 38.0	1.4 37.3	2.8 35.9	4.1 34.6	5.5 33.2	6.9 31.8	8.3 30.4	9.6 29.1	11.0 27.7	12.4 26.3	13.8 24.9
1300	0.6 38.1	1.3 37.4	2.5 36.2	3.8 34.9	5.1 33.6	6.3 32.4	7.6 31.1	8.9 29.8	10.2 28.5	11.4 27.3	12.7 26.0
1400	0.6 38.1	1.2 37.5	2.4 36.3	3.5 35.2	4.7 34.0	5.9 32.8	7.1 31.6	8.3 30.4	9.4 29.3	10.6 28.1	11.8 26.9
1500	0.6 38.1	1.1 37.6	2.2 36.5	3.3 35.4	4.4 34.3	5.5 33.2	6.6 32.1	7.7 31.0	8.8 29.9	9.9 28.8	11.0 27.7
1600	0.5 38.2	1.0 37.7	2.1 36.6	3.1 35.6	4.1 34.6	5.2 33.5	6.2 32.5	7.2 31.5	8.3 30.4	9.3 29.4	10.3 28.4
1700	0.5 38.2	1.0 37.7	1.9 36.8	2.9 35.8	3.9 34.8	4.9 33.8	5.8 32.9	6.8 31.9	7.8 30.9	8.7 30.0	9.7 29.0
1800	0.5 38.2	0.9 37.8	1.8 36.9	2.8 35.9	3.7 35.0	4.6 34.1	5.5 33.2	6.4 32.3	7.3 31.4	8.3 30.5	9.2 29.5
1900	0.4 38.3	0.9 37.8	1.7 37.0	2.6 36.1	3.5 35.2	4.3 34.4	5.2 33.5	6.1 32.6	6.9 31.8	7.8 30.9	8.7 30.0
2000	0.4 38.3	0.8 37.9	1.7 37.0	2.5 36.2	3.3 35.4	4.1 34.6	5.0 33.7	5.8 32.9	6.6 32.1	7.4 31.3	8.3 30.4

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 K

TIPS

- After removing chip from the sealed bag, use in **≤24 h**.
- Open the lid (gasket attached) of the assembled chip and lay flat for loading.
- When loading the chip, 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.

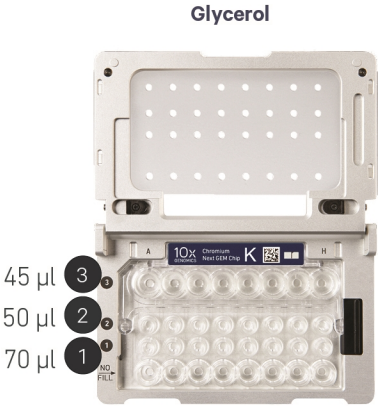


a. Add 50% glycerol solution to each unused well

(if loading less than 8 samples/chip)

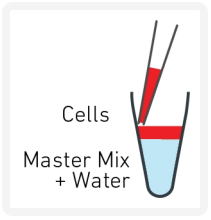
- **70 µl** in each unused well in row labeled 1
- **50 µl** in each unused well in row labeled 2
- **45 µl** in each unused well in row labeled 3

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



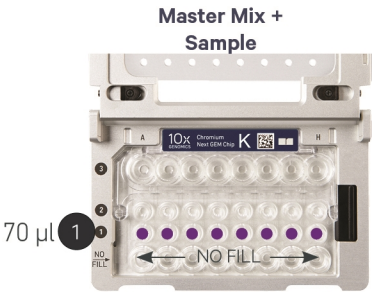
b. Prepare Master Mix + Cell suspension

- Refer to the Cell Suspension Volume Calculator Table.
- Add the appropriate volume of nuclease-free water to Master Mix.
- Add corresponding volume of single cell suspension to Master Mix. *Total of 75 µl in each tube.*
  Gently pipette mix the cell suspension before adding to the Master Mix.



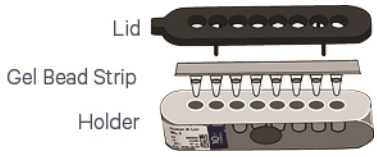
c. Load Row Labeled 1

- Gently pipette mix the Master Mix + Cell Suspension.
- 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.



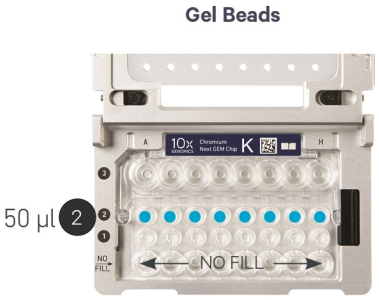
d. Prepare Gel Beads

- 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 & the liquid levels are even.
- Place the Gel Bead strip back in the holder. Secure the holder lid.



e. Load Row Labeled 2

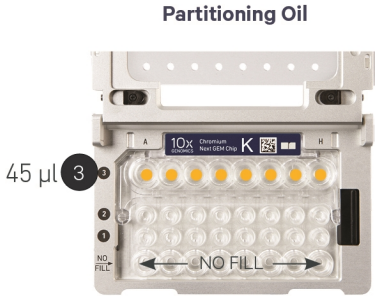
- Puncture the foil seal of the Gel Bead tubes.
- Slowly aspirate **50 µl** Gel Beads.
- Dispense into the wells in **row labeled 2** without introducing bubbles.
- Wait **30 sec**.



f. Load Row Labeled 3

- Dispense **45 µl** Partitioning Oil into the wells in **row labeled 3** from a reagent reservoir.

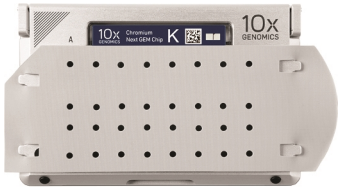
Failure to add Partitioning Oil to the row labeled 3 will prevent GEM generation and can damage the instrument.



g. Prepare for Run

- Close the lid (gasket already attached). DO NOT touch the smooth side of the gasket. DO NOT press down on the top of the gasket. Keep horizontal to avoid wetting the gasket.

*Run the chip in Chromium Controller or X/iX **immediately** after loading the Partitioning Oil.*



1.3 Run the Chromium Controller or X/iX

Firmware Version 4.0 or higher is required in the Chromium Controller or the Chromium Single Cell Controller used for this protocol.

If using Chromium Controller

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

Chromium Controller

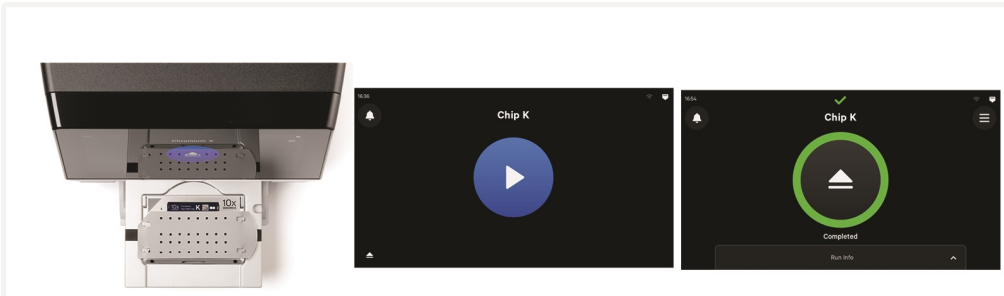
b. Place the assembled chip with the gasket in the tray, ensuring that the chip stays horizontal. Press the button to retract the tray.

c. Press the play button.

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

If Using Chromium X/iX

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



a. Press the eject button on the Chromium X 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

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. Visually compare the remaining volume in rows labeled 1-2. Abnormally high volume in one well relative to other wells may indicate 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 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**.

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** *hh:mm:ss*
1	53°C	00:45:00
2	85°C	00:05:00
3	4°C	Hold

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 & cDNA Amplification

2.0 Get Started 52

2.1 Post GEM-RT Cleanup – Dynabeads 53

Step Overview (steps 2.2 & 2.3) 56

2.2 cDNA Amplification 57

2.3 cDNA Cleanup – SPRIselect 58

2.3A Pellet Cleanup for V(D)J & 5' Gene Expression Library 59

2.3B Transferred Supernatant Cleanup for Cell Surface Protein and BEAM Library 59

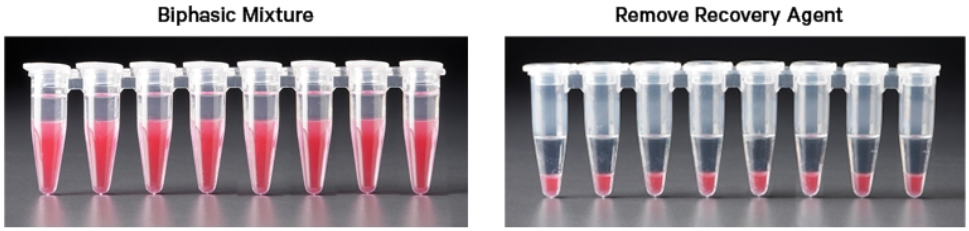
2.4 Post cDNA Amplification QC & Quantification 60

2.0 Get Started

Action	Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature				
☐ ◯	**Reducing Agent B**	2000087	Thaw, vortex, verify no precipitate, centrifuge briefly.	−20°C
☐	**Feature cDNA Primers 5** *Verify name & PN*	2000812	Thaw, vortex, 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
☐	**Beckman Coulter SPRIselect Reagent**	—	Manufacturer's recommendations.	—
☐	**Agilent Bioanalyzer High Sensitivity Kit** *If used for QC & quantification*	—	Manufacturer's recommendations.	—
☐	**Agilent TapeStation ScreenTape & Reagents** *If used for QC & quantification*	—	Manufacturer's recommendations.	—
☐	**Qubit dsDNA HS Assay Kit** *If used for QC & quantification*	—	Manufacturer's recommendations.	—
Place on Ice				
☐ ◯	**Amp Mix**	2000047 /2000103	Vortex, centrifuge briefly.	−20°C
Thaw at 65°C				
☐	**Cleanup Buffer**	2000088	Thaw for 10 min at 65°C at max speed on a thermomixer. Verify no visible crystals. Cool to room temperature.	−20°C
Obtain				
☐ ◯	**Recovery Agent**	220016	—	Ambient

Action	Item	10x PN	Preparation & Handling	Storage
☐	**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.	Ambient

2.1 Post GEM-RT Cleanup – Dynabeads



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

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.

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.

Before using Dynabeads MyOne SILANE to prepare the Dynabeads Cleanup Mix:

Vortex the Dynabeads thoroughly (≥**30 sec**) **immediately** before adding to the mix.

- Aspirate full liquid volume in the Dynabead tube with a pipette tip to verify that beads have not settled in the bottom of the tube. If clumps are present, pipette mix to resuspend completely. DO NOT centrifuge before adding to the 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
⬤	**Cleanup Buffer**	2000088	182	801	1602
	Dynabeads MyOne SILANE	2000048	8	35	70
◯	**Reducing Agent B**	2000087	5	22	44
	Total		**200**	**880**	**1760**

d. Vortex and add **200 µl** to each tube. Pipette mix 10x (pipette set to 200 µl).

e. Incubate **10 min** at **room temperature** (keep caps open).

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
◯	**Reducing Agent B**	2000087	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 10x Magnetic Separator•**Low** position (magnet•**Low)**.

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

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

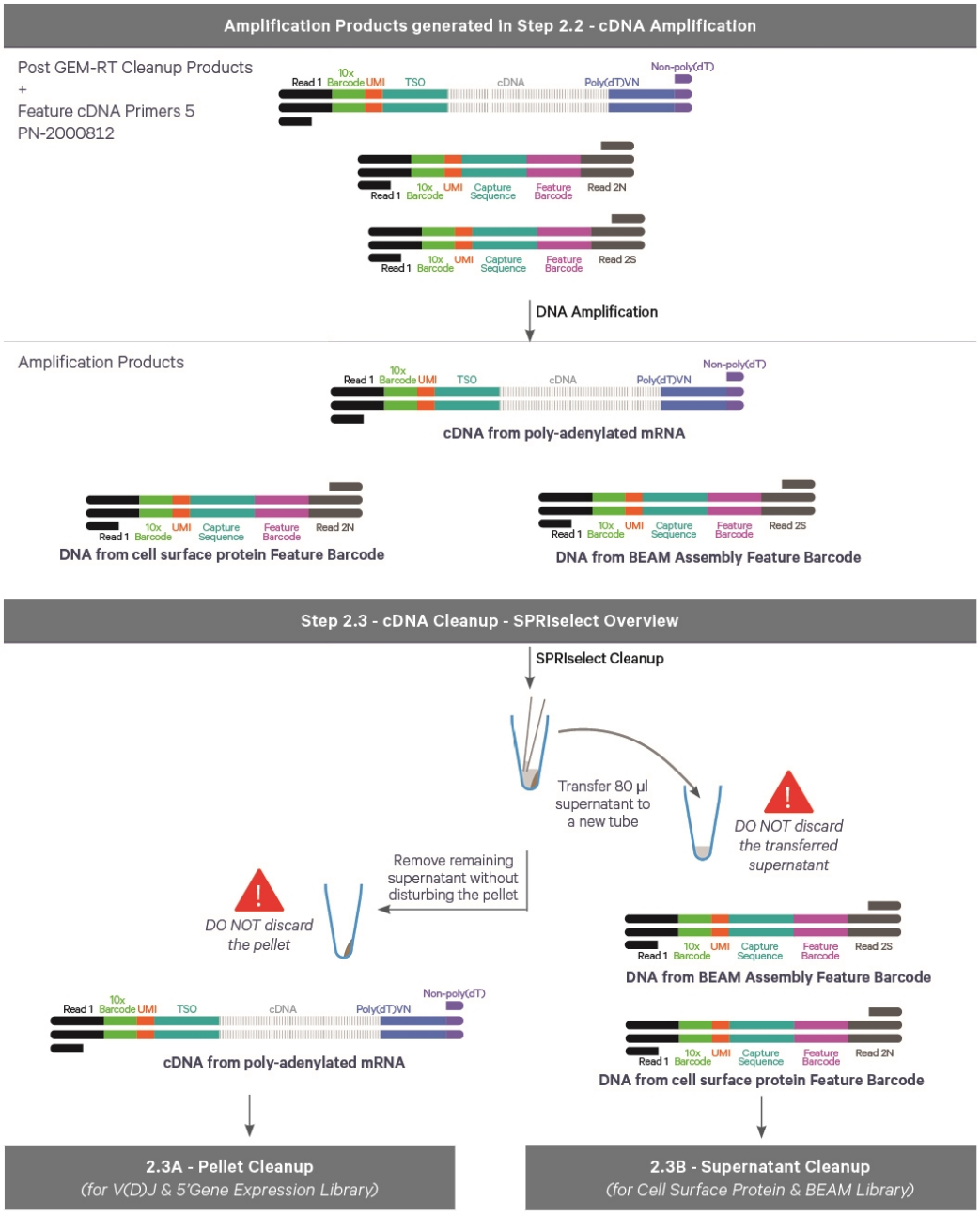
p. Pipette mix (pipette set to 30 µl) without introducing bubbles. Pipette mix 15x. If beads still appear clumpy, continue pipette mixing until fully resuspended.

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 Overview (steps 2.2 & 2.3)



2.2 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)**
○	**Amp Mix** *Retrieve from Single Cell 5' GEM Kit*	2000047/ 2000103	50	220	440
●	**Feature cDNA Primers 5** *Verify name & PN*	2000812	15	66	132
	Total		**65**	**286**	**572**

b. Add **65 µl** cDNA Amplification Reaction Mix to **35 µl** sample (Post-GEM-RT Cleanup, step 2.1s)

c. 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**
105°C	100 µl	~25-50 min
Step	**Temperature**	**Time** *hh:mm:ss*
1	98°C	00:00:45
2	98°C	00:00:20
3	63°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 **Recommended starting point for cycle number optimization.** The optimal cycle number is a trade-off between generating sufficient final mass for libraries & minimizing PCR amplification artifacts.

Targeted Cell Recovery	**Total Cycles Low RNA Content** *(e.g. Primary Cells)*	**Total Cycles High RNA Content** *(e.g. Cell Lines)*
500–2,000	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 **–20°C** for **≤1 week**, or proceed to the next

step.

2.3 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 140 µl).

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

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

d. Transfer and save **80 µl** supernatant in a new tube strip without disturbing the pellet. Maintain at **room temperature**. DO NOT discard the transferred supernatant (cleanup for Cell Surface Protein and BEAM library construction; step 2.3B).

e. Remove the remaining supernatant from the pellet without disturbing the pellet. DO NOT discard the pellet (cleanup for V(D)J and 5' Gene Expression library construction). **Immediately** proceed to Pellet Cleanup (step 2.3A).

2.3A Pellet Cleanup for V(D)J & 5' Gene Expression Library

a. Add **200 µl** 80% ethanol to the pellet while still on the magnet•**High**. Wait **30 sec**.

b. Remove the ethanol.

c. **Repeat** steps a and b for a total of 2 washes.

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

e. Remove any remaining ethanol. Air dry for **2 min**. DO NOT exceed **2 min** as this will decrease elution efficiency.

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

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

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

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

j. Store at **4°C** for up to **72 h** or at **−20°C** for up to **4 weeks**, or proceed to step 2.4 followed by steps 3-5 for V(D)J & 5' Gene Expression Library Construction.

2.3B Transferred Supernatant Cleanup for Cell Surface Protein and BEAM Library

a. Vortex to resuspend the SPRIselect reagent. Add **70 µl** SPRIselect reagent (**2.0X**) to 80 µl of the transferred supernatant and pipette mix 15x (pipette set to 140 µl).

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

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

d. Remove supernatant.

e. Add **300 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**. DO NOT exceed **2 min** as this will decrease elution efficiency.

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 directly to step 6 for BEAM Library Construction and step 7 for Cell Surface Protein Library Construction.

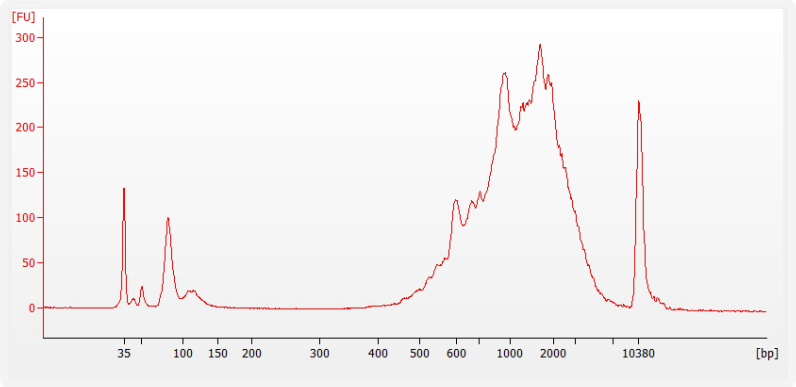
2.4 Post cDNA Amplification QC & Quantification

a. Run **1 µl** undiluted sample from Pellet Cleanup (step 2.3A) on an Agilent Bioanalyzer High Sensitivity chip.

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

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

Representative Trace for PBMCs



See example calculation in the following page.

Alternate Quantification Methods

Agilent TapeStation

LabChip Qubit Fluoromter

Qubit dsDNA HS Assay Kit

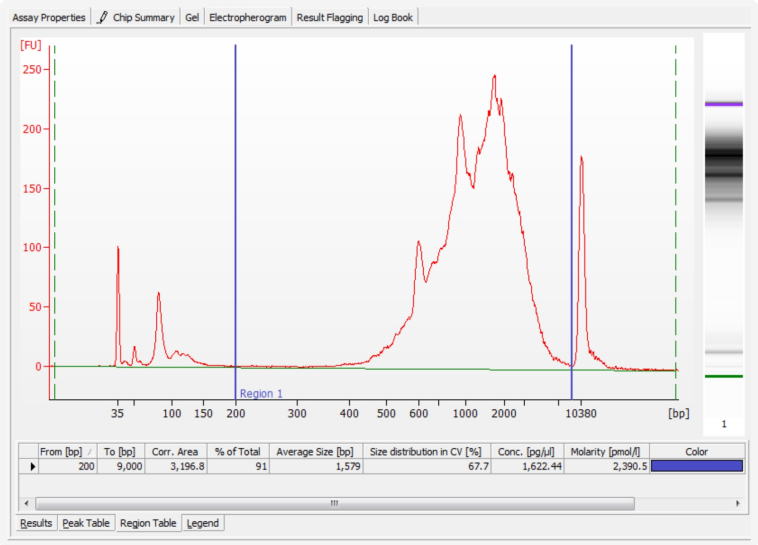
See Appendix for representative traces.

- LabChip Traces on page 110
- Agilent TapeStation Traces on page 111

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: Multiply the cDNA concentration [pg/µl] reported via Agilent 2100 Expert Software by dilution factor and divide by 1000 to obtain the total cDNA yield in ng/µl.

Example Calculation of cDNA Total Yield

Concentration: **1622.44 pg/µl** Dilution Factor: **1**

Total cDNA Yield

$$= \frac{\text{Conc'n (pg/µl) x Dilution Factor}}{\text{1000 (pg/ng)}}$$

$$= \frac{\text{1622.44 (pg/µl) x 1}}{\text{1000 (pg/ng)}} = \mathbf{1.6\ ng/µl}$$

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

Volume for 50 ng

$$= \frac{\text{50 ng}}{\text{1.6 ng/µl}}$$

$$= \mathbf{31.25\ µl}$$

- 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 (as in above example), carry ONLY 20 µl sample into library construction
- 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.

Step 3:

V(D)J Amplification from cDNA

3.0 Get Started 64

3.1 V(D)J Amplification 1 66

3.2 Post V(D)J Amplification 1 Cleanup Double Sided - SPRIselect 67

3.3 V(D)J Amplification 2 68

3.4 Post V(D)J Amplification 2 Cleanup Double Sided - SPRIselect 69

3.5 Post V(D)J Amplification QC & Quantification 70

3.0 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 amplification products)				
☐	**Human T Cell Mix 1 v2**	2000242	Thaw, vortex, centrifuge briefly	–20°C
☐	**Human T Cell Mix 2 v2**	2000246	Thaw, vortex, centrifuge briefly	–20°C
☐	**Human B Cell Mix 1 v2**	2000254	Thaw, vortex, centrifuge briefly	–20°C
☐	**Human B Cell Mix 2 v2**	2000255	Thaw, vortex, centrifuge briefly	–20°C
For Mouse Samples (Choose B or T-cell primers based on desired amplification products)				
☐	**Mouse T Cell Mix 1 v2**	2000256	Thaw, vortex, centrifuge briefly	–20°C
☐	**Mouse T Cell Mix 2 v2**	2000257	Thaw, vortex, centrifuge briefly	–20°C
☐	**Mouse B Cell Mix 1 v2**	2000258	Thaw, vortex, centrifuge briefly	–20°C
☐	**Mouse B Cell Mix 2 v2**	2000259	Thaw, vortex, centrifuge briefly	–20°C
For All Samples				
☐	**Beckman Coulter SPRIselect Reagent**	—	Manufacturer's recommendations.	—
☐	**Qubit dsDNA HS Assay Kit** *If used for quantification*	—	Manufacturer's recommendations.	—
☐	**Agilent Bioanalyzer High Sensitivity Kit** *If used for QC and quantification*	—	Manufacturer's recommendations.	—
☐	**Agilent TapeStation ScreenTape & Reagents** *If used for QC and quantification*	—	Manufacturer's recommendations.	—
Place on Ice				
☐	**Amp Mix** *Retrieve from Single Cell V (D)J Amplification Kits*	2000047	Vortex, centrifuge briefly.	–20°C
Obtain				

Action	Item	10x PN	Preparation & Handling	Storage
☐	**Qiagen Buffer EB**	—	Manufacturer's recommendations.	Ambient
☐	**10x Magnetic Separator**	230003	See Tips & Best Practices.	Ambient
☐	**Prepare 80% Ethanol** *Prepare 15 ml for 8 reactions.*	—	Prepare fresh.	Ambient

3.1 V(D)J Amplification 1

a. Place a tube strip on ice and transfer **2 µl** sample (post cDNA Amplification & QC, step 2.3 cDNA Cleanup – SPRIselect on page 58) to the same tube.

b. Prepare V(D)J Amplification 1 Reaction Mix on ice. Vortex and centrifuge briefly.

V(D)J Amplification 1 Rxn Mix *Add reagents in the order listed*		**PN**	**1X (µl)**	**4X + 10% (µl)**	**8X + 10% (µl)**
○	**Amp Mix**	2000047	50	220	440
●	**T Cell Mix 1 v2**	Human 2000242/ Mouse 2000256	48	211.2	422.4
	or	or			
●	**B Cell Mix 1 v2**	Human 2000254/ Mouse 2000258			
	Total		**98**	**431.2**	**862.4**

c. Add **98 µl** V(D)J Amplification 1 Reaction Mix to each tube containing **2 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** *hh:mm:ss*
1	98°C	00:00:45
2	98°C	00:00:20
3	62°C	00:00:30
4	72°C	00:01:00
5 ⚠ Different cycle numbers for T & B cells	T Cell: Go to Step 2, 11x (total 12 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.

3.2 Post V(D)J Amplification 1 Cleanup Double Sided – SPRIselect

a. Vortex to resuspend SPRIselect Reagent. Add **50 µl** SPRIselect Reagent **(0.5X)** to each sample. Pipette mix 15x (pipette set to 140 µl).

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

c. Place tube strip 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 **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.

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.

3.3 V(D)J Amplification 2

a. Prepare V(D)J Amplification 2 Reaction Mix on ice. Vortex and centrifuge briefly.

V(D)J Amplification 2 Reaction Mix *Add reagents in the order listed*		**PN**	**1X (µl)**	**4X + 10% (µl)**	**8X + 10% (µl)**
○	Amp Mix	2000047	50	220	440
●	T Cell Mix 2 v2	Human 2000246/ Mouse 2000257	15	66	132
	or	or			
●	B Cell Mix 2 v2	Human 2000255/ Mouse 2000259			
	Total		65	286	572

b. Add **65 µl** V(D)J Amplification 2 Reaction Mix to each tube containing **35 µl** sample.

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	~25-30 min
Step	**Temperature**	**Time** *hh:mm:ss*
1	98°C	00:00:45
2	98°C	00:00:20
3	62°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 **e.** Store at **4°C** for up to **72 h** or proceed to the next step.

3.4 Post V(D)J Amplification 2 Cleanup Double Sided – 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 tube strip 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.

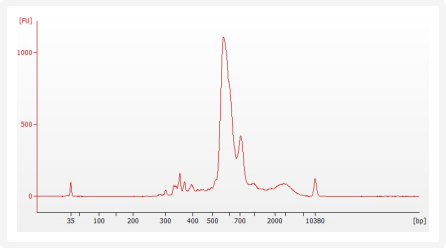
3.5 Post V(D)J Amplification QC & Quantification

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

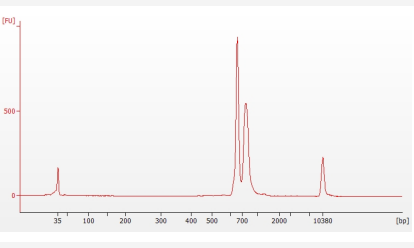
Samples of RNA-rich cells may require additional dilution in nuclease-free water. The number of distinct peaks may vary. Higher molecular weight product (2,000-9,000 bp) may be present. This does not affect sequencing.

Representative Traces

PBMCs amplified for TCR



PBMCs amplified for BCR



Determine yield for each sample. See example calculation in the following page.

Alternate Quantification Methods

Agilent TapeStation

LabChip

Qubit Fluorometer and Qubit dsDNA HS Assay Kit

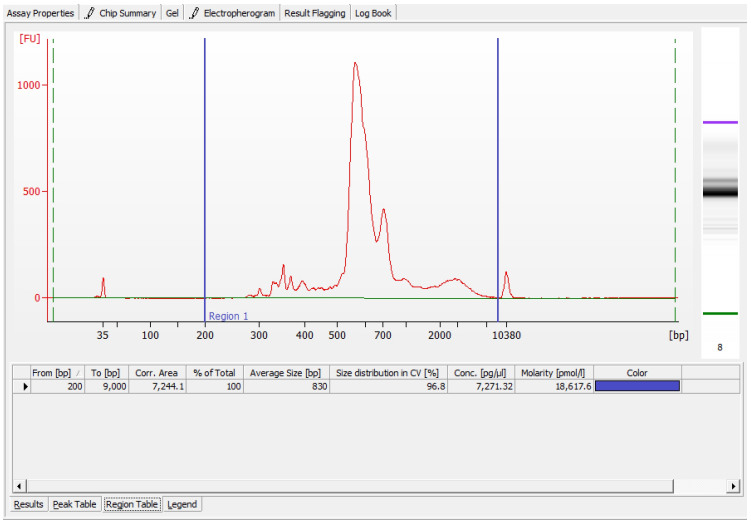
See Appendix for representative traces.

- LabChip Traces on page 110
- Agilent TapeStation Traces on page 111

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: Multiply the diluted sample concentration [pg/µl] reported via Agilent 2100 Expert Software by the dilution factor and divide by 1000 to obtain the total V(D)J amplified product concentration in ng/µl.

Example Calculation of cDNA Total Yield Example Calculation of V(D)J Amplified Product Concentration

Concentration: **7271.32 pg/µl** Dilution Factor: **5**

V(D)J Amplified Product Concentration

$$= \frac{\text{Conc'n (pg/µl) x Dilution Factor}}{\text{1000 (pg/ng)}}$$

$$= \frac{\text{7271.32 (pg/µl) x 5}}{\text{1000 (pg/ng)}} = \mathbf{36.35\ ng/µl}$$

Example Calculation for Carrying Forward 50 ng Sample for V(D)J Library Construction

Volume for 50 ng

$$= \frac{\text{50 ng}}{\text{36.35 ng/µl}}$$

$$= \mathbf{1.37\ µl}$$

V(D)J Library Construction Sample =1.37 µl + 18.63 µl nuclease-free water =20 µl total

If <50 ng available, carry forward 20 µl sample (2-50 ng) into V(D)J Library Construction. DO NOT exceed a mass of 50 ng in the 20 µl carry forward volume.

Step 4:

V(D)J Library Construction

4.0 Get Started 74

4.1 Fragmentation, End Repair & A-tailing 76

4.2 Adaptor Ligation 77

4.3 Post Ligation Cleanup – SPRIselect 77

4.4 Sample Index PCR 78

4.5 Post Sample Index PCR Cleanup – SPRIselect 78

4.6 Post Library Construction QC 79

4.0 Get Started

Action	Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature				
☐ ●	**Fragmentation Buffer**	2000091	Thaw, vortex, verify no precipitate, centrifuge briefly	−20°C
☐ ●	**Adaptor Oligos**	2000094	Thaw, vortex, centrifuge briefly.	−20°C
☐ ●	**Ligation Buffer**	2000092	Thaw, vortex, verify no precipitate, centrifuge briefly.	−20°C
☐ ⚠	**Dual Index Plate TT Set A** Verify name & PN. Use indicated plate only	3000431	—	−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 & 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				
☐ ●	**Fragmentation Enzyme** *Ensure that Fragmentation Buffer and Fragmentation Enzyme from the same kit are used together. Lots are matched for optimal performance*	2000090/ 2000104	Centrifuge briefly.	−20°C
☐ ●	**DNA Ligase**	220131/ 220110	Centrifuge briefly.	−20°C
☐ ○	**Amp Mix**	2000047/2000103	Vortex, centrifuge briefly.	−20°C

Action	Item	10x PN	Preparation & Handling	Storage
Obtain				
☐	**Qiagen Buffer EB**	—	Manufacturer's recommendations.	Ambient
☐	**10x Magnetic Separator**	230003	See Tips & Best Practices.	Ambient
☐	**Prepare 80% Ethanol** *Prepare 15 ml for 8 reactions*	—	Prepare fresh.	Ambient

4.1 Fragmentation, End Repair & A-tailing

a. Determine the volume for **50 ng** mass of sample (see example calculation at Step 3.5). Dispense the sample 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 hh:mm:ss**
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	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Nuclease-free Water	-	15	66	132
Fragmentation Buffer	2000091	5	22	44
Fragmentation Enzyme	2000090/ 2000104	10	44	88
Total		**30**	**132**	**264**

e. Add **30 µl** Fragmentation Mix to each **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.

4.2 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)**
Ligation Buffer	2000092	20	88	176
DNA Ligase	220110/ 220131	10	44	88
Adaptor Oligos	2000094	20	88	176
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** *hh:mm:ss*
1	20°C	00:15:00
2	4°C	Hold

4.3 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.

4.4 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-3000431 Dual Index Plate TT Set A well ID) used.

b. Add **50 µl** Amp Mix (PN-2000047/2000103) to **30 µl** sample.

c. Add **20 µl** of an individual Dual Index TT Set A to each sample and record the well ID used. 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	~30 min
Step	**Temperature**	**Time** *hh:mm:ss*
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, 7x (total 8 cycles)	
6	72°C	00:01:00
7	4°C	Hold

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

4.5 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.

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

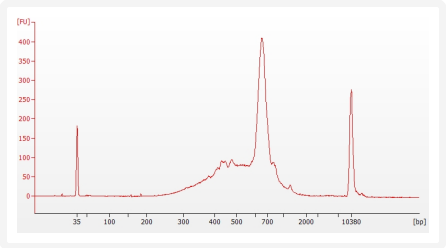
4.6 Post Library Construction QC

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

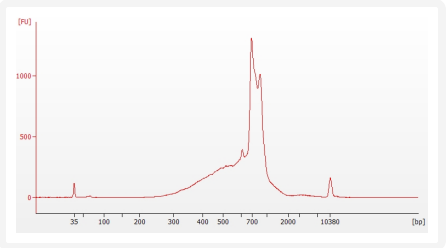
b. Determine the average fragment size from the Bioanalyzer trace. This will be used as the insert size for library quantification.

Representative Traces

PBMCs amplified for TCR



PBMCs amplified for BCR



Alternate QC Method

Agilent TapeStation

LabChip

See Appendix for:

- Post Library Construction Quantification on page 109
- LabChip Traces on page 110
- Agilent TapeStation Traces on page 111

Step 5:

5' Gene Expression Library Construction

5.0 Get Started 81

5.1 GEX Fragmentation, End Repair & A-tailing 82

5.2 GEX Post Fragmentation, End Repair & A-tailing Double Sided – SPRIselect 83

5.3 GEX Adaptor Ligation 84

5.4 GEX Post Ligation Cleanup – SPRIselect 84

5.5 GEX Sample Index PCR 85

5.6 Post Sample Index PCR Double Sided Size Selection – SPRIselect 86

5.7 Post Library Construction QC 87

5.0 Get Started

Action	Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature				
☐ ●	**Fragmentation Buffer**	2000091	Thaw, vortex, verify no precipitate, centrifuge.	–20°C
☐ ●	**Adaptor Oligos**	2000094	Thaw, vortex, centrifuge briefly.	–20°C
☐ ●	**Ligation Buffer**	2000092	Thaw, vortex , verify no precipitate, centrifuge briefly.	–20°C
☐ ⚠	**Dual Index Plate TT Set A** *Verify name & PN. Use indicated plate only*	3000431	—	–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 & 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				
☐ ●	**Fragmentation Enzyme** *Ensure that Fragmentation Buffer and Fragmentation Enzyme from the same kit are used together. Lots are matched for optimal performance*	2000090 /2000104	Centrifuge briefly.	–20°C
☐ ●	**DNA Ligase**	220110/220131	Centrifuge briefly.	–20°C
☐ ○	**Amp Mix**	2000047 /2000103	Vortex, centrifuge briefly.	–20°C
☐	**KAPA Library Quantification Kit for Illumina Platforms**	—	Manufacturer's recommendations.	—

Action	Item	10x PN	Preparation & Handling	Storage
Obtain				
☐	**Qiagen Buffer EB**	—	Manufacturer's recommendations.	Ambient
☐	**10x Magnetic Separator**	230003	See Tips & Best Practices.	Ambient
☐	**Prepare 80% Ethanol** *Prepare 15 ml for 8 reactions.*	—	Prepare fresh.	Ambient

5.1 GEX Fragmentation, End Repair & A-tailing

a. Determine the volume for **50 ng** mass of sample (see example calculation at Step 2.4). Dispense the sample 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** *hh:mm:ss*
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**	2000091	5	22	44
● **Fragmentation Enzyme**	2000090/ 2000104	10	44	88
Total		**30**	**132**	**264**

e. Add **30 µl** Fragmentation Mix to each **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 GEX Post Fragmentation, End Repair & A-tailing Double Sided – SPRIselect

a. Vortex to resuspend SPRIselect reagent. Add **30 µl** SPRIselect **(0.6X)** reagent 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 supernatant.

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 80 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** until the solution clears.

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

n. Remove from the magnet. Add **50.5 µl** Buffer EB to each sample. 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.

5.3 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)**
Ligation Buffer	2000092	20	88	176
DNA Ligase	220110/ 220131	10	44	88
Adaptor Oligos	2000094	20	88	176
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** *hh:mm:ss*
1	20°C	00:15:00
2	4°C	Hold

5.4 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.

5.5 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-3000431 Dual Index Plate TT Set A well ID) used.

b. Add **50 µl** Amp Mix (PN-2000047/2000103) to **30 µl** sample.

c. Add **20 µl** of an individual Dual Index TT Set A to each sample and record the well ID used. 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	~30 min
Step	**Temperature**	**Time** *hh:mm:ss*
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 below for # of 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 2.4 to determine the mass input into Library Construction.

Recommended Cycle Numbers

cDNA Input	Total Cycles
1-25 ng	16
26-50 ng	14

STOP

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

5.6 Post Sample Index PCR Double Sided Size Selection – SPRIselect

a. Vortex to resuspend the 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 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 the SPRIselect reagent. Add **20 µl** SPRIselect Reagent **(0.8X)** to each transferred supernatant. Pipette mix 15x (pipette set to 150 µl).

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

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

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

i. With the tube still in 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 remaining ethanol. DO NOT over-dry beads to ensure maximum elution efficiency.

n. Remove 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.

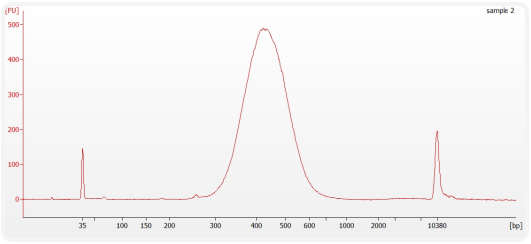
q. Transfer **35 µl** to a new tube strip.

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

5.7 Post Library Construction QC

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

Representative Trace



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

Alternate QC Method

Agilent TapeStation

LabChip

See Appendix for:

- Post Library Construction Quantification on page 109
- LabChip Traces on page 110
- Agilent TapeStation Traces on page 111

Step 6:

Barcode Enabled Antigen Mapping (BEAM) Library Construction

6.0 Get Started 89

6.1 Sample Index PCR 90

6.2 Post Sample Index PCR Size Selection - SPRIselect 91

6.3 Post Library Construction QC 92

6.0 Get Started

Action	Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature				
☐ ⚠	**Dual Index Plate TS Set A** *Verify name & PN. Use indicated plate only*	3000511	—	–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 & Reagents** *If used for QC and quantification*	—	Manufacturer's recommendations.	—
Place on Ice				
☐ ◯	**Amp Mix** *Retrieve from 5' Feature Barcode Kit*	2000047	Centrifuge briefly.	–20°C
☐	**KAPA Library Quantification Kit for Illumina Platforms**	—	Manufacturer's recommendations.	—
Obtain				
☐	**Qiagen Buffer EB**	—	Manufacturer's recommendations.	Ambient
☐	**10x Magnetic Separator**	230003	See Tips & Best Practices.	Ambient
☐	**Prepare 80% Ethanol** *Prepare 20 ml for 8 reactions.*	—	Prepare fresh.	Ambient

6.1 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-3000511 Dual Index Plate TS 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	—	25	110	220
◯	**Amp Mix** *Retrieve from 5' Feature Barcode Kit*	2000047	50	220	440
	Total		**75**	**330**	**660**

c. Transfer **ONLY 5 µl** from the Transferred Supernatant Cleanup (step 2.3B) to a new tube strip.

- Note that only **5 µl** sample transfer is sufficient for generating BEAM library.
- The remaining sample can be stored at **4°C** for up to **72 h** or at **–20°C** for up to **4 weeks**, for generating additional libraries.

d. Add **75 µl** Sample Index PCR Mix to **5 µl** Transferred Supernatant Cleanup sample.

e. Add **20 µl** of an individual Dual Index TS Set A to each sample and record the well ID used. Pipette mix 5x (pipette set to 90 µl). Centrifuge briefly.

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

Lid Temperature	Reaction Volume	Run Time
105°C	100 µl	~30 min
Step	**Temperature**	**Time** *hh:mm:ss*
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, repeat 11X for a total of 12 cycles	
6	72°C	00:01:00
7	4°C	Hold

6.2 Post Sample Index PCR Size Selection – SPRIselect

a. Vortex to resuspend the SPRIselect reagent. Add **120 µl** SPRIselect Reagent **(1.2X)** 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. Remove the supernatant.

d. Add **300 µl** 80% ethanol to the pellet. Wait 30 sec.

e. Remove the ethanol.

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

g. Remove the ethanol.

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

i. Remove remaining ethanol. Air dry for **1 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.

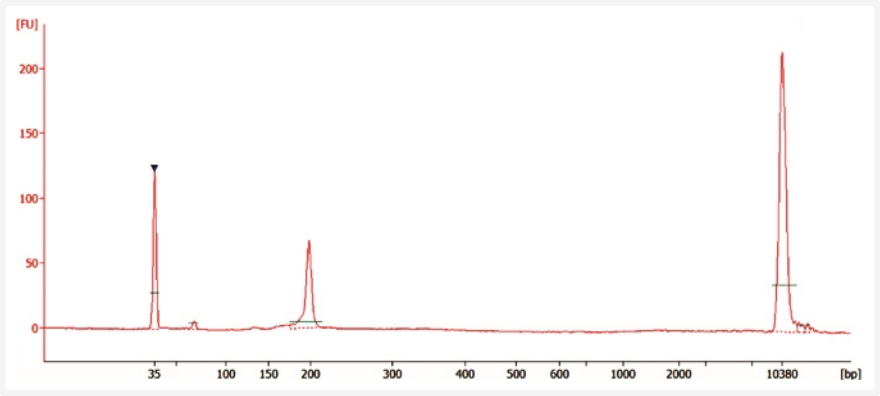
STOP

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

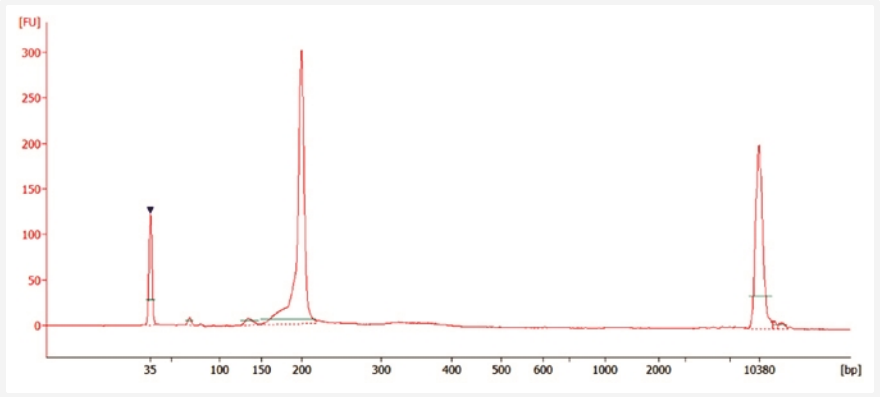
6.3 Post Library Construction QC

Run **1 µl** sample at 1:5 dilution on an Agilent Bioanalyzer High Sensitivity chip.

Representative Trace BEAM-T Library



Representative Trace BEAM-Ab Library



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

Alternate QC Method

- Agilent TapeStation
- LabChip

See Appendix for:

- Post Library Construction Quantification on page 109
- LabChip Traces on page 110
- Agilent TapeStation Traces on page 111

Step 7:

Cell Surface Protein Library Construction

7.0 Get Started 94

7.1 Sample Index PCR 95

7.2 Post Sample Index PCR Size Selection – SPRIselect 96

7.3 Post Library Construction QC 97

7.0 Get Started

Action	Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature				
☐ ⚠	**Dual Index Plate TN Set A** *Verify name & PN. Use indicated plate only*	3000510	—	–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 & Reagents** *If used for QC and quantification*	—	Manufacturer's recommendations.	—
Place on Ice				
☐ ◯	**Amp Mix** *Retrieve from 5' Feature Barcode Kit*	2000047	Centrifuge briefly.	–20°C
☐	**KAPA Library Quantification Kit for Illumina Platforms**	—	Manufacturer's recommendations.	—
Obtain				
☐	**Qiagen Buffer EB**	—	Manufacturer's recommendations.	Ambient
☐	**10x Magnetic Separator**	230003	See Tips & Best Practices.	Ambient
☐	**Prepare 80% Ethanol** *Prepare 20 ml for 8 reactions.*	—	Prepare fresh.	Ambient

7.1 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-3000510 Dual Index Plate TN 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	—	25	110	220
◯	**Amp Mix** *Retrieve from 5' Feature Barcode Kit*	2000047	50	220	440
	Total		**75**	**330**	**660**

c. Transfer **ONLY 5 µl** from the Transferred Supernatant Cleanup (step 2.3B) to a new tube strip.

- Note that only 5 **µl** sample transfer is sufficient for generating Cell Surface Protein library.
- The remaining sample can be stored at **4°C** for up to **72 h** or at **–20°C** for up to **4 weeks**, for generating additional libraries.

d. Add **75 µl** Sample Index PCR Mix to **5 µl** Transferred Supernatant Cleanup sample.

e. Add **20 µl** of an individual Dual Index TN Set A to each sample and record the well ID used. Pipette mix 5x (pipette set to 90 µl). Centrifuge briefly.

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

Lid Temperature	Reaction Volume	Run Time
105°C	100 µl	~35 min
Step	**Temperature**	**Time** *hh:mm:ss*
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, repeat 13X for a total of 14 cycles*	
6	72°C	00:01:00
7	4°C	Hold

**Optimization of cycle number may be needed based on target protein expression levels and number of antibodies used for labeling.*

7.2 Post Sample Index PCR Size Selection – SPRIselect

a. Vortex to resuspend the SPRIselect reagent. Add **120 µl** SPRIselect Reagent **(1.2X)** 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. Remove the supernatant.

d. Add **300 µl** 80% ethanol to the pellet. Wait 30 sec.

e. Remove the ethanol.

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

g. Remove the ethanol.

h. Centrifuge briefly. Place on the magnet•**Low**. Remove remaining ethanol.

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

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

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

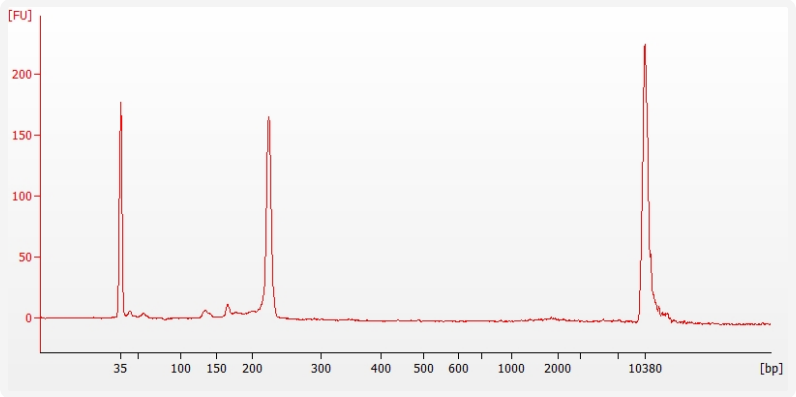
l. Transfer **35 µl** to a new tube strip.

m. Store at **4°C** for up to **72 h** or at **−20°C** for **long-term** storage.

7.3 Post Library Construction QC

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

Representative Trace



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

Alternate QC Method

- Agilent TapeStation
- LabChip

See Appendix for:

- Post Library Construction Quantification on page 109
- LabChip Traces on page 110
- Agilent TapeStation Traces on page 111

Step 8:

Sequencing

Sequencing Libraries 99

Illumina Sequencer Compatibility 100

Sample Indices 100

Library Sequencing Depth & Run Parameters 100

Library Loading 101

Library Pooling 101

Data Analysis and Visualization 102

Sequencing Libraries

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

TruSeq Read 1, TruSeq Read 2, Nextera Read 2 (Read 2N), and small RNA Read 2 (Read 2S) are all standard Illumina sequencing primer sites. TruSeq Read 1 and TruSeq Read 2 are used in paired-end sequencing of V(D)J and 5' Gene Expression libraries. TruSeq Read 1 and Nextera Read 2 (Read 2N) are used for paired-end sequencing of Cell Surface Protein library. TruSeq Read 1 and small RNA Read 2 (Read 2S) are used for paired-end sequencing of BEAM library. Sequencing these libraries produce a standard Illumina BCL data output folder.

Chromium Single Cell V(D)J Dual Index Library

Chromium Single Cell 5' Gene Expression Dual Index Library

Chromium Single Cell Barcode Enabled Antigen Mapping (BEAM) Library

Chromium Single Cell 5' Cell Surface Protein Library

Illumina Sequencer Compatibility

The compatibility of the listed sequencers has been verified by 10x Genomics. Some variation in assay performance is expected based on sequencer choice. For more information about performance variation, visit the 10x Genomics Support website.

- MiSeq
- NextSeq 500/550/2000
- HiSeq 2500 (Rapid Run)
- HiSeq 3000/4000
- NovaSeq

Sample Indices

Each sample index in the Dual Index Kit TT Set A (PN-1000215), Dual Index Kit TN Set A (PN-1000250), and Dual Index Kit TS Set A (PN-1000251) contains a mix of one unique i7 and one unique i5 sample index. If multiple samples are pooled in a sequence lane, the sample index name (i.e. the Dual Indexplate well ID) is needed in the sample sheet used for generating FASTQs with "cellranger mkfastq". If multiple libraries are pooled in a sequence lane, a separate sample index is needed with each library (see Sample Indices in Sample Index PCR on page 39).

Library Sequencing Depth & Run Parameters

Parameter	Description
Sequencing Depth	Minimum 5,000 read pairs/cell for V(D)J library Minimum 20,000 read pairs/cell for 5' Gene Expression library* Minimum 5,000 read pairs/cell for Cell Surface Protein library Minimum 5,000 read pairs/cell for BEAM library
Sequencing Type	Paired-end, dual indexing
Sequencing Read	Recommended Number of Cycles
Read 1 i7 Index i5 Index Read 2	26 cycles 10 cycles 10 cycles 90 cycles

**Sequence data from the 5' Gene Expression library is required for analysis in Cell Ranger, as it is used for cell calling. If detailed characterization of Gene Expression data is not desired, the sequencing depth may be reduced to 5,000 read pairs/cell, which is typically sufficient for cell calling. The minimum Gene Expression sequencing depth needed for accurate cell calling may vary based*

on the sample type and data quality.

Library Loading

Once quantified and normalized, 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	150*/300	1
NextSeq 2000	650	1

** Use 150 pM loading concentration for Illumina XP workflow.*

Library Pooling

V(D)J, 5' Gene Expression, Cell Surface Protein, and BEAM libraries may be pooled for sequencing, taking into account the differences in cell number and per-cell read depth requirements between each library.

Libraries	Sequencing Depth (read pairs per cell)	Library Pooling Ratio	PhiX*
Example 1			
V(D)J library	5,000	1	1%
5' Gene Expression library	20,000	4	
Cell Surface Protein library	5,000	1	
BEAM library	5,000	1	
Example 2			
V(D)J library	5,000	1	1%
5' Gene Expression library	50,000	10	
Cell Surface Protein library	5,000	1	
BEAM library	5,000	1	
Example 3			
V(D)J library	5,000	1	10%
5' Gene Expression library	5,000	1	
Cell Surface Protein library	5,000	1	
BEAM library	5,000	1	

*10% PhiX is recommended when pooling Gene Expression, V(D)J, BEAM, and Cell Surface Protein libraries at a ratio of 1:1:1:1. DO NOT sequence BEAM libraries alone.

Data Analysis and Visualization

Sequencing data may be analyzed using Cell Ranger and visualized using Loupe Browser. Key features for these tools are listed below. For detailed product-specific information, visit the 10x Genomics Support website.

Cell Ranger

Cell Ranger is a set of analysis pipelines that processes Chromium Single Gene Expression data to align reads, generate Feature Barcode matrices and perform clustering and gene expression analysis.

- Input: Base call (BCL) and FASTQ
- Output: BAM, MEX, CSV, HDF5, Web Summary, .cloupe/.loupe
- Operating System: Linux

Loupe Browser

Loupe Browser is an interactive data visualization tool that requires no prior programming knowledge.

- Input: .cloupe
- Output: Data visualization, including t-SNE and UMAP projections, custom clusters, differentially expressed genes
- Operating System: MacOS, Windows

Loupe V(D)J Browser

Loupe V(D)J Browser is an interactive data visualization and exploration tool for immune cells that requires no prior programming knowledge.

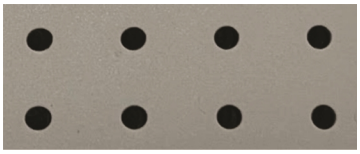
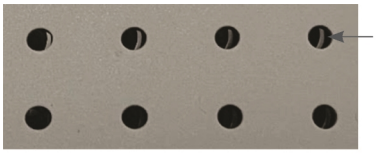
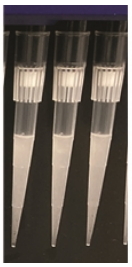
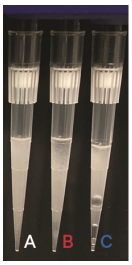
- Input: .vloupe
- Output: Clonotype distribution plot, Filtered Clonotype lists, Immune Receptor nucloetide and amino acid sequences
- Operating System: MacOS, Windows

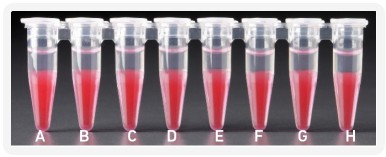
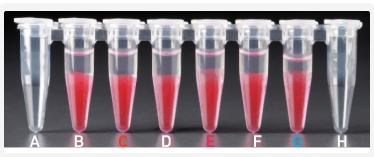
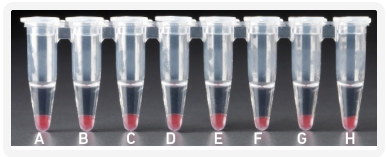
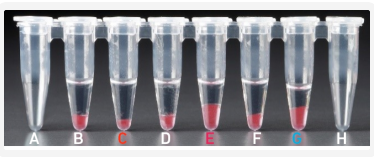
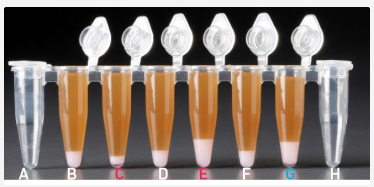
Troubleshooting

GEM Generation & Barcoding 104

Chromium Controller Errors 106

GEM Generation & Barcoding

STEP	NORMAL	Impacted
1.2 **Load Chromium Next GEM Chip K**		
	Gasket holes are aligned with the sample and gel bead wells.	Gasket holes are misaligned with the gel bead wells. Open and close the chip holder slowly once.
1.4d **After Chip K is removed from the instrument 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
1.4e **Transfer GEMs from Chip K Recovery Wells**		 Adequate emulsion volume (no clog or wetting failure) Wetting failure Low emulsion volume (clog)
	All liquid levels are similar in volume and opacity without air trapped in the pipette tips.	Pipette tip A shows normal GEM generation, pipette tip B indicates a wetting failure, and pipette tip C shows a clog and wetting failure.

STEP	NORMAL	Impacted
2.1a **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 d **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.

Consult the Best Practices to Minimize Chromium Next GEM Chip Clogs and Wetting Failures (Technical Note CG000479) for more information.

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. Network Error

f. Update Error

Consult the Chromium X Series (X/iX) User Guide (CG000396) for additional information and follow the Chromium X touchscreen prompts for execution. The Chromium X touchscreen will guide the user through recoverable errors.

Appendix

Post Library Construction Quantification 109
LabChip Traces 110
Agilent TapeStation Traces 111
Oligonucleotide Sequences 112

Post Library Construction Quantification

a. Thaw KAPA Library Quantification Kit for Illumina Platforms.

b. Dilute **2 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.

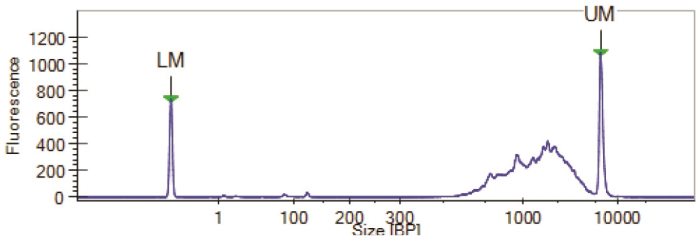
Step	Temperature	Run Time
1	95°C	00:03:00
2	95°C	00:00:05
3	67°C Read Signal	00:00:30
4	Go to Step 2, 29X (Total 30 cycles)	

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.

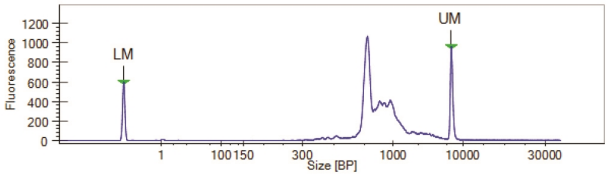
LabChip Traces

DNA High Sensitivity Reagent Kit was used.

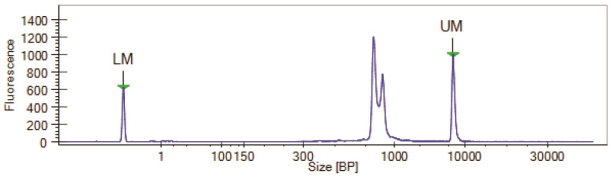
Protocol Step 2.4 – cDNA QC & Quantification



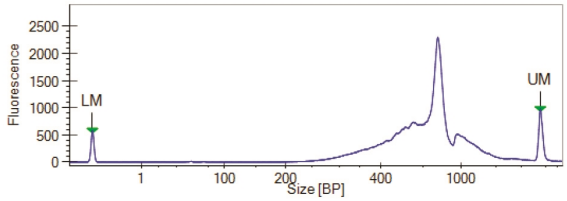
Protocol Step 3.5 – Post TCR Amplification



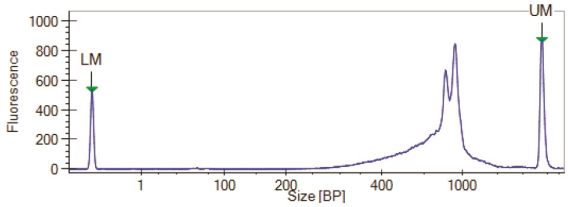
Protocol Step 3.5 – Post BCR Amplification



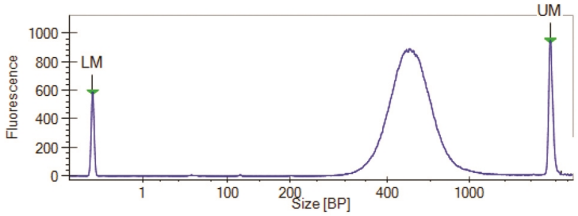
Protocol Step 4.6 – Post Library Construction (PBMCs amplified for TCR)



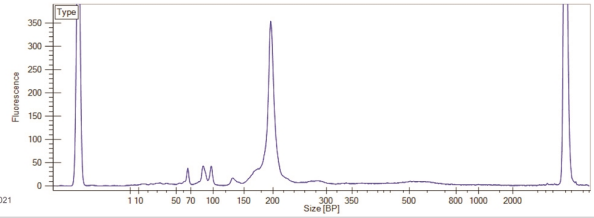
Protocol Step 4.6 – Post Library Construction (PBMCs amplified for BCR)



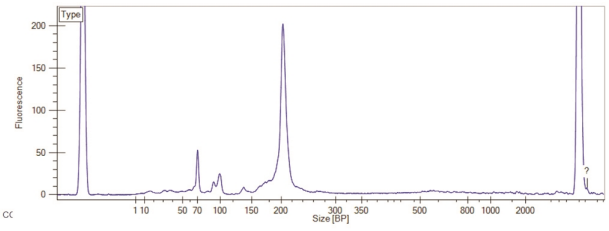
Protocol Step 5.7 – GEX Post Library Construction



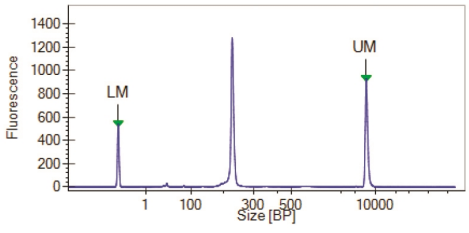
Protocol Step 6.3 – Post Library Construction (BEAM-Ab)



Protocol Step 6.3 – Post Library Construction (BEAM-T)



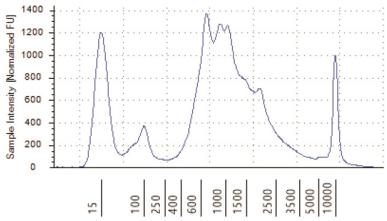
Protocol Step 7.3 – Post Library Construction (Cell Surface Protein)



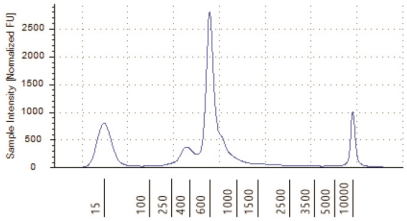
Agilent TapeStation Traces

Agilent TapeStation High Sensitivity D5000 ScreenTape was used.

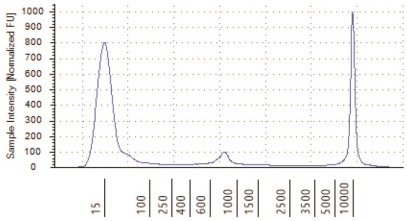
Protocol Step 2.4 – cDNA QC & Quantification



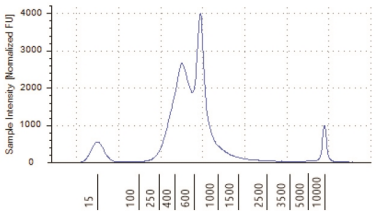
Protocol Step 3.5 – Post TCR Amplification



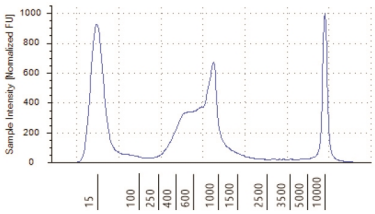
Protocol Step 3.5 – Post BCR Amplification



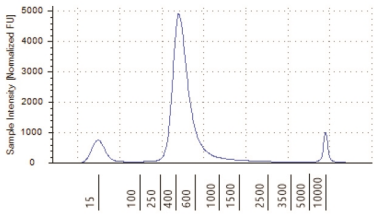
Protocol Step 4.6 – Post Library Construction (PBMCs amplified for TCR)



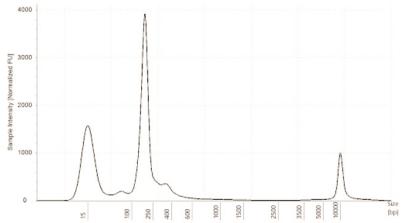
Protocol Step 4.6 – Post Library Construction (PBMCs amplified for BCR)



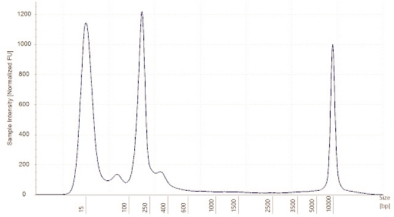
Protocol Step 5.7 – GEX Post Library Construction



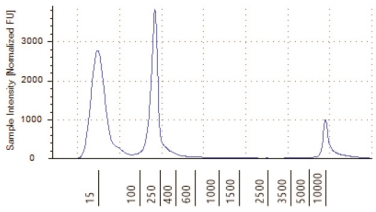
Protocol Step 6.3 – Post Library Construction (BEAM-Ab)



Protocol Step 6.3 – Post Library Construction (BEAM-T)



Protocol Step 7.3 – Post Library Construction (Cell Surface Protein)



Oligonucleotide Sequences

Protocol Step 1.5 – GEM-RT Incubation

Gel Bead Primer

5'-CTACACGACGCTCTTCCGATCT-N16-N10-TTTCTTATATrGrGrG-3'

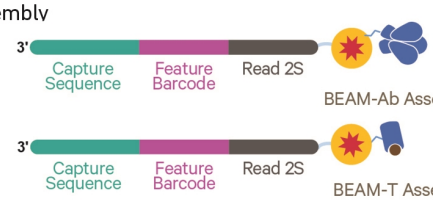
Poly-dT RT Primer
PN-2000007

5'-AAGCAGTGGTATCAACGCAGAGTACTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTVN-3'

Cell Surface Protein Feature Barcode

3'-AAAGAATATACCC-N9-N15-N10-GACAGAGAATATGTGTAGAGGC-5'

BEAM-Ab or BEAM-T Assembly Feature Barcode



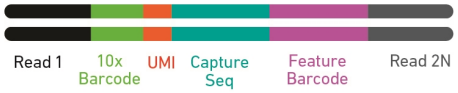
3'-AAAGAATATACCC-N15-ACCTTAAGAGCCCACGGTTCC-5'

GEM-RT Products

cDNA from poly-adenylated mRNA

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

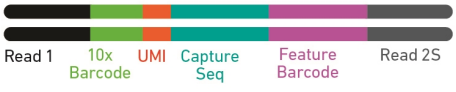
DNA from cell surface protein Feature Barcode



5'-CTACACGACGCTCTTCCGATCT-N16-N10-TTTCTTATATGGG-N9-N15-N10-CTGTCTCTTATACACATCTCCG-3'

3'-GATGTGCTGCGAGAAGGCTAGA-N16-N10-AAAGAATATACCC-N9-N15-N10-GACAGAGAATATGTGTAGAGGC-5'

DNA from BEAM-Ab or BEAM-T Assembly Feature Barcode



5'-CTACACGACGCTCTTCCGATCT-N16-N10-TTTCTTATATGGG-N15-TGGAATTCTCGGGTGCCAAGG-3'

3'-GATGTGCTGCGAGAAGGCTAGA-N16-N10-AAAGAATATACCC-N15-ACCTTAAGAGCCCACGGTTCC-5'

Protocol Step 2.2 – cDNA Amplification

Feature cDNA Primers 5 -2000812

Amplifies cDNA

Partial Read 1

Forward Primer:

5'-CTACACGACGCTCTTCCGATCT-3'

Non-poly(dT)

Reverse Primer:

5'-AAGCAGTGGTATCAACGCAGAG-3'

Amplifies DNA from cell surface protein Feature Barcode

Partial Read 1

Forward Primer:

5'-CTACACGACGCTCTTCCGATCT-3'

Read 2N

Reverse Primer:

5'-CTCGTGGGCTCGGAGATGTG-3'

Amplifies DNA from BEAM-Ab or BEAM-T Assembly Feature Barcode

Partial Read 1

Forward Primer:

5'-CTACACGACGCTCTTCCGATCT-3'

Read 2S

Reverse Primer:

5'-CCTTGGCACCCGAGAATTCCA-3'

Amplified Products

Amplified cDNA from poly-adenylated mRNA

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

Amplified DNA from cell surface protein Feature Barcode

5'-CTACACGACGCTCTTCCGATCT-N16-N10-TTTCTTATATGGG-N9-N15-N10-CTGTCTCTTATACACATCTCCGAGCCCACGAG-3'
3'-GATGTGCTGCGAGAAGGCTAGA-N16-N10-AAAGAATATACCC-N9-N15-N10-GACAGAGAATATGTGTAGAGGCTCGGGTGCTC-5'

Amplified DNA from BEAM-Ab or BEAM-T Assembly Feature Barcode

5'-CTACACGACGCTCTTCCGATCT-N16-N10-TTTCTTATATGGG-N15-TGGAATTCTCGGGTGCCAAGG-3'
3'-GATGTGCTGCGAGAAGGCTAGA-N16-N10-AAAGAATATACCC-N15-ACCTTAAGAGCCCACGGTTCC-5'

Protocol Step 3.1 – V(D)J Amplification 1			
Human T Cell Mix 1 v2 PN-2000242	Forward Primer: PCR Primer 5'-GATCTACACTCTTTCCCTACACGACGC-3'	Reverse Outer Primers: 5'-TGAAGGCGTTTGCACATGCA-3' 5'-TCAGGCAGTATCTGGAGTCATTGAG-3	Outer Primer
Human B Cell Mix 1 v2 PN-2000254	Forward Primer: PCR Primer 5'-GATCTACACTCTTTCCCTACACGACGC-3'	Reverse Outer Primers: 5'-CAGGGCACAGTCACATCCT-3' 5'-TGCTGGACCACGCATTTGTA-3' 5'-GGTTTTGTTGTCGACCCAGTCT-3' 5'-TTGTCCACCTTGGTGTTGCT-3' 5'-CATGACGTCCTTGGAAGGCA-3' 5'-TGTGGGACTTCCACTG-3' 5'-TTCTCGTAGTCTGCTTTGCTCAG-3'	Outer Primer
Mouse T Cell Mix 1 v2 PN-2000256	Forward Primer: PCR Primer 5'-GATCTACACTCTTTCCCTACACGACGC-3'	Reverse Outer Primers: 5'-CTGGTTGCTCCAGGCAATGG-3' 5'-TGTAGGCCTGAGGGTCCGT-3'	Outer Primer
Mouse B Cell Mix 1 v2 PN-2000258	Forward Primer: PCR Primer 5'-GATCTACACTCTTTCCCTACACGACGC-3'	Reverse Outer Primers: 5'-TCAGCACGGGACAAACTCTTCT-3' 5'-GCAGGAGACAGACTCTTCTCCA-3' 5'-AACTGGCTGCTCATGGTGT-3' 5'-TGGTGCAAGTGTGGTTGAGGT-3' 5'-TGGTCACTTGGCTGGTGGTG-3' 5'-CACTTGGCAGGTGAACTGTTTTCT-3' 5'-AACCTTCAAGGATGCTCTTGGGA-3' 5'-GGACAGGGATCCAGAGTTCCA-3' 5'-AGGTGACGGTCTGACTTGGC-3' 5'-GCTGGACAGGGCTCCATAGTT-3' 5'-GGCACCTTGTCCAATCATGTTCC-3' 5'-ATGTCGTTCATACTCGTCCTTGGT-3'	Outer Primer

Protocol Step 3.3 – V(D)J Amplification 2			
Human T Cell Mix 2 v2 PN-2000246	Forward Primer: PCR Primer 5'-GATCTACACTCTTTCCCTACACGACGC-3'	Reverse Inner Primers: 5'-AGTCTCTCAGCTGGTACACG-3' 5'-TCTGATGGCTCAAACACAGC-3'	Inner Primer
Human B Cell Mix 2 v2 PN-2000255	Forward Primer: PCR Primer 5'-GATCTACACTCTTTCCCTACACGACGC-3'	Reverse Inner Primers: 5'-GGGAAGTTTCTGGCGGTCA-3' 5'-GGTGGTACCCAGTTATCAAGCAT-3' 5'-GTGTCCCAGGTCACCATCAC-3' 5'-TCCTGAGGACTGTAGGACAGC-3' 5'-CACGCTGCTCGTATCCGA-3' 5'-TAGCTGCTGGCCGC-3' 5'-GCGTTATCCACCTTCCACTGT-3'	Inner Primer
Mouse T Cell Mix 2 v2 PN-2000257	Forward Primer: PCR Primer 5'-GATCTACACTCTTTCCCTACACGACGC-3'	Reverse Inner Primers: 5'-AGTCAAAGTCGGTGAACAGGCA-3' 5'-GGCCAAGCACACGAGGGTA-3'	Inner Primer
Mouse B Cell Mix 2 v2 PN-2000259	Forward Primer: PCR Primer 5'-GATCTACACTCTTTCCCTACACGACGC-3'	Reverse Inner Primers: 5'-TACACACCAGTGTGGCCTT-3' 5'-CAGGCCACTGTCACACCACT-3' 5'-CAGGTCACATTCATCGTGCCG-3' 5'-GAGGCCAGCACAGTGACCT-3' 5'-GCAGGGAAGTTCACAGTGCT-3' 5'-CTGTTTGAGATCAGTTTGCCATCCT-3' 5'-TGCGAGGTGGCTAGGTACTTG-3' 5'-CCCTTGACCAGGCATCC-3' 5'-AGGTCACGGAGGAACCAGTTG-3' 5'-GGCATCCCAGTGTCACCGA-3' 5'-AGAAGATCCACTTCACCTTGAAC-3' 5'-GAAGCACACGACTGAGGCAC-3'	Inner Primer

V(D)J Amplification Product

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

Protocol Step 4.2 – Adaptor Ligation (for V(D)J Library Construction)

Adaptor (Read 2) PN-220026

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

Ligation Product

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

Protocol Step 4.4 – Sample Index PCR (for V(D)J Library Construction)

Dual Indexing

Dual Index Kit TT Set A PN-1000215

Forward Primer:

5'–AATGATACGGCGACCACCGAGATCTACAC–N10–ACACTCTTTCCCTACACGACGCTC–3'

Reverse Primer:

5'–CAAGCAGAAGACGGCATACGAGAT–N10–GTGACTGGAGTTCAGACGTGT–3'

Sample Index PCR Product

5'–AATGATACGGCGACCACCGAGATCTACAC–N10 –ACACTCTTTCCCTACACGACGCTCTTCCGATCT–N16–N10–TTTCTTATATGGG–Insert–AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC–N10–ATCTCGTATGCCGTCTTCTGCTTG–3'
3'–TTACTATGCCGCTGGTGGCTCTAGATGTG–N10–TGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA–N16–N10–AAAGAATATACCC–Insert–TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG–N10–TAGAGCATACGGCAGAAGACGAAC–5'

Protocol Step 5.3 –GEX Adaptor Ligation (for 5' Gene Expression (GEX) Library Construction)

Adaptor (Read 2) PN-220026

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

Ligation Product

5'–CTACACGACGCTCTTCCGATCT–N16–N10–TTTCTTATATGGG–cDNA_Insert–AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC–3'
3'–GATGTGCTGCGAGAAGGCTAGA–N16–N10–AAAGAATATACCC–cDNA_Insert–TCTAGCCTTCTCG–5'

Protocol Step 5.5 – Sample Index PCR (for 5' Gene Expression (GEX) Library Construction)

Dual Indexing

Dual Index Kit
TT Set A
PN-1000215

Forward Primer:

5'-AATGATACGGCGACCACCGAGATCTACAC-N10-ACACTCTTTCCCTACACGACGCTC-3'

Reverse Primer:

5'-CAAGCAGAAGACGGCATACGAGAT-N10-GTGACTGGAGTTCAGACGTGT-3'

Sample Index PCR Product

5'-AATGATACGGCGACCACCGAGATCTACAC-N10 -ACACTCTTTCCCTACACGACGCTCTTCCGATCT-N16-N10-TTTCTTATATGGG-cDNA_Insert-AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC-N10-ATCTCGTATGCCGTCTTCTGCTTG-3'
3'-TTACTATGCCGCTGGTGGCTCTAGATGTG-N10-TGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-N16-N10-AAAGAATATACCC-cDNA_Insert-TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG-N10-TAGAGCATACGGCAGAAGACGAAC-5'

Protocol Step 6.1 – Sample Index PCR (for BEAM Library Construction)

Dual Indexing

Dual Index Kit
TS Set A
PN-1000251

Forward Primer:

5'-AATGATACGGCGACCACCGAGATCTACAC-N10-ACACTCTTTCCCTACACGACGCTC-3'

Reverse Primer:

5'-CAAGCAGAAGACGGCATACGAGAT-N10-GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA-3'

Sample Index PCR Product

5'-AATGATACGGCGACCACCGAGATCTACAC-N10 -ACACTCTTTCCCTACACGACGCTCTTCCGATCT-N16-N10-TTTCTTATATGGG-N15-TGGAATTCTCGGGTGCCAAGGAACTCCAGTCAC-N10-ATCTCGTATGCCGTCTTCTGCTTG-3'
3'-TTACTATGCCGCTGGTGGCTCTAGATGTG-N10-TGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-N16-N10-AAAGAATATACCC-N15-ACCTTAAGAGCCCACGGTTCCTTGAGGTCAGTG-N10-TAGAGCATACGGCAGAAGACGAAC-5'

Protocol Step 7.1 – Sample Index PCR (for Cell Surface Protein Library Construction)

Dual Indexing

Dual Index Kit
TN Set A
PN-1000250

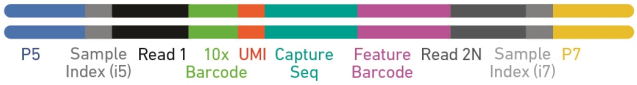
Forward Primer:

5'-AATGATACGGCGACCACCGAGATCTACAC-N10-ACACTCTTTCCCTACACGACGCTC-3'

Reverse Primer:

5'-CAAGCAGAAGACGGCATACGAGAT-N10-GTCTCGTGGGCTCGG-3'

Sample Index PCR Product



5'-AATGATACGGCGACCACCGAGATCTACAC-N10 -ACACTCTTTCCCTACACGACGCTCTTCCGATCT-N16-N10-TTTCTTATATGGG-N9-N15-N10-CTGTCTCTTATACACATCTCCGAGCCCACGAGAC-N10-ATCTCGTATGCCGTCTTCTGCTTG-3'
3'-TTACTATGCCGCTGGTGGCTCTAGATGTG-N10-TGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-N16-N10-AAAGAATATACCC-N9-N15-N10-GACAGAGAATATGTGTAGAGGCTCGGGTGCTCTG-N10-TAGAGCATACGGCAGAAGACGAAC-5'