

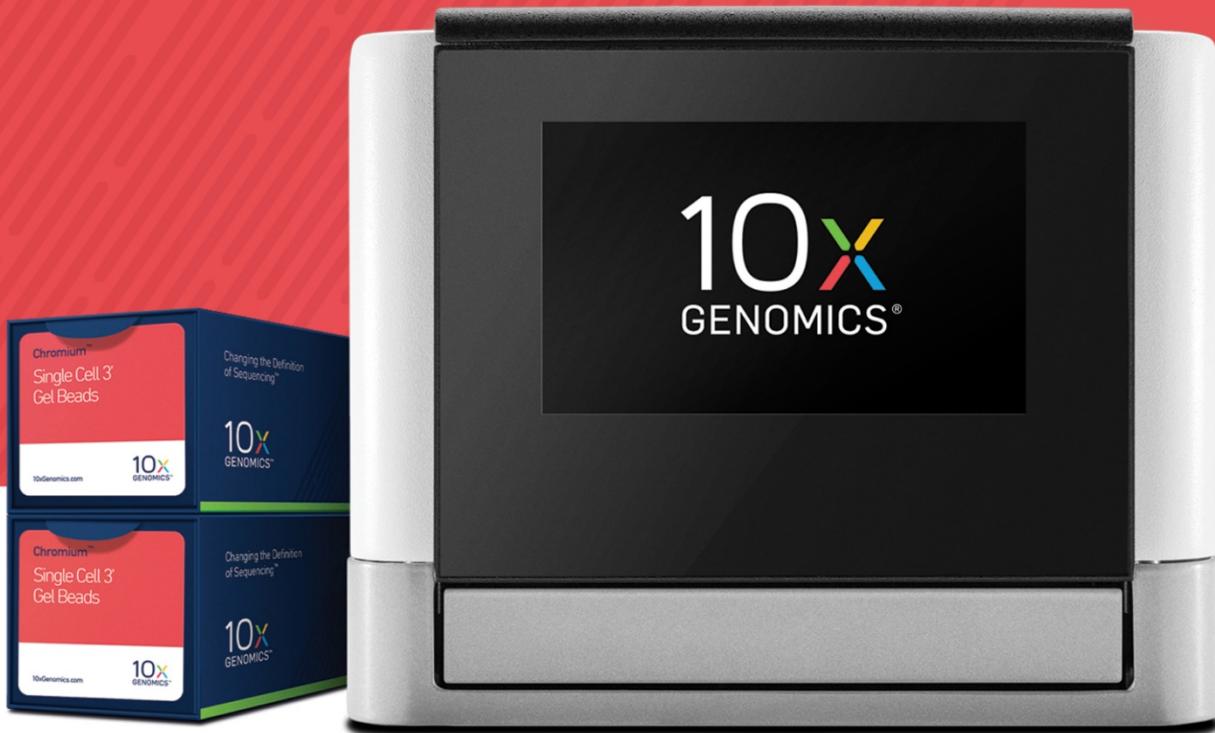
Chromium™ Single Cell 3' Reagent Kits v2 User Guide

FOR USE WITH

Chromium™ Single Cell 3' Library & Gel Bead Kit v2 PN-120237

Chromium™ Single Cell 3' Chip Kit v2 PN-120236

Chromium™ i7 Multiplex Kit PN-120262



Notices

Manual Part Number

CG00052 Rev A

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Introduction

Chromium™ Single Cell 3' Reagent Kits v2 - Components

Chromium™ Accessories

Additional Kits, Reagents & Equipment

Recommended Thermal Cyclers

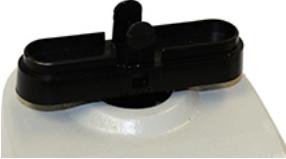
Chromium™ Single Cell 3' Reagent Kits v2 – Components

CRITICAL!

Parts from Chromium™ Single Cell 3' Reagents Kits v2 are NOT interchangeable with parts from earlier Chromium™ Single Cell 3' Reagent Kits, despite the same or similar names.

Product	Description	#	Part Number
Chromium™ Single Cell 3' Library Kit v2 (store at -20°C)			120234
Reagents Module 1	RT Reagent Mix RT Enzyme Mix Additive A RT Primer Buffer Sample Clean Up 1 Amplification Master Mix cDNA Primer Mix cDNA Additive	1	220089
Reagents Module 2	Fragmentation Enzyme Blend Fragmentation Buffer Ligation Buffer DNA Ligase Adaptor Mix SI-PCR Primer Amplification Master Mix	1	220107
Chromium™ Single Cell 3' Gel Bead Kit v2 (store at -80°C)			120235
	Single Cell 3' Gel Beads	2	220104
Chromium™ Single Cell 3' Chip Kit v2 (store at ambient temperature)			120236
	Single Cell 3' Chips Gaskets Partitioning Oil Recovery Agent	1 1 5 6	230027 370017 220088 220016
Chromium™ i7 Multiplex Kit (store at -20°C)			120262
	Chromium™ i7 Sample Index Plate	1	220103

Chromium™ Accessories

Product	Description	Part Number
10x™ Vortex Adapter	The 10x Vortex Adapter attaches to the top of a standard laboratory vortexer and enables users to vortex Gel Bead Strips.	 330002
10x™ Chip Holder	<p>The 10x Chip Holder encases the Chromium Chips and holds them in the correct position in the Chromium Controller. The 10x Gasket fits over the top of the 10x Chip Holder before inserting the assembly in the Chromium Controller.</p> <p>The 10x Chip Holder lid also conveniently flips over to become a stand, holding the Chromium Chip at the ideal 45° angle for removing GEMs from the Recovery Wells after a Chromium Controller run.</p> <p>Squeeze the black sliders on the back side of the 10x Chip Holder together to unlock the lid and return the 10x Chip Holder to a flat position.</p>  	330019
10x™ Magnetic Separator	The 10x Magnetic Separator offers two positions of the magnets relative to the 8-tube strip inserted, depending on its orientation. Simply flip the 10x Magnetic Separator over to switch between the magnets being High or Low.	230003

Additional Kits, Reagents & Equipment

Supplier	Description	Part Number (US)
Qiagen	Buffer EB	19086
Thermo Fisher Scientific	DynaBeads® MyOne™ Silane Beads*	37002D
	Nuclease-Free Water	AM9937
	Low TE Buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA)	12090-015
Sigma	Ethanol, Pure (200 Proof, anhydrous)	459836-500ML
Beckman Coulter	SPRIselect Reagent Kit*	B23318
USA Scientific	TempAssure PCR 8-tube strip* (alternate to Eppendorf product)	14024700
Eppendorf	twin.tec® 96-Well PCR Plate* Semi-skirted§	951020362
	twin.tec® 96-Well PCR Plate* Divisible, unskirted§	0030133374
	twin.tec® 96-Well PCR Plate* Unskirted§	0030133390
	ThermoMixer C®	5382000015
	SmartBlock 1.5 ml, Thermoblock for 24 Reaction Vessels	5360000038
	DNA LoBind Tubes, 0.5 ml*	022431005
	DNA LoBind Tubes, 1.5 ml*	022431021
	DNA LoBind Tubes, 2.0 ml*	022431048
	PCR Tubes 0.2 ml 8-tube strips* (alternate to USA Scientific product)	951010022
Bio-Rad	Heat Sealing Foil, PCR clean (alternate to Bio-Rad product)	0030127854
	PX1 PCR Plate Sealer**	1814000
	Optical Flat 8-Cap Strips	TCS0803
	Pierceable Foil Heat Seal**	1814040
	10% Tween 20	1610781
KAPA Biosystems	Microseal 'B' Adhesive Seals	MSB1001
	Illumina Library Quantification Kit	KK4824
-	qPCR instrument and compatible consumables	-
VWR	Vortex Mixer*	10153-838
	Divided Polystyrene Reservoirs**	41428-958
Agilent†	2100 Bioanalyzer Laptop Bundle	G2943CA
	High Sensitivity DNA Kit	5067-4626
	4200 TapeStation	G2291aa
	High Sensitivity D1000 ScreenTape	5067-5584
	High Sensitivity D1000 Reagents	5067-5585
Ricca Chemical Company	Glycerin (glycerol), 50% (v/v) Aqueous Solution	3290-32
Table continued on next page		

INTRODUCTION

Supplier	Description	Part Number (US)
Rainin	Tips LTS 20UL Filter RT-L10FLR	17007957
	Tips LTS 200UL Filter RT-L200FLR*	17007961
	Tips LTS 1ML Filter RT-L1000FLR	17007954
	Tips LTS W-O 1MLUL Fltr RT-L1000WFLR	17014297
	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
	Pipet-Lite LTS Pipette L-200XLS+	17014391
	Pipet-Lite LTS Pipette L-1000XLS+	17014382
	Pipet-Lite Multi Pipette L8-10XLS+	17013802
	Pipet-Lite Multi Pipette L8-20XLS+	17013803
	Pipet-Lite Multi Pipette L8-50XLS+	17013804
	Pipet-Lite Multi Pipette L8-200XLS+	17013805

*No substitutions are allowed. Items have been validated by 10x Genomics® and are required for Single Cell 3' workflow, training and system operations. §Eppendorf twin.tec® brand PCR plates are required to ensure stability of GEM emulsions, but the specific model should be selected based on compatibility with thermal cycler in use. **Substituting materials may adversely affect system performance and are not supported.

†Either Bioanalyzer or TapeStation needed for cDNA quantitation and quality control.

PCR 8-tube strips

USA Scientific TempAssure PCR 8-tube strip and Eppendorf PCR Tubes 0.2 ml 8-tube strips have been validated by 10x Genomics®.

If USA Scientific or Eppendorf 8-tube strips are not available in your region, alternatives are MicroAmp® and BIoplastics 8-tube strips and caps.

Recommended Thermal Cyclers

Thermal cyclers used with the Single Cell 3' Protocol must support uniform heating of 105 µl emulsion volumes. Thermal cyclers recommended for use with the Single Cell 3' Protocol are:

- Bio-Rad C1000 Touch™ Thermal Cycler with 96-Deep Well Reaction Module (PN-1851197)
- Eppendorf MasterCycler® Pro (PN North America 950030010, International 6321 000.019)
- Thermo Fisher Veriti® 96-Well Thermal Cycler (PN-4375786)

The Single Cell 3' Reagent Kit Protocol

Stepwise Objectives

Steps & Timing

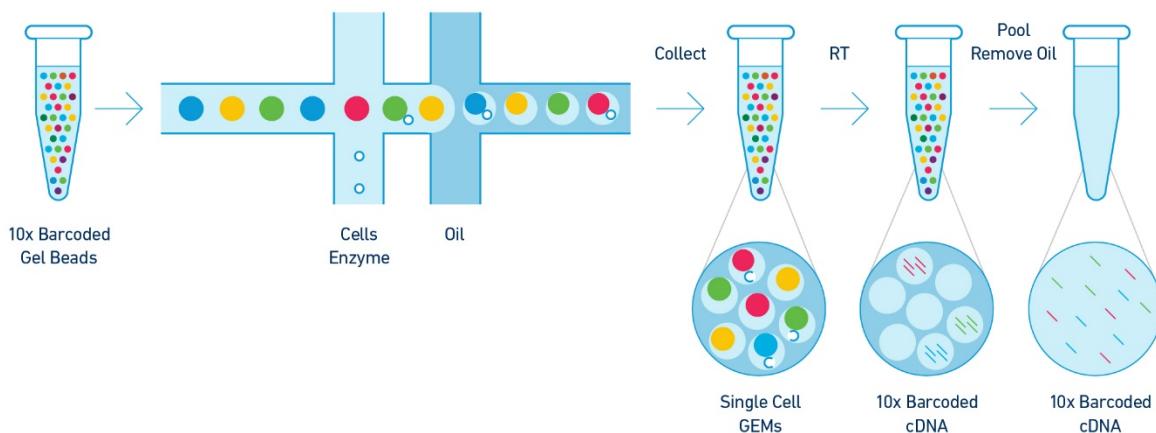
The Single Cell 3' Reagent Kit Protocol – Stepwise Objectives

Step 1 – GEM Generation & Barcoding

The Single Cell 3' Protocol upgrades short read sequencers to deliver a scalable microfluidic platform for 3' digital gene expression profiling of 500 – 10,000 individual cells per sample. The 10x™ GemCode™ Technology samples a pool of ~ 750,000 barcodes to separately index each cell's transcriptome. It does so by partitioning thousands of cells into nanoliter-scale Gel Bead-In-EMulsions (GEMs), where all generated cDNA share a common 10x Barcode. Libraries are generated and sequenced from the cDNA and the 10x Barcodes are used to associate individual reads back to the individual partitions.

To achieve single cell resolution, the cells are delivered at a limiting dilution, such that the majority (~90-99%) of generated GEMs contains no cell, while the remainder largely contain a single cell.

Upon dissolution of the Single Cell 3' Gel Bead in a GEM, primers containing (i) an Illumina R1 sequence (read 1 sequencing primer), (ii) a 16 bp 10x Barcode, (iii) a 10 bp randomer and (iv) a poly-dT primer sequence are released and mixed with cell lysate and Master Mix. Incubation of the GEMs then produces barcoded, full-length cDNA from poly-adenylated mRNA. After incubation, the GEMs are broken and the pooled fractions are recovered.



Step 2 – Post GEM-RT Cleanup & cDNA Amplification

Silane magnetic beads are used to remove leftover biochemical reagents and primers from the post GEM reaction mixture. Full-length, barcoded cDNA is then amplified by PCR to generate sufficient mass for library construction.

Step 3 – Library Construction

Enzymatic Fragmentation and Size Selection are used to optimize the cDNA amplicon size prior to library construction. R1 (read 1 primer sequence) are added to the molecules during GEM incubation. P5, P7, a sample index and R2 (read 2 primer sequence) are added during library construction via End Repair, A-Tailing, Adaptor Ligation and PCR. The final libraries contain the P5 and P7 primers used in Illumina bridge amplification.

Step 4 – Sequencing Libraries

The Single Cell 3' Protocol produces Illumina-ready sequencing libraries. A Single Cell 3' Library comprises standard Illumina paired-end constructs which begin and end with P5 and P7. The Single Cell 3' 16 bp 10x™

INTRODUCTION The Single Cell 3' Reagent Kit Protocol

Barcode and 10 bp randomer is encoded in Read 1, while Read 2 is used to sequence the cDNA fragment. Sample index sequences are incorporated as the i7 index read. Read 1 and Read 2 are standard Illumina sequencing primer sites used in paired-end sequencing.

Final Library Structure:



Single Cell 3' Library Analysis

Sequencing a Single Cell 3' Library produces a standard Illumina BCL data output folder. The BCL data will include the paired-end Read 1 (containing the 16 bp 10x™ Barcode and 10 bp Randomer) and Read 2 and the sample index in the i7 index read.

The Cell Ranger™ analysis pipelines perform secondary analysis and visualization. In addition to performing standard analysis steps such as demultiplexing, alignment, and gene counting, Cell Ranger™ leverages the 10x Barcodes to generate expression data with single-cell resolution. This data type enables applications including cell clustering, cell type classification, and differential gene expression at a scale of hundreds to millions of cells.

The Single Cell 3' Reagent Kit Protocol – Steps & Timing

	Bench Time	Instrumentation Time	Stop & Store Options
	Cell Preparation Sample Dependent 1 - 1.5 h		
1 h	Reagent Prep - 20 min Loading Single Cell 3' Chip - 10 min Transferring GEMs - 3 min	GEM Generation - 6.5 min	
2 h		GEM-RT Incubation - 55 min	STOP 4°C ≤72 h
3 h	Post GEM-RT Recovery - 10 min Cleanup - Silane Beads - 35 min cDNA Amplification Prep - 5 min	cDNA Amplification - 30 - 45 min	STOP 4°C ≤72 h
4 h	Cleanup - SPRiselect - 20 min	QC & Quantification - 50 min	STOP 4°C ≤72 h or -20°C ≤1 week
5 h	Frag, End Repair & A-tailing Prep - 10 min	Fragmentation, End Repair & A-tailing Incubation - 40 min	
6 h	Double Sided SPRiselect Sizing - 25 min Adaptor Ligation Prep - 10 min	Adaptor Ligaton Incubation - 15 min	
7 h	Cleanup - SPRiselect - 20 min Sample Index PCR Prep - 10 min	Sample Index PCR - 20 - 40 min	STOP 4°C ≤72 h
8 h	Double Sided SPRiselect Sizing - 25 min	QC - 50 min	STOP 4°C ≤72 h or -20°C long-term
		qPCR Quantification*	

*qPCR Quantification (~ 1 h total) time not included.

Protocol Step 1

GEM Generation & Barcoding

Partition input cells across tens of thousands
of GEMs for lysis and barcoding

1

1. GEM Generation & Barcoding

Tips

Importance of Emulsion-safe Plastic Consumables

Some plastics can interact with and destabilize GEMs. It is therefore critical to use validated emulsion-safe plastic consumables when handling GEMs. 10x Genomics® has validated Eppendorf twin.tec® PCR plates and Rainin LTS Low retention pipette tips as GEM-compatible plastics. Substituting these materials can adversely affect performance.

Importance of Loading Cell Concentration

The recommended starting point for a new sample type is to load ~1700 cells into each reaction, recovering approximately 1000 cells, to achieve an expected multiplet rate of approximately 0.8%. Loading fewer cells per reaction will result in a lower multiplet rate while loading more cells per reaction will elevate the multiplet rate (see table below).

Multiplet Rate (%)	# of Cells Loaded	# of Cells Recovered
~0.4%	~870	~500
~0.8%	~1700	~1000
~1.6%	~3500	~2000
~2.3%	~5300	~3000
~3.1%	~7000	~4000
~3.9%	~8700	~5000
~4.6%	~10500	~6000
~5.4%	~12200	~7000
~6.1%	~14000	~8000
~6.9%	~15700	~9000
~7.6%	~17400	~10000

Best Practices for Handling Single Cell 3' Chips

The generation of GEMs occurs in channels that are narrower than the typical human hair (i.e. <100 µm). Care should be taken to avoid introduction of particles, fibers or clumped cells into these channels. Prepare reagents and load the chips in a positive-pressure laminar flow hood, and filter the single cell suspension before addition to the Master Mix whenever possible. Minimize exposure of reagents, chips, and gaskets to sources of particles and fibers, such as open reagent reservoirs, laboratory wipes, frequently opened flip-cap tubes, clothing that easily sheds fibers, and dusty surfaces. The presence of excess Partitioning Oil in recovered GEMs from the Single Cell 3' Chip after running the Chromium™ Controller may indicate that a clog occurred. See Practical Tips & Troubleshooting for more information (Section 5).

Pay particular attention to the timing of loading and running chips. Steps should be executed successively without pauses or delays. When multiple chips are to be used, load and run the chips in series, collecting the GEMs from the one chip before loading the next.

PROTOCOL STEP 1 GEM Generation & Barcoding

All input wells (rows 1, 2, and 3) of unused channels on a chip should be filled with a 50% volume/volume aqueous solution of glycerol before loading the used wells with reagents. See Practical Tips & Troubleshooting (Section 5) for information on purchasing or preparing a 50% glycerol solution.

When removing a chip from the box and inserting it into a Chip Holder, avoid contacting the bottom surface of the chip with gloved hands and other surfaces. Frictional charging can interfere with correct priming of the channels, potentially leading to either clogs or wetting failures. See Practical Tips & Troubleshooting (Section 5) for the definition of these failures.

Minimize the distance that a loaded chip has to be moved to reach the instrument. When transferring to the Chromium™ Controller, keep the chip horizontal to prevent wetting the gasket with oil, which depletes the input volume and may adversely affect the quality of the resulting emulsion.

Best Practices – Preparing & Handling Reagents & Master Mixes

- Cover Partitioning Oil tubes and reservoirs to minimize evaporation.
- Ensure that reagents are fully thawed and thoroughly mixed before use. Keep all enzyme components and Master Mixes on ice during setup and promptly move reagents back to the recommended storage temperature when possible.
- Assemble Master Mix on ice and keep cold until Single Cell 3' Chip loading.
- For tips on processing fewer than 8 reactions, see Practical Tips & Troubleshooting (Section 5).

Best Practices – Handling Gel Beads

- Equilibrate the Single Cell 3' Gel Beads Strip to room temperature before use.
- Store any unused Single Cell 3' Gel Beads at -80°C and avoid more than 4 freeze-thaw cycles.
- Never store Single Cell 3' Gel Beads at -20°C.
- Pierce the Gel Bead Strip foil seals with pipette tips without engaging the plunger.
- Upon initial Gel Bead Strip foil seal puncture, the pipette tips should extend no more than 2 mm below the seal. Then, raise the tips above the foil seal and depress the plunger. Lower the tips to the bottom of the wells and widen the opening by gently rocking the tips back and forth, keeping the plunger engaged. This technique will maximize recovery of Gel Beads for optimal performance.

Best Practices – Loading the Single Cell 3' Chip

- Wait >30 sec between loading the Master Mix and loading the Gel Beads to ensure proper priming of the channels. Vortex the Single Cell 3' Gel Bead Strip for 30 sec AFTER loading the Master Mix to ensure that the correct time has passed before loading the Gel Beads. Do not exceed 120 sec between loading Master Mix and Gel Beads.
- When aspirating Gel Beads from the Gel Bead Strip or emulsion from the Recovery Wells, pipet slowly to avoid introducing air bubbles and leave the pipette tips in the wells for an additional 5 sec after the aspiration stops to allow pressure to equilibrate.
- When dispensing Gel Beads into the Single Cell 3' Chip, wait for the remainder to drain into the bottom of the pipette tips and dispense again to ensure complete transfer.
- Start GEM generation immediately after Single Cell 3' Chip loading. Do not exceed 120 sec between loading the chip and starting the run.

Best Practices – GEM Recovery

- Retrieve GEMs immediately after the completion of a run.
- When dispensing GEMs into the PCR plate, wait for the remainder to drain into the bottom of the pipette tips and dispense again to ensure complete transfer.
- Immediately place the plate of recovered GEMs on a chilled metal block resting on ice.

Getting Started!

Equilibrate to room temperature before use:

Item	Part Number	Storage Location
Single Cell 3' Gel Beads <i>Equilibrate to room temperature 30 min before loading the Single Cell 3' Chip</i>	220104	-80°C
● RT Reagent Mix <i>Vortex and verify no precipitate</i>	220089	-20°C
● RT Primer <i>Provided as lyophilized oligos; after resuspension, store unused primers at -80°C</i>	310354	-20°C
● Additive A <i>Vortex, verify no precipitate, centrifuge briefly</i>	220074	-20°C

Resuspend:

Briefly centrifuge the tube containing the lyophilized RT Primer and then resuspend the oligo by adding 40 µl of low TE. Vortex for 15 sec at full speed, centrifuge briefly, and leave at room temperature for at least 30 min

50% glycerol solution:

If processing fewer than 8 reactions, see Practical Tips & Troubleshooting (Section 5) for information on purchasing or generating 50% glycerol solution

Place on ice:

Item	Part Number	Storage Location
○ RT Enzyme Mix <i>Maintain on ice, centrifuge briefly before adding to Master Mix</i>	220079	-20°C
Chilled Metal Block	-	-

Obtain:

Item	Part Number	Storage Location
Partitioning Oil	220088	Ambient temperature
Single Cell 3' Chip(s)	230027	Ambient temperature
10x™ Gasket(s)	370017	Ambient temperature
10x Chip Holder	330019	Ambient temperature

Plate sealer:

Set the Bio-Rad® PX1 Plate Sealer to seal at 185°C for 6 sec. Keep heat block external to sealer until plate sealing step

Cell Suspension Volume Calculator Table

Volume of Cell Suspension Stock per reaction (μ l) Volume of Nuclease-free Water per reaction (μ l)											
Cell Stock Concentration (Cells/ μ l)	Targeted Cell Recovery										
	500 cells	1000 cells	2000 cells	3000 cells	4000 cells	5000 cells	6000 cells	7000 cells	8000 cells	9000 cells	10000 cells
100	8.7 25.1	17.4 16.4	n/a								
200	4.4 29.5	8.7 25.1	17.4 16.4	26.1 7.7	n/a						
300	2.9 30.9	5.8 28.0	11.6 22.2	17.4 16.4	23.2 10.6	29.0 4.8	n/a	n/a	n/a	n/a	n/a
400	2.2 31.6	4.4 29.5	8.7 25.1	13.1 20.8	17.4 16.4	21.8 12.1	26.1 7.7	30.5 3.4	n/a	n/a	n/a
500	1.7 32.1	3.5 30.3	7.0 26.8	10.4 23.4	13.9 19.9	17.4 16.4	20.9 12.9	24.4 9.4	27.8 6.0	31.3 2.5	n/a
600	1.5 32.4	2.9 30.9	5.8 28.0	8.7 25.1	11.6 22.2	14.5 19.3	17.4 16.4	20.3 13.5	23.2 10.6	26.1 7.7	29.0 4.8
700	1.2 32.6	2.5 31.3	5.0 28.8	7.5 26.3	9.9 23.9	12.4 21.4	14.9 18.9	17.4 16.4	19.9 13.9	22.4 11.4	24.9 8.9
800	1.1 32.7	2.2 31.6	4.4 29.5	6.5 27.3	8.7 25.1	10.9 22.9	13.1 20.8	15.2 18.6	17.4 16.4	19.6 14.2	21.8 12.1
900	1.0 32.8	1.9 31.9	3.9 29.9	5.8 28.0	7.7 26.1	9.7 24.1	11.6 22.2	13.5 20.3	15.5 18.3	17.4 16.4	19.3 14.5
1000	0.9 32.9	1.7 32.1	3.5 30.3	5.2 28.6	7.0 26.8	8.7 25.1	10.4 23.4	12.2 21.6	13.9 19.9	15.7 18.1	17.4 16.4
1100	0.8 33.0	1.6 32.2	3.2 30.6	4.7 29.1	6.3 27.5	7.9 25.9	9.5 24.3	11.1 22.7	12.7 21.1	14.2 19.6	15.8 18.0
1200	0.7 33.1	1.5 32.4	2.9 30.9	4.4 29.5	5.8 28.0	7.3 26.6	8.7 25.1	10.2 23.7	11.6 22.2	13.1 20.8	14.5 19.3
1300	0.7 33.1	1.3 32.5	2.7 31.1	4.0 29.8	5.4 28.4	6.7 27.1	8.0 25.8	9.4 24.4	10.7 23.1	12.0 21.8	13.4 20.4
1400	0.6 33.2	1.2 32.6	2.5 31.3	3.7 30.1	5.0 28.8	6.2 27.6	7.5 26.3	8.7 25.1	9.9 23.9	11.2 22.6	12.4 21.4
1500	0.6 33.2	1.2 32.6	2.3 31.5	3.5 30.3	4.6 29.2	5.8 28.0	7.0 26.8	8.1 25.7	9.3 24.5	10.4 23.4	11.6 22.2
1600	0.5 33.3	1.1 32.7	2.2 31.6	3.3 30.5	4.4 29.5	5.4 28.4	6.5 27.3	7.6 26.2	8.7 25.1	9.8 24.0	10.9 22.9
1700	0.5 33.3	1.0 32.8	2.0 31.8	3.1 30.7	4.1 29.7	5.1 28.7	6.1 27.7	7.2 26.6	8.2 25.6	9.2 24.6	10.2 23.7
1800	0.5 33.3	1.0 32.8	1.9 31.9	2.9 30.9	3.9 29.9	4.8 29.0	5.8 28.0	6.8 27.0	7.7 26.1	8.7 25.1	9.7 24.1
1900	0.5 33.3	0.9 32.9	1.8 32.0	2.7 31.1	3.7 30.1	4.6 29.2	5.5 28.3	6.4 27.4	7.3 26.6	8.2 25.6	9.2 24.6
2000	0.4 33.4	0.9 32.9	1.7 32.1	2.6 31.2	3.5 30.3	4.4 29.5	5.2 28.6	6.1 27.7	7.0 26.8	7.8 26.0	8.7 25.1

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

GEM Generation & Barcoding

1.1. Preparing Single Cell Master Mix

Volumes for 8 reactions are listed in all reagent tables and include 10% excess (i.e. 8.8X).

To set up a different number of reactions (n), multiply the indicated 1 reaction volumes by $n.n$ (for example, multiply by 4.4 to set up 4 reactions with 10% excess).

NOTE

The volume of Nuclease-Free Water per reaction is specified in the Cell Suspension Volume Calculator Table. Note the corresponding volume of cell suspension per reaction for use in step 1.2d.

- Prepare Master Mix on ice. Add reagents in the order shown below. Pipette mix 15 times and centrifuge briefly. **Do not add Single Cell Suspension at this point.**

Master Mix	Part Number	1 rxn (μl)	8 rxns + 10% excess (μl)
Nuclease-Free Water	-	From Table	Multiply by 8.8X
● RT Reagent Mix	220089	50.0	440.0
● RT Primer	310354	3.8	33.4
● Additive A	220074	2.4	21.1
○ RT Enzyme Mix	220079	10.0	88.0
Total	-	Sum	Multiply by 8.8X

- Place the Master Mix on a chilled metal block resting on ice.

1.2. Loading the Single Cell 3' Chip

- Place a Single Cell 3' Chip in a 10x™ Chip Holder. Handle the chip by its edges, taking care to avoid touching its bottom surface. See Practical Tips & Troubleshooting (Section 5) for tips on assembly.

CRITICAL!

The order in which the wells of Single Cell 3' Chips are loaded is critical for optimal performance. Always load the rows in the labeled order: 1 followed by 2, then 3.

- If processing fewer than 8 samples per Single Cell 3' Chip, first add the following volumes of 50% glycerol solution to each unused well:
 - 90 μl** in the row labeled 1
 - 40 μl** in the row labeled 2
 - 270 μl** in the row labeled 3

CRITICAL!

Do not add 50% glycerol solution to Recovery Wells (row labeled ▲). Do not use Partitioning Oil or any other solution as a substitute for 50% glycerol solution.

- Dispense the **calculated total volume (μl)** of Master Mix into each well of an 8-tube strip on a chilled metal block resting on ice.

PROTOCOL STEP 1 GEM Generation & Barcoding

CRITICAL!

The next step is critical to recovering the maximum number of cells. It is important to ensure that all the cells in the sample are suspended and that the sample is homogenous when adding the cell suspension volume to the Master Mix. To do this, estimate the volume of the cell suspension and set the pipette at half that volume for pipette mixing. When withdrawing the volume of cell suspension from the tube, place the pipette tip near the center of the suspension volume. Cells settle at different rates, so it is important to mix directly before taking the sample.

- d) Gently pipette mix the tube containing the washed and diluted cells. Add the appropriate **volume (μl)** of **single cell suspension** (from the Cell Suspension Volume Calculator Table) to each well of the tube strip containing the Master Mix.

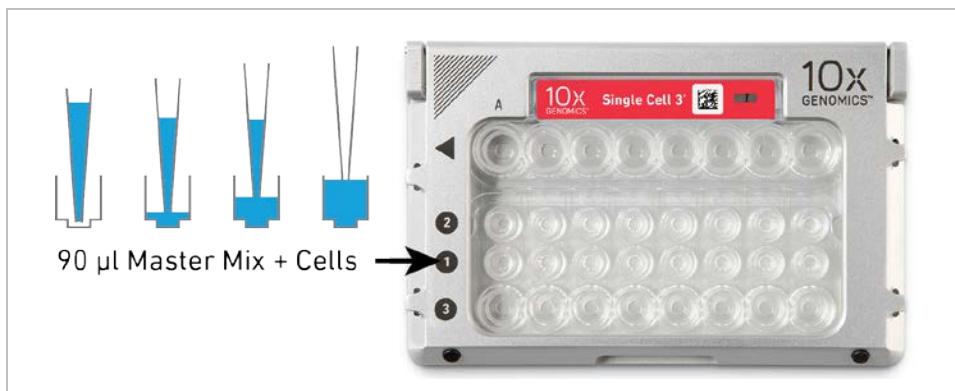
NOTE

The total volume of the combined cells and Master Mix is 100 μl in each well.

- e) With a pipette set to 90 μl, gently pipette mix the combined cells and Master Mix 5 times while keeping the tube strip on a chilled metal block resting on ice.
- f) Without discarding the pipette tips, transfer **90 μl** Master Mix containing cells to the wells in the **row labeled 1**, taking care not to introduce bubbles. To do this, place the tips into the bottom center of the wells and raise the tips slightly above the bottom before slowly dispensing the Master Mix containing cells.

CRITICAL!

Pipette slowly. Raising and depressing the pipette plunger should each take 2 sec. Raise the pipette tips at the same rate as the liquid level is rising in the well, keeping the tip slightly submerged.



- g) Snap the Single Cell 3' Gel Bead Strip into a 10x™ Vortex Adapter and vortex for **30 sec**.
A 30 sec wait while vortexing the Single Cell 3' Gel Bead Strip is required to ensure proper priming of the Master Mix containing cells in the Single Cell 3' Chip. Then, immediately load the Single Cell 3' Gel Beads.
- h) Remove the Single Cell 3' Gel Bead Strip and flick in a sharp, downward motion to ensure maximum recovery. Confirm that there are no bubbles at the bottom of the tube and that liquid levels are uniform.

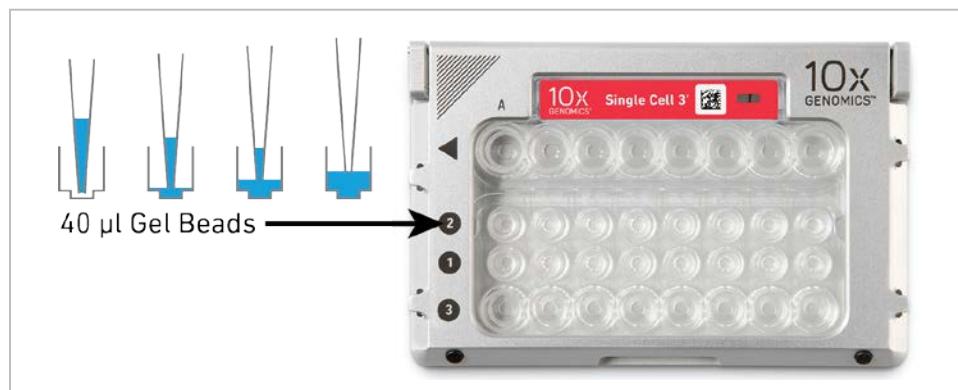
NOTE

Pipette Single Cell 3' Gel Beads slowly as they have a viscosity similar to high-concentration glycerol.

PROTOCOL STEP 1 GEM Generation & Barcoding

- i) Carefully puncture the foil seal and slowly aspirate **40 µl** Single Cell 3' Gel Beads, taking care not to introduce air bubbles.

NOTE
Only puncture the foil of a number of wells in the Single Cell 3' Gel Bead Strip equal to the number of samples that will be processed.
- j) Slowly dispense the Single Cell 3' Gel Bead suspension **into the bottom of the wells in the row labeled 2**, taking care not to introduce bubbles. To do this, place the tips into the bottom center of the wells and raise the tips slightly above the bottom before slowly dispensing the Gel Beads.



CRITICAL!

Raise the pipette tips at the same rate as the liquid level is rising in the wells, keeping the tip slightly submerged. Confirm that the pipette tips do not contain leftover Gel Beads. If necessary, wait for the remaining Gel Beads to drain into the bottom of the pipette tips and dispense into the wells without introducing bubbles.

- k) Pipette a total volume of **270 µl** of Partitioning Oil into the wells in the **row labeled 3** by **pipetting two aliquots of 135 µl** from a reagent reservoir. Do not add Partitioning Oil to any unused input wells that already contain 50% glycerol solution.



CRITICAL!

Failure to add Partitioning Oil can damage the Chromium™ Controller or the Chromium Single Cell Controller.

- l) Attach the 10x™ Gasket. The notched cut should be at the top left corner. Ensure the 10x Gasket holes are aligned with the wells. Avoid touching the smooth side of the 10x

PROTOCOL STEP 1 GEM Generation & Barcoding

Gasket and do not press down on the top of the 10x™ Gasket after attachment. Keep the assembly horizontal to avoid wetting the 10x Gasket with Partitioning Oil.

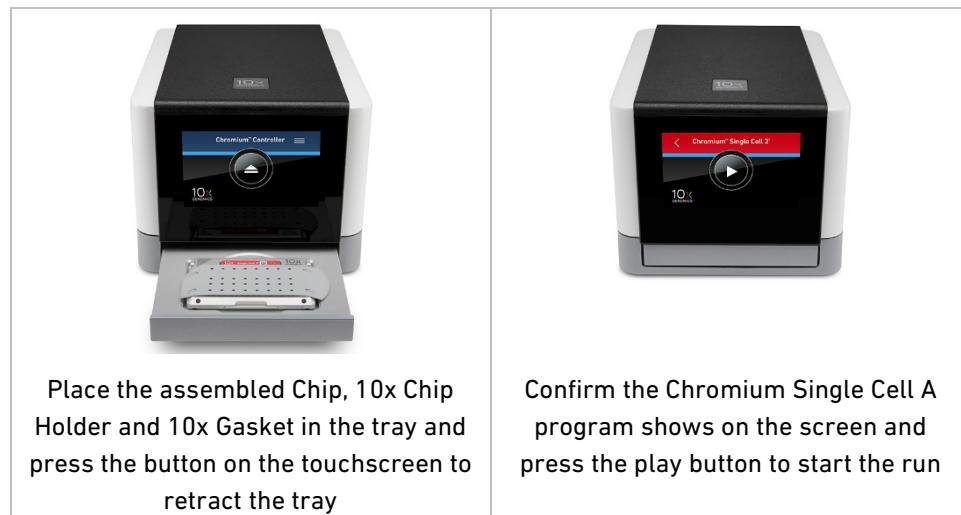


1.3. Running the Chromium™ Controller

NOTE

The same instructions apply to the Chromium Single Cell Controller.

- a) Press the button on the touchscreen of the Chromium Controller to eject the tray.
- b) Place the assembled Chip, 10x Chip Holder and 10x Gasket on the tray.
- c) Press the button on the touchscreen again to retract the tray. Confirm the Chromium Single Cell A program shows on screen and press the play button to begin the run.
- d) At the completion of the run (~6.5 min), the Chromium Controller will chime. Proceed immediately to the next step.



1.4. Transferring GEMs

- a) Maintain an Eppendorf twin.tec® 96-Well PCR plate for GEM transfer on a chilled metal block resting on ice.
- b) Press the eject button to eject the tray and remove the Single Cell 3' Chip. Remove and discard the 10x™ Gasket. Press the button to retract the empty tray (or the tray will automatically close after 40 sec).
- c) Open the 10x Chip Holder and fold the lid back until it clicks to expose the wells at a 45-degree angle.
- d) Check for volume uniformity in the Gel Bead, Sample, and Partitioning Oil wells remaining in the Single Cell 3' Chip.

**NOTE**

Abnormally high volume in any of the wells may indicate that a clog occurred during GEM generation.

- e) Slowly aspirate **100 µL** GEMs from the lowest points of the Recovery Wells (**row labeled ◀**) without creating a seal between the tips and the bottom of the wells. Avoid introducing air bubbles.

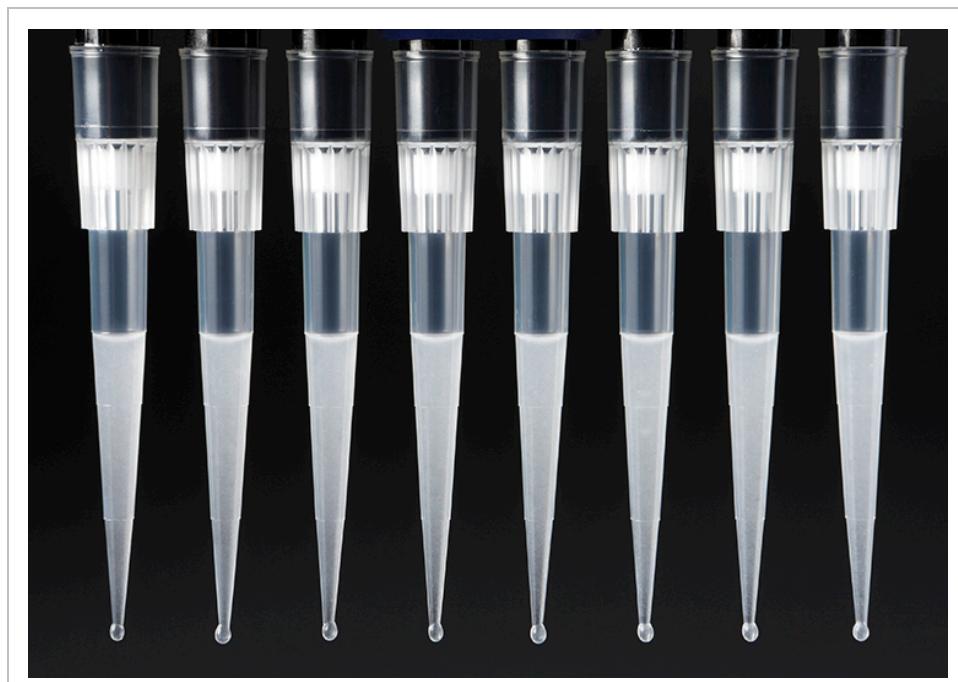
PROTOCOL STEP 1 GEM Generation & Barcoding



NOTE

Pipette GEMs slowly as they have a high viscosity. If a tip aspirates excessive air the sample may be compromised.

- f) Withdraw pipette tips from the wells and verify that there is no air in the tips. GEMs should appear opaque and uniform across all channels.



NOTE

The presence of excess Partitioning Oil (clear) in the pipette tips indicates a potential clog during GEM generation.

PROTOCOL STEP 1 GEM Generation & Barcoding

- g) Over the course of ~20 sec, dispense the GEMs into the Eppendorf® twin-tec 96-Well PCR plate (on a chilled metal block resting on ice) with the pipette tips **against the sidewalls of the wells**. (See Practical Tips & Troubleshooting, Section 5). Keep the tips above the liquid level to minimize GEMs lost on the outside of the tips.



NOTE

Check the volume uniformity of the GEMs and the Partitioning Oil in the PCR plate. A clog occurred if the Partitioning Oil volume in one or more wells is increased compared to other wells. See Practical Tips & Troubleshooting for more information (Section 5).

- h) If multiple Single Cell 3' Chips are run back-to-back, keep plate containing recovered GEMs on ice and seal the plate wells containing GEMs with Strip Caps before proceeding to load reagents into the next chip. Avoid storing the GEMs on ice for more than 1 h.
- i) Discard the used Single Cell 3' Chip. Push the black sliders on the back of the 10x™ Chip Holder toward the middle to release the lock and close the lid.

1.5. GEM-RT Incubation

- a) If necessary, remove the strip caps from the PCR plate with recovered GEMs. Check that the Plate Sealer plate block is at room temperature.
- b) Seal the plate with pierceable foil heat seal at **185°C** for **6 sec** and promptly remove.
- c) Load the sealed PCR plate into a thermal cycler that can accommodate at least 100 µl reaction volume and proceed with the following incubation protocol.

NOTE

A reaction volume of 125 µl is the preferred setting on the Bio-Rad C1000 Touch™ Thermal Cycler. If using an alternate thermal cycler, the highest reaction volume setting should be used.

Lid Temperature	Reaction Volume	Run Time
53°C	125 µl	~55 min
Step	Temperature	Time
1	53°C	45:00
2	85°C	5:00
3	4°C	Hold

- d) Place PCR plate on a chilled metal block resting on ice after completion of the thermal cycler program.
- e) Store in the PCR plate at **4°C** for up to **72 h** before proceeding to Post GEM-RT Cleanup.

STOP

Protocol Step 2

Post GEM-RT Cleanup & cDNA Amplification

Isolate and amplify cDNA for library construction

2

2. Post GEM-RT Cleanup & cDNA Amplification

Tips

Best Practices

Ensure that the reagents are fully thawed and thoroughly mixed before use. During the bead-based cleanup steps, ensure that the samples are thoroughly mixed with the Silane beads or the SPRIselect Reagent to achieve optimal recovery. Always use fresh preparations of 80% Ethanol.

Best Practices – Post cDNA Amplification Reaction QC

Agilent Bioanalyzer analysis is the recommended method for ensuring successful cDNA amplification before proceeding into library construction. Accurate quantification of cDNA at this step is necessary to determine Total Sample Index Cycles for the Sample Index PCR (step 3.5).

Getting Started!

Equilibrate to room temperature before use:

Item	Part Number	Storage Location
● Additive A <i>Vortex, verify no precipitate, centrifuge briefly</i>	220074	-20°C
DynaBeads® MyOne™ Silane beads	-	Manufacturer's recommendation
Beckman Coulter SPRiselect Reagent	-	Manufacturer's recommendation
Agilent Bioanalyzer High Sensitivity Kit	-	Manufacturer's recommendation
● cDNA Primer Mix	220106	-20°C
● cDNA Additive	220067	-20°C

Place on ice:

Item	Part Number	Storage Location
● Amplification Master Mix	220125	-20°C

Obtain:

Item	Part Number	Storage Location
○ Recovery Agent	220016	Ambient temperature
Qiagen Buffer EB	-	Manufacturer's recommendation
Bio-Rad 10% Tween 20	-	Manufacturer's recommendation
10x™ Magnetic Separator	230003	Ambient temperature

Thaw at 65°C:

Item	Part Number	Storage Location
● Buffer Sample Clean Up 1	220020	-20°C

Thaw Buffer Sample Clean Up 1 for 10 min at 65°C at max speed on a thermomixer. Verify there are no visible crystals. Let cool to room temperature.

Prepare 80% Ethanol (15 ml for 8 samples)

Post GEM-RT Cleanup & cDNA Amplification

2.1. Post GEM-RT Cleanup – Silane DynaBeads

- a) Remove the foil seal and add **125 µl** Recovery Agent to each well containing post incubation GEMs. Do not pipette mix or vortex the biphasic mixture. Wait **60 sec** and then transfer the entire volume to an 8-tube strip.

NOTE

After transferring the initial volume to a tube strip, aqueous phase recovery can be maximized by lightly sealing the plate with a Microseal® 'B' Adhesive Seal and spinning in a plate centrifuge at 1200 rpm for 30 sec. The collected volumes can be combined with those previously transferred into the tube strip.

- b) The recovered biphasic mixture contains distinct Recovery Agent/Partitioning Oil (pink) and aqueous phases (clear), with no persisting emulsion (opaque).



NOTE

A decrease in the aqueous phase indicates that a clog occurred during GEM generation.

- c) Slowly remove **125 µl** Recovery Agent/Partitioning Oil (pink) from the bottom of the tubes and discard. Be careful not to aspirate any of the clear aqueous sample.

A small volume of Recovery Agent/Partitioning Oil will remain.

NOTE

Do not aspirate the aqueous solution during Recovery Agent/Partitioning Oil removal. Should aspiration of the aqueous solution occur, return the solution to the tube strip, reduce removal volume by 5 µl, and reattempt removal.



PROTOCOL STEP 2 Post GEM-RT Cleanup & cDNA Amplification

- d) Vortex DynaBeads MyOne Silane beads until fully resuspended. Prepare DynaBeads Cleanup Mix by adding reagents in the order shown below. Vortex mix thoroughly.

DynaBeads Cleanup Mix	Part Number	1X (μ l)	8 rxns + 10% excess (μ l)
Nuclease-Free Water	-	9	79
● Buffer Sample Clean Up 1	220020	182	1602
DynaBeads MyOne Silane	-	4	35
● Additive A	220074	5	44
Total	-	200	1760

- e) Immediately add **200 μ l** DynaBeads Cleanup Mix to each sample. Pipette mix 5 times (pipette set to 200 μ l) and incubate at room temperature for **10 min**.



- f) Prepare Elution Solution I by adding reagents in the order shown below. Vortex mix thoroughly and centrifuge briefly.

Elution Solution I	Part Number	1 rxn (μ l)	10 rxns (μ l)
Buffer EB	-	98	980
10% Tween 20	-	1	10
● Additive A	220074	1	10
Total	-	100	1000

- g) After the 10 min incubation step is complete, place the tube strip into a 10x™ Magnetic Separator in the **High** position until the supernatant is clear.

A white interface may appear between the aqueous solution and Recovery Agent layers. This is normal.

- h) Carefully remove and discard the supernatant.
i) Add **150 μ l** freshly prepared 80% ethanol **twice** to the pellet while on the magnet for a total volume of 300 μ l and stand for **30 sec**.

NOTE

PROTOCOL STEP 2 Post GEM-RT Cleanup & cDNA Amplification

- j) Carefully remove and discard the ethanol wash.
- k) Add **200 µl** 80% ethanol to the pellet and stand for **30 sec.**
- l) Carefully remove and discard the ethanol wash.
- m) Centrifuge the tube strip briefly and return it to a 10x™ Magnetic Separator in the **Low** position.
- n) Remove and discard any remaining ethanol and allow the samples to air dry for **1 min.**
- o) Remove the tube strip from the magnet and add **35.5 µl** Elution Solution I. Pipette mix thoroughly until beads are fully resuspended (pipette set to 30 µl to avoid introducing air bubbles).

NOTE

Silane Dynabeads can be difficult to resuspend due to residual reagents from the GEM reaction. To aid resuspension, the tube strip can be capped, vortexed for 5 sec and then centrifuged briefly prior to incubation.

- p) Incubate at room temperature for **1 min.**
- q) Place the tube strip in a 10x Magnetic Separator in the **Low** position until the solution is clear.
- r) Transfer **35 µl** of eluted sample to a new tube strip.

2.2. cDNA Amplification Reaction

- a) Prepare cDNA Amplification Reaction Mix on ice. Add reagents in the order shown below. Vortex mix and centrifuge briefly. **Do not add Purified GEM-RT Product at this point.**

cDNA Amplification Reaction Mix	Part Number	1 rxn (μ l)	8 rxns + 10% excess (μ l)
Nuclease-Free Water	-	8	70
● Amplification Master Mix	220125	50	440
● cDNA Additive	220067	5	44
● cDNA Primer Mix	220106	2	18
Total	-	65	572

- b) Add **65 μ l** cDNA Amplification Reaction Mix to each tube containing **35 μ l** of purified GEM-RT product.
- c) Pipette mix 15 times (pipette setting 90 μ l) and centrifuge briefly.
- d) Cap and load the tube strip into a thermal cycler that can accommodate at least 100 μ l reaction volume and proceed with the following incubation protocol.

Lid Temperature	Reaction Volume	Run Time
105°C	100 μ l	~30-45 min
<hr/>		
Step	Temperature	Time
1	98°C	3:00
2	98°C	0:15
3	67°C	0:20
4	72°C	1:00
5	Go to Step 2, see table below for # of cycles	
6	72°C	1:00
7	4°C	Hold

NOTE

The optimal number of cycles for the cDNA amplification reaction is a trade-off between generating sufficient mass for the subsequent library construction steps and minimizing PCR amplification artifacts. If large numbers of cells are sampled, the total number of cDNA amplification cycles should be reduced. The following table is a recommended starting point for optimization.

Targeted Cell Recovery	Total cDNA Amplification Cycles
<2000	14
2000 – 6000	12
6000 – 10000	10
>10000	8



- e) Store the samples at **4°C** in a tube strip for up to **72 h** or proceed directly to SPRIselect Cleanup.

2.3. Post cDNA Amplification Reaction Cleanup – SPRIselect

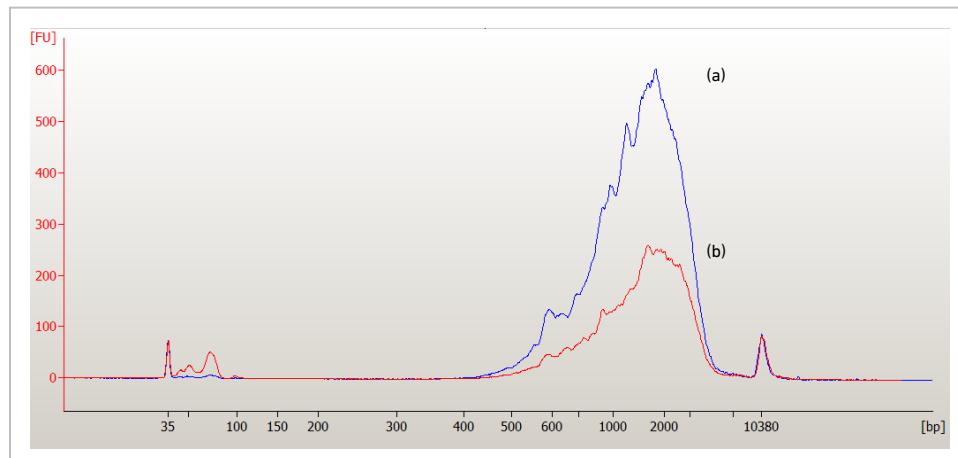
Repeat

- a) Vortex the SPRIselect Reagent until fully resuspended. Add **60 µl** SPRIselect Reagent (**0.6X**) to each sample in the tube strip and pipette mix 15 times (pipette set to 150 µl).
- b) Incubate the tube strip at room temperature for **5 min**.
- c) Place the tube strip in a 10x™ Magnetic Separator in the **High** position until the solution is clear.
- d) Carefully remove and discard the supernatant.
- e) Add **200 µl** 80% ethanol to the pellet and stand for **30 sec**.
- f) Carefully remove and discard the ethanol wash.
- g) **Repeat** steps e and f for a total of 2 washes.
- h) Centrifuge the tube strip briefly and return it to a 10x Magnetic Separator in the **Low** position.
- i) Remove and discard any remaining ethanol and allow the samples to air dry for **2 min**. **Do not exceed 2 min** as this will lead to decreased elution efficiency.
- j) Remove the tube strip from the 10x Magnetic Separator and add **40.5 µl** Buffer EB.
- k) Pipette mix 15 times and incubate at room temperature for **2 min**.
- l) Place the tube strip in a 10x Magnetic Separator in the **High** position until the solution is clear.
- m) Transfer **40 µl** of sample to a new tube strip and cap the sample wells.
- n) Store the samples at **4°C** in a tube strip for up to **72 h** or at **-20°C** for up to a **week**, or proceed directly to Post cDNA Amplification QC.



2.4. Post cDNA Amplification Reaction QC & Quantification

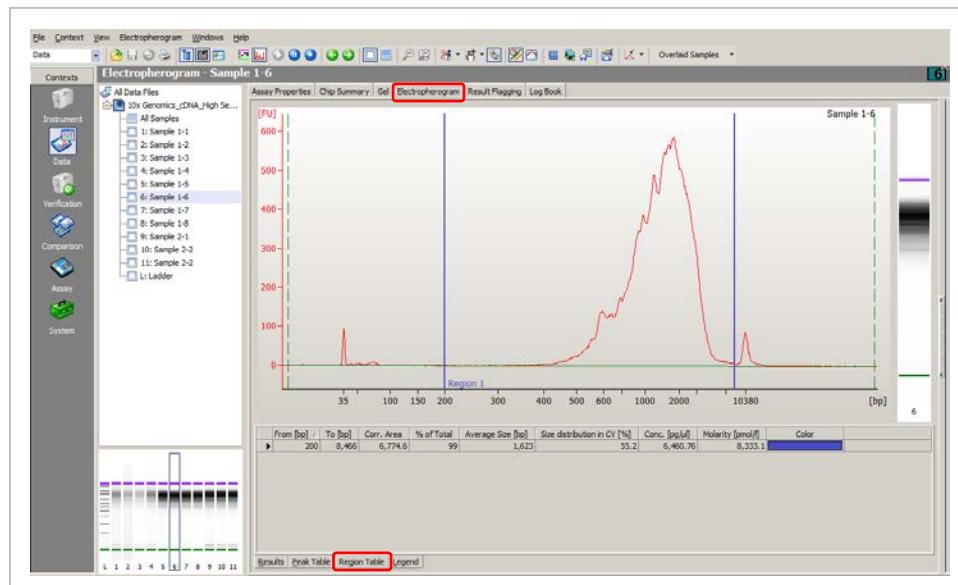
- a) Run **1 μ l** of sample at a dilution of 1 part sample:5 parts Nuclease-Free Water on the Agilent Bioanalyzer High Sensitivity chip for qualitative analysis (trace a). Traces should resemble the overall shape of the sample electropherograms shown below.



NOTE

If the input cells are particularly RNA-poor (< 1 pg total RNA/cell), it may be necessary to run 1 μ l of undiluted product (trace b). Lower molecular weight product (between 35 – 150 bp) may be present. This is normal and does not affect sequencing or application performance.

- b) To determine the cDNA yield per sample, under the "Electropherogram" view choose the "Region Table" tab on the Agilent 2100 Expert Software.
c) Manually select the region encompassing ~200 – ~9000 bp.

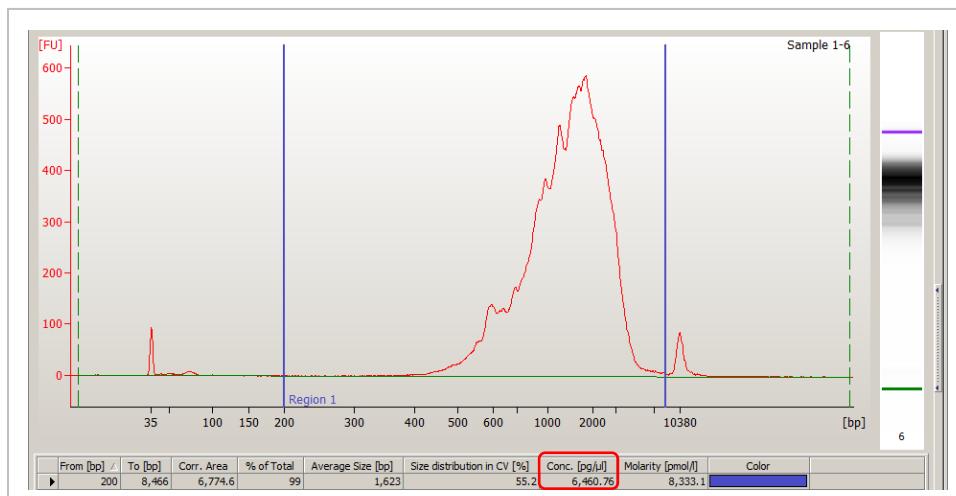


PROTOCOL STEP 2 Post GEM-RT Cleanup & cDNA Amplification

- d) Multiply the cDNA concentration [pg/ μ l] reported via the Agilent 2100 Expert Software by the elution volume (40 μ l) of the post cDNA Amplification Reaction Clean Up sample (taking any dilution factors into account) and then divide by 1000 to obtain the total cDNA yield in ng.

NOTE

This concentration will be used in step 3.5 to determine the appropriate number of Sample Index PCR cycles to generate a sufficient concentration of final library whilst minimizing PCR amplification artifacts.



Example Calculation of cDNA Total Yield:

Agilent 2100 Expert Concentration: 6460.76 [pg/ μ l]

Dilution Factor used to run the Agilent 2100 Bioanalyzer: 6

Post cDNA Amplification Clean Up Elution Volume: 40 μ l

$$\begin{aligned} \text{Total cDNA Yield: } &= \frac{\text{Concentration } \left(\frac{\text{pg}}{\mu\text{l}} \right) \times \text{Elution Volume } (\mu\text{l}) \times \text{Dilution Factor}}{1000 \left(\frac{\text{pg}}{\text{ng}} \right)} \\ &= \frac{6460.76 \left(\frac{\text{pg}}{\mu\text{l}} \right) \times 40 (\mu\text{l}) \times 6}{1000 \left(\frac{\text{pg}}{\text{ng}} \right)} \\ &= 1550 \text{ ng} \end{aligned}$$

Refer to step 3.5 for the appropriate number of Sample Index PCR cycles.

Protocol Step 3

Library Construction

Insert P5, P7, Read2, and Sample Index to
prepare for sequencing

3

3. Library Construction

Tips

General

The final Single Cell 3' Libraries contain the P5 and P7 primers used in Illumina bridge amplification PCR. The Single Cell 3' Barcode and Read 1 (primer site for sequencing read 1) is added to the molecules during the GEM-RT incubation. The P5 primer, Read 2 (primer site for sequencing read 2), Sample Index and P7 primer will be added during library construction. The protocol is designed to support library construction from a wide range of cDNA amplification yields spanning at least 2 ng to >2 µg without modification.

Best Practices – Reagents

Ensure that the reagents are fully thawed and thoroughly mixed before use. Keep all enzyme components and Master Mixes on ice during setup and promptly move back to the recommended storage temperature when possible.

Best Practices – Enzymatic Fragmentation

Ensure that Enzymatic Fragmentation reactions are prepared on a chilled metal block resting 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.

Best Practices – Double Sided Size Selection – SPRI Select

After aspirating the desired volume of SPRIselect Reagent, examine the pipette tip(s) before dispensing to ensure that the correct volume is being transferred. Pipette mix thoroughly as insufficient mixing of sample and SPRIselect Reagent will lead to inconsistent size selection results. Always use fresh preparations of 80% Ethanol.

Best Practices – Sample Index PCR

Choose the appropriate sample index sets to ensure that no sample indices overlap in a multiplexed sequencing run. Each sample index set is base-balanced to avoid monochromatic signal issues when it is the sole sample loaded on an Illumina sequencer. The sample index sets can therefore be used in any combination.

Getting Started!

Equilibrate to room temperature before use:

Item	Part Number	Storage Location
○ Fragmentation Buffer <i>Vortex, verify no precipitate, centrifuge briefly</i>	220108	-20°C
● Ligation Buffer <i>Vortex, verify no precipitate, centrifuge briefly</i>	220109	-20°C
● Adaptor Mix	220026	-20°C
● SI-PCR Primer	220111	-20°C
Chromium™ i7 Sample Index Plate	220103	-20°C
Beckman Coulter SPRIselect Reagent	-	Manufacturer's recommendation
Agilent Bioanalyzer High Sensitivity Kit <i>If used for QC</i>	-	Manufacturer's recommendation
Agilent TapeStation ScreenTape and Reagents <i>If used for QC</i>	-	Manufacturer's recommendation

Obtain:

Item	Part Number	Storage Location
Qiagen Buffer EB	-	Manufacturer's recommendation
10x™ Magnetic Separator	230003	Ambient temperature

Place on ice:

Item	Part Number	Storage Location
● Fragmentation Enzyme Blend	220107	-20°C
● DNA Ligase	220110	-20°C
● Amplification Master Mix	220125	-20°C
Chilled Metal Block	-	-
Kapa DNA Quantification Kit for Illumina Platforms	-	Manufacturer's recommendation

Prepare 80% Ethanol (20 ml for 8 samples)

Library Construction

3.1. Fragmentation, End Repair & A-tailing

NOTE

It is important to ensure Enzymatic Fragmentation reactions are prepared on a chilled metal block resting on ice and are inserted into a pre-cooled (4°C) thermal cycler.

- Prepare a thermal cycler with the following incubation protocol and initiate the **4°C** pre-cool block step prior to assembling the Fragmentation Mix.

Lid Temperature	Reaction Volume	Run Time
65°C	50 µl	~35 min
Step	Temperature	Time
Pre-cool block	4°C	Hold
End Repair	32°C	5:00
A-tailing	65°C	30:00
Hold	4°C	Hold

- Vortex the Fragmentation Buffer. Verify there is no precipitate before proceeding.
- Prepare the Fragmentation Mix on ice. Add the reagents in the order shown below. Mix thoroughly and centrifuge briefly.

Fragmentation Mix	Part Number	1 rxn (µl)	8 rxns + 10% excess (µl)
● Fragmentation Enzyme Blend	220107	10	88
○ Fragmentation Buffer	220108	5	44
Total	-	15	132

- Dispense **15 µl** Fragmentation Mix into each well of an 8-tube strip on a chilled metal block resting on ice.
- Add **35 µl** purified cDNA to each well of the tube strip containing the Fragmentation Mix. Pipette mix 15 times (pipette set to 35 µl) and centrifuge briefly before returning the tube strip to a chilled metal block resting on ice.
- Transfer the chilled tube strip into the pre-cooled thermal cycler (**4°C**) and press "SKIP" to initiate the Fragmentation protocol.

3.2. Post Fragmentation, End Repair & A-tailing Double Sided Size Selection – SPRI Select

NOTE

After aspirating the desired volume of SPRIselect Reagent, examine the pipette tip(s) before dispensing to ensure that the correct volume is being transferred. Pipette mix thoroughly as insufficient mixing of sample and SPRIselect Reagent will lead to inconsistent size selection results.

- Vortex the SPRIselect Reagent until fully resuspended. Add **30 µl** SPRIselect Reagent (**0.6X**) to each sample in the tube strip and pipette mix 15 times (pipette set to 75 µl).
- Incubate the tube strip at room temperature for **5 min**.
- Place the tube strip in a 10x™ Magnetic Separator in the **High** position until the solution is clear.

**CRITICAL!**

DO NOT discard supernatant.

- Transfer **75 µl** supernatant to a new tube strip and discard the previous tube strip.
- Add **10 µl** SPRIselect Reagent (**0.8X**) to each sample in the tube strip and pipette mix 15 times (pipette set to 75 µl).
- Incubate the tube strip at room temperature for **5 min**.
- Place the tube strip in a 10x Magnetic Separator in the **High** position until the solution is clear.



- Carefully remove and discard **80 µl** supernatant.

Due to the low volume of the SPRIselect Reagent used in this step it is crucial to not discard any of the beads with the supernatant. To ensure that beads are not discarded 5 µl of supernatant is left in each well of the tube strip. This will not impact the effectiveness of the size selection.

NOTE

- With the tube strip still in a 10x Magnetic Separator, add **125 µl** 80% ethanol to the pellet and stand for **30 sec**.

PROTOCOL STEP 3 Library Construction

- j) Carefully remove and discard the ethanol wash.
- Repeat**
- k) **Repeat** steps i and j for a total of two washes.
- l) Briefly centrifuge the tube strip and return it to the 10x™ Magnetic Separator in the **Low** position. Carefully remove and discard the remaining ethanol wash.

NOTE

Due to the low volume of SPRiselect Reagent used in this size selection it is crucial not to over-dry the beads. Proceed directly into resuspension with Buffer EB without waiting for the beads to dry to ensure maximum elution efficiency.

- m) Remove the tube strip from the 10x Magnetic Separator and add **50.5 µl** Buffer EB. Pipette mix 15 times.
- n) Incubate the tube strip at room temperature for **2 min**.
- o) Place the tube strip in a 10x Magnetic Separator in the **High** position until the solution is clear.
- p) Transfer **50 µl** of sample to a new tube strip and cap the sample wells.

3.3. Adaptor Ligation

- a) Prepare Adaptor Ligation Mix by adding the reagents in the order shown below. Mix thoroughly and centrifuge briefly.

Adaptor Ligation Mix	Part Number	1 rxn (µl)	8 rxns + 10% excess (µl)
Nuclease-Free Water	-	17.5	154
● Ligation Buffer	220109	20	176
● DNA Ligase	220110	10	88
● Adaptor Mix	220026	2.5	22
Total	-	50	440

- b) Add **50 µl** Adaptor Ligation Mix to each tube containing **50 µl** sample from the Post Fragmentation, End Repair and A-tailing Size Selection. Pipette mix 15 times (pipette set to 50 µl) and centrifuge briefly.
- c) Incubate in a thermal cycler with the following protocol.

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

- d) Proceed immediately to the next step.

3.4. Post Ligation Cleanup – SPRIselect

- a) Vortex the SPRIselect Reagent until fully resuspended. Add **80 µl** SPRIselect Reagent (**0.8X**) to each sample in the tube strip and pipette mix 15 times (pipette set to 150 µl).
- b) Incubate the tube strip at room temperature for 5 min.
- c) Place the tube strip in a 10x™ Magnetic Separator in the **High** position until the solution is clear.
- d) Carefully remove and discard the supernatant.
- e) Add **200 µl** 80% ethanol to the pellet and stand for **30 sec.**
- f) Carefully remove and discard the ethanol wash.
- Repeat**
- g) **Repeat** steps e and f for a total of 2 washes.
- h) Centrifuge the tube strip briefly and return it to a 10x Magnetic Separator in the **Low** position.
- i) Remove and discard any remaining ethanol and allow the samples to air dry for **2 min**. Do not exceed 2 min as this will lead to decreased elution efficiency.
- j) Remove the tube strip from the 10x Magnetic Separator and add **30.5 µl** Buffer EB. Pipette mix 15 times.
- k) Incubate the tube strip at room temperature for **2 min**.
- l) Place the tube strip in a 10x™ Magnetic Separator in the **Low** position until the solution is clear.
- m) Transfer **30 µl** of sample to a new tube strip.

3.5. Sample Index PCR

NOTE

Choose the appropriate sample index sets to ensure that no sample indices overlap in a multiplexed sequencing run. Record the 10x Sample Index name (Chromium™ i7 Sample Index plate well ID) used, especially if running more than one sample.

- a) Prepare Sample Index PCR Mix by adding the reagents in the order shown below. Mix thoroughly and centrifuge briefly.

Sample Index PCR Mix	Part Number	1 rxn (µl)	8 rxns + 10% excess (µl)
Nuclease-Free Water	-	8	70
Amplification Master Mix	220125	50	440
SI-PCR Primer	220111	2	18
Total	-	60	528

- b) Add **60 µl** Sample Index PCR Mix to each tube containing **30 µl** purified Post Ligation sample.
- c) Add **10 µl** of an individual Chromium i7 Sample Index to each well and record their assignment. Pipette mix 15 times (pipette set to 90 µl) and centrifuge briefly.

PROTOCOL STEP 3 Library Construction

- d) Index the library DNA in a thermal cycler with the following protocol.

Lid Temperature	Reaction Volume	Run Time
105°C	100 µl	~25-40 min
Step	Temperature	Time
1	98°C	0:45
2	98°C	0:20
3	54°C	0:30
4	72°C	0:20
5	Go to step 2, see table below for # of cycles	
6	72°C	1:00
7	4°C	Hold

NOTE

The optimal number of cycles for the Sample Index PCR reaction is a trade-off between generating sufficient final mass for sequencing and minimizing PCR amplification artifacts.

The following table is a recommended starting point for optimization. Input into Library Construction refers to the cDNA quantification result from step 2.4.

Input into Library Construction	Total Sample Index Cycles
0 – 150 ng	14
150 – 500 ng	12
500 – 1000 ng	10
1000 – 1500 ng	8
>1500 ng	7



- e) Store the tube strip at **4°C** for up to **72 h** or proceed directly to Post Sample Index PCR Double Sided Size Selection.

3.6. Post Sample Index Double Sided Size Selection – SPRI Select

NOTE

After aspirating the desired volume of SPRIselect Reagent, examine the pipette tip(s) before dispensing to ensure that the correct volume is being transferred. Pipette mix thoroughly as insufficient mixing of sample and SPRIselect Reagent will lead to inconsistent size selection results.

- a) Vortex the SPRIselect Reagent until fully resuspended. Add **60 µl** SPRIselect Reagent (**0.6X**) to each sample in the tube strip and pipette mix 15 times (pipette set to 150 µl).
- b) Incubate the tube strip at room temperature for **5 min**.
- c) Place the tube strip in a 10x™ Magnetic Separator in the **High** position until the solution is clear.

CRITICAL!

DO NOT discard supernatant.

- d) Transfer **150 µl** supernatant to a new tube strip and discard the previous tube strip.
- e) Add **20 µl** SPRIselect Reagent (**0.8X**) to each sample in the tube strip and pipette mix 15 times (pipette set to 150 µl).
- f) Incubate the tube strip at room temperature for **5 min**.
- g) Place the tube strip in a 10x Magnetic Separator in the **Low** position until the solution is clear.
- h) Carefully remove and discard **165 µl** supernatant.

NOTE

Due to the low volume of the SPRIselect Reagent used in this step it is crucial to not discard any of the beads with the supernatant. To ensure that beads are not discarded 5 µl of supernatant is left in each well of the tube strip. This will not impact the effectiveness of the size selection.

Repeat

- i) With the tube strip still in a 10x Magnetic Separator, add **200 µl** 80% ethanol to the pellet and stand for **30 sec**.
- j) Carefully remove and discard the ethanol wash.
- k) **Repeat** steps i and j for a total of two washes.
- l) Briefly centrifuge the tube strip and return it to the 10x Magnetic Separator in the **Low** position. Carefully remove and discard the remaining ethanol wash.

NOTE

Due to the low volume of SPRIselect Reagent used in this size selection it is crucial not to over-dry the beads. Proceed directly into resuspension with Buffer EB without waiting for the beads to dry to ensure maximum elution efficiency.

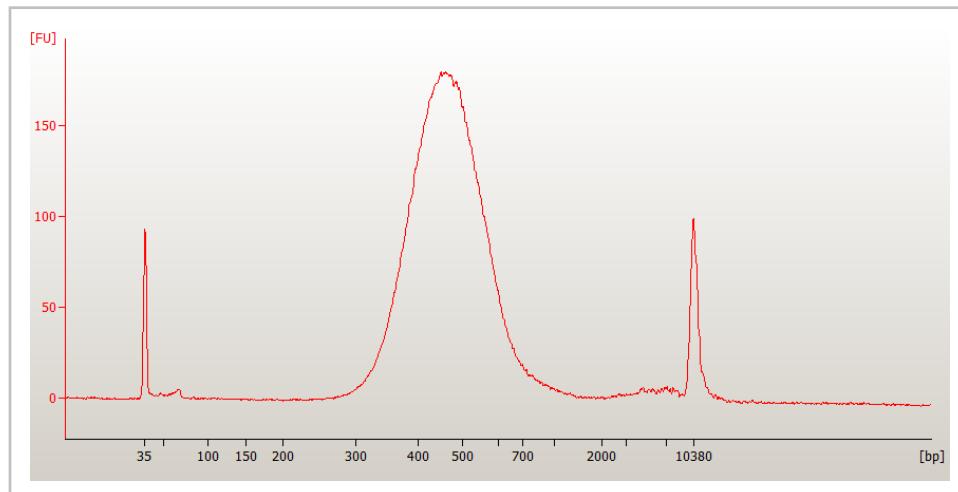
- m) Remove the tube strip from the 10x Magnetic Separator and add **35.5 µl** Buffer EB. Pipette mix 15 times.
- n) Incubate the tube strip at room temperature for **2 min**.
- o) Place the tube strip in a 10x Magnetic Separator in the **High** position until the solution is clear.
- p) Transfer **35 µl** of sample to a new tube strip and cap the sample wells.
- q) Store the tube strip at **4°C** for up to **72 h** or at **-20°C** for **long-term** storage.



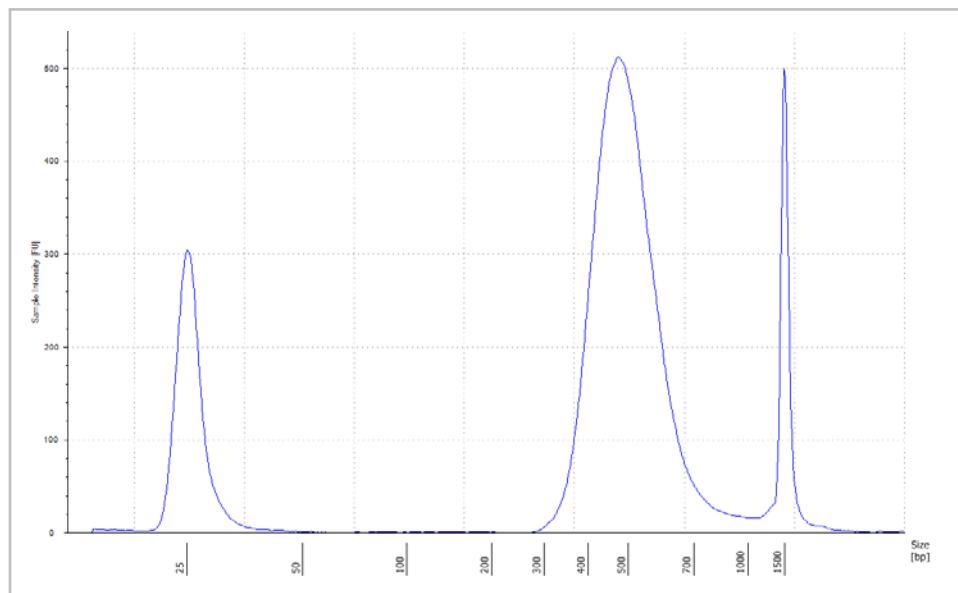
3.7. Post Library Construction QC

- a) **EITHER** Run 1 μ l of sample at 1:10 dilution on the Agilent Bioanalyzer High Sensitivity chip for qualitative analysis. Traces should resemble the overall shape of the sample electropherogram shown below.

A 1:10 dilution ratio is typically sufficient to avoid over-loading the High Sensitivity DNA Chip. For samples of particularly RNA-rich cells, additional dilution may be required to QC the library.



- b) **OR** Run 1 μ l of sample at 1:10 dilution on the Agilent TapeStation High Sensitivity D1000 ScreenTape for qualitative analysis. Traces should resemble the overall shape of the sample electropherogram shown below.



3.8. Post Library Construction Quantification

NOTE

Typically a series of 1:40,000, 1:200,000, 1:1,000,000 and 1:5,000,000 of the completed Single Cell 3' library is required to fall within the dynamic range of the assay.

- a) Thaw Kapa DNA Quantification Kit for Illumina platforms.
- b) Dilute **1 µl** of sample with Nuclease-Free Water to appropriate dilutions that fall within the linear detection range of the Kapa DNA Quantification Kit.
- 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	1 rxn (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** of sample dilutions and **4 µl** DNA Standards to appropriate wells. Centrifuge the PCR plate briefly.
- f) Run DNA Quantification Cycling Protocol with data acquisition at Step 3.

Step	Temperature	Time
1	95°C	3:00
2	95°C	0:05
3	67°C	0:30
4	Go to Step 2, 29X (for 30 cycles in total)	

- g) Follow the manufacturer's recommendations for qPCR analysis. The average fragment size derived from the Bioanalyzer/TapeStation trace from step 3.7 is used as the insert size for accurate library quantification in qPCR.

Protocol Step 4

Sequencing

Sequencing prepared libraries



4. Sequencing Libraries

A Single Cell 3' Library comprises standard Illumina paired-end constructs which begin and end with P5 and P7. The Single Cell 3' v2 16 bp 10x™ Barcodes are encoded at the start of Read 1, while sample index sequences are incorporated as the i7 index read. Read 1 and Read 2 are standard Illumina sequencing primer sites used in paired-end sequencing. Read 1 is used to sequence 16 bp 10x Barcodes and 10 bp randomer, while Read 2 is used to sequence the cDNA fragment.



Each sample index provided in the Single Cell 3' Sample Index Kit combines 4 different sequences in order to balance across all 4 nucleotides.

4.1. Sequencing Depth Recommendations

- a) The technical performance of Single Cell 3' libraries is driven by sequencing coverage per cell. 50,000 raw reads per cell is recommended.
- b) Adjust loading concentrations according to Illumina specifications.
- c) The following are supported sequencing platforms for Single Cell 3' libraries.

Platform
MiSeq
NextSeq 500/550
HiSeq 2500 (Rapid Run and High Output)
HiSeq 3000/4000

4.2. Sequencing Run Parameters

- a) Single Cell 3' libraries use standard Illumina sequencing primers for both sequencing and index reads, and require no custom primers.
- b) Single Cell libraries must be run using paired-end sequencing with dual indexing. The supported number of cycles for each read is shown below.

Sequencing Read	Recommended Number of Cycles
Read 1	26 cycles
i7 Index	8 cycles
i5 Index	0 cycles
Read 2	98 cycles

4.3. Sample Indices

- a) Sample Indices are a mix of four oligos. The 10x™ Sample Index sequence is not needed for the sample sheet, but the 10x Sample Index name (Chromium™ i7 Sample Index plate well ID) is needed if running more than one sample.

4.4. Loading Single Cell 3' Libraries

- a) Once quantified and normalized, Single Cell 3' Libraries should be denatured and diluted as recommended for Illumina sequencing platforms. Refer to Illumina documentation for Denaturing and Diluting Libraries.
- b) Refer to the 10x Genomics® support website for further information.

Practical Tips & Troubleshooting

Processing Fewer than 8 Reactions

Assembling a Chip, 10x™ Chip Holder & 10x™ Gasket

Pipetting Gel Beads

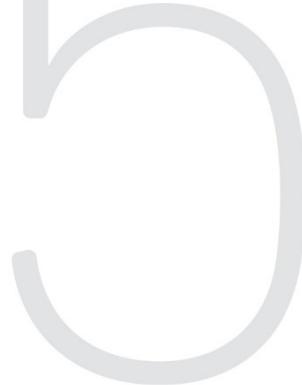
Pipetting GEMs

50% Glycerol Solution

Failure Modes during GEM Generation

Chromium™ Controller Errors

Glossary of Terms



5. Practical Tips & Troubleshooting

5.1. Processing Fewer than 8 Reactions

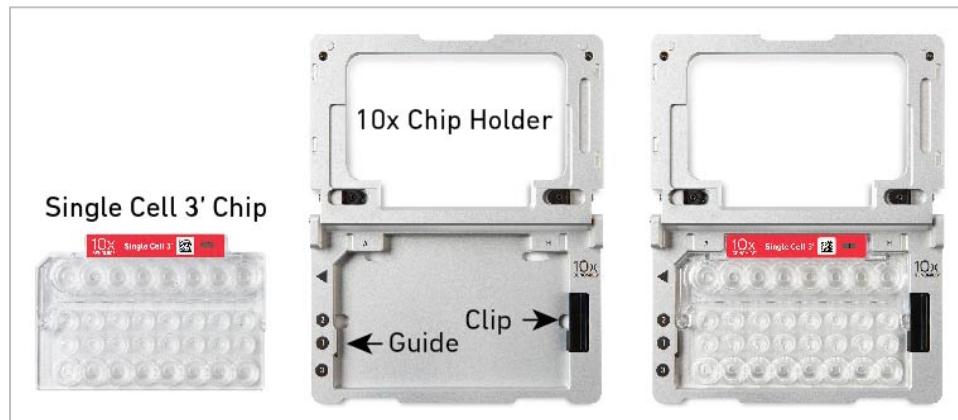
- a) Puncture foil seals in the Gel Bead Strip as needed for a run.
- b) **Store any unused Gel Beads at -80°C and avoid more than 4 freeze-thaw cycles.**
- c) **Never store Gel Beads at -20°C.**
- d) Reagent volumes should be calculated with a 10% excess of 1 rxn values quoted in the protocol. e.g. For 3 samples, multiply the 1 rxn volume quoted in the protocol by 3.3 to determine the suitable volume to prepare. Using larger reagent excesses may reduce the total number of reactions that can be run using one kit.
- e) Store any unused RT Primer at -80°C for future use.

5.2. Assembling a Chip, 10x™ Chip Holder & 10x™ Gasket

NOTE

Always handle the Chromium™ Chip by its edges and avoid touching its bottom surface. Once the chip is in the holder, keep the assembly horizontal at all times to avoid wetting the 10x Gasket with Partitioning Oil.

- a) Align the notch on the upper left corner of the Chromium Chip with the notch on the 10x Chip Holder and insert the left-hand side of the Chromium Chip under the guide.
- b) Depress the right-hand side of the Chromium Chip until the spring-loaded clip engages the Chromium Chip.

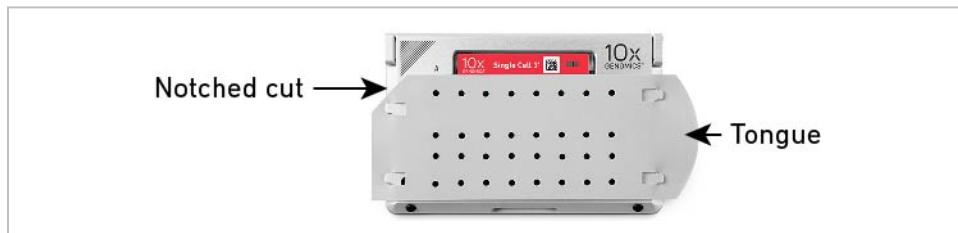


- c) Close the hinged lid of the 10x Chip Holder. After loading the Chromium Chip, the 10x Chip Holder should lay flat on the bench top with the lid closed.

PRACTICAL TIPS & TROUBLESHOOTING



- d) Position the assembly so that the Partitioning Oil wells (row labeled 3) are toward you and identify the rows labeled 1, 2 and 3 for correct addition of the reagents.
- e) After the reagents have been added, attach a 10x™ Gasket by holding the tongue (curved end, to the right) and hook it on the left-hand tabs of the 10x Chip Holder. Gently pull the 10x Gasket toward the right and hook it on the two right-hand tabs. Avoid touching the smooth side of the 10x Gasket and do not press down on the top of the 10x Gasket after attachment. Keep the assembly horizontal to avoid wetting the 10x Gasket with Partitioning Oil.



[Click back to Loading the Single Cell 3' Chip](#)

5.3. Pipetting Gel Beads

- a) After vortexing, remove the Gel Bead Strip from the 10x™ Vortex Adapter and flick the Gel Bead Strip in a sharp, downward motion to ensure maximum Gel Bead recovery. Confirm that there are no bubbles at the bottom of the tube.
- b) Best practices for recovering adequate volume of Gel Beads from the Gel Bead Strip include the following:
 - i. Set a pipette to the volume being pipetted and, without engaging the plunger, puncture the foil seal on the Gel Bead Strip. The pipette tips should extend no more than 2 mm below the seal.
 - ii. Once the holes are formed, raise the pipette tips above the seal and engage the plunger.
 - iii. Lower the tips to the bottom of the wells and widen the opening by gently rocking the tips back and forth, keeping the plunger engaged. Widening the foil seal opening allows the pipette tips to reach the bottom of the Gel Bead Strip wells. This is important for recovering the full volume of Gel Beads required for optimal performance.
 - iv. With the pipette tips still in the Gel Bead Strip, very slowly aspirate the required volume of Gel Beads. After aspiration stops, leave the pipette tips in the wells for an additional 5 sec to allow pressure to equilibrate.



- c) If the required volume of beads cannot be recovered, place the pipette tips against the sidewalls of the Gel Bead Strip wells and slowly dispense the Gel Beads back into the strip. Take care not to introduce bubbles into the wells and verify that the pipette tips contain no leftover Gel Beads. Attempt to withdraw the full volume of beads again by pipetting slowly.

5.4. Pipetting GEMs

- a) After the completion of a Chromium™ Controller run, the hinged lid of the 10x™ Chip Holder is folded back to expose the wells at a 45-degree angle. The GEMs should be aspirated from the lowest points of the Recovery Wells (row labeled ◀) without creating a seal between the tips and the bottom of the wells. Avoid introducing air bubbles.



- b) When transferring the GEMs from the Single Cell 3' Chip after the Chromium Controller run into the Eppendorf® twin-tec 96-Well PCR plate (on a chilled metal block resting on ice), the pipette tips should be positioned against the side walls of the wells as shown below.



[Click back to Loading the Single Cell 3' Chip](#)

5.5. 50% Glycerol Solution

It is critical to add glycerol in a ~50% volume/volume aqueous solution in all unused wells in Rows labeled 1, 2 and 3 of the Single Cell 3' Chip prior to running the Chromium™ Controller or the Chromium Single Cell Controller. 50% glycerol solution can be purchased: Ricca Chemical Company, Glycerin (glycerol), 50% (v/v) Aqueous Solution, PN-3290-32

Alternatively, 50% glycerol solution can be made from a stock solution of glycerol as follows:

- a) Mix an equal volume of water and 99% Glycerol, Molecular Biology Grade.
- b) Filter through a 0.2 µm filter.
- c) Store at -20°C in 1 ml LoBind tubes.
- d) 50% glycerol solution should be equilibrated to room temperature before use.

5.6. Failure Modes during GEM Generation

Reagent Clogs

GEM reagents are manufactured in a cleanroom environment to minimize the level of particles and fibers that could clog microfluidic channels during GEM generation and therefore reduce technical performance.

To avoid clogs, it is also important for users to minimize exposure of reagents, chips, and gaskets to sources of particles and fibers such as open reagent reservoirs, laboratory wipes, frequently opened flip-cap tubes, clothing that easily sheds fibers, and dusty surfaces.

There are several ways to identify if a clog has occurred as outlined below. If any of the following occur, take a picture and send it to support@10xgenomics.com for further assistance. If a channel clogs during GEM generation, it is recommended that the sample be remade.

Wetting Failures

Once reagents are added to the Chromium Chip wells, they immediately flow into and prime the microfluidic channels on the chip. Incorrect priming can result in wetting failures, in which polydisperse, millimeter-scale droplets are formed instead of a uniform GEM.

To minimize the occurrence of wetting failures, it is critical to add reagents in the stipulated order and to wait 30 sec between addition of Master Mix and addition of Gel Beads.

There are several ways to identify if a wetting failure has occurred as outlined below. If any of the following occur, take a picture and send it to support@10xgenomics.com for further assistance. If a wetting failure occurs during GEM generation, it is recommended that the sample be remade.

Normal Operations	Reagent Clogs or Wetting Failures
 <p data-bbox="204 572 775 665">After the Chromium™ Single Cell 3' Chip is removed from the Chromium Controller and the wells exposed:</p> <p data-bbox="204 677 791 739">All Recovery Well levels are similar in volume and opacity.</p>	 <p data-bbox="827 572 1421 665">After the Chromium Single Cell 3' Chip is removed from the Chromium Controller and the wells exposed:</p> <p data-bbox="827 677 1351 739">Recovery Well G indicates a reagent clog has occurred in this channel.</p> <p data-bbox="827 751 1388 844">Recovery Wells C and E indicate a wetting failure has occurred in these channels. There is an absence of emulsion.</p> <p data-bbox="827 857 1274 889">Recovery Wells B, D, and F are normal.</p> <p data-bbox="827 901 1411 963">Note 50 % glycerol solution was entered into wells A and H.</p>
 <p data-bbox="204 1444 747 1505">After aspirating the GEMs from the Chromium Single Cell 3' Chip Recovery Wells:</p> <p data-bbox="204 1518 780 1579">All liquid levels are similar in volume and opacity. There is also no air in the pipette tips.</p>	 <p data-bbox="827 1444 1370 1505">After aspirating the GEMs from the Chromium Single Cell 3' Chip Recovery Wells:</p> <p data-bbox="827 1518 1421 1685">Pipette tip G indicates a reagent clog has occurred in this channel. There is excess Partitioning Oil (clear) and air in the pipette tip. In some reagent clog cases, there is only 5-10 µl excess Partitioning Oil (and no air) in the pipette tip.</p> <p data-bbox="827 1698 1421 1816">Pipette tips C and E indicate a wetting failure has occurred in these channels. Pipette tip C contains partially emulsified GEMs. Emulsion is absent in pipette tip E.</p> <p data-bbox="827 1828 1230 1860">Pipette tips B, D, and F are normal.</p> <p data-bbox="827 1873 1204 1902">Pipette tips A and H are missing.</p>

Normal Operations	Reagent Clogs or Wetting Failures
	
<p>After transfer of the GEMs + Recovery Agent to a tube strip:</p> <p>All liquid levels are similar in the aqueous sample volume (clear) and Recovery Agent/Partitioning Oil (pink).</p>	<p>After transfer of the GEMs + Recovery Agent to a tube strip:</p> <p>Tube G indicates a reagent clog has occurred in this channel. There is a decreased volume of aqueous sample (clear) when compared to normal channels B, D, and F.</p> <p>Tubes C and E indicate a wetting failure has occurred in these channels. There can be an abnormal volume of Recovery Agent/Partitioning Oil (pink) when compared to normal channels B, D, and F.</p> <p>Tubes A and H are empty.</p>
	
<p>After removing the designated volume of Recovery Agent/Partitioning Oil:</p> <p>All liquid volumes are similar in the aqueous sample volume (clear) and residual Recovery Agent/Partitioning Oil (pink).</p>	<p>After removing the designated volume of Recovery Agent/Partitioning Oil:</p> <p>Tube G indicates a reagent clog has occurred in this channel. There is a decreased volume of aqueous sample (clear). There is also a greater residual volume of Recovery Agent/Partitioning Oil (pink) when compared to normal channels B, D, and F.</p> <p>Tubes C and E indicate a wetting failure has occurred in these channels. There is an abnormal residual volume of Recovery Agent/Partitioning Oil (pink) when compared to normal channels B, D, and F.</p> <p>Tubes A and H are empty.</p>

Normal Operations	Reagent Clogs or Wetting Failures
 <p>A B C D E F G H</p> <p>After addition of DynaBeads Cleanup Mix: All liquid volumes are similar after addition of the DynaBeads Cleanup Mix.</p>	 <p>A B C D E F G H</p> <p>After addition of DynaBeads Cleanup Mix: Tube G indicates a reagent clog has occurred in this channel. There is an abnormal ratio of Dynabeads Cleanup Mix (brown) to Recovery Agent/Partitioning Oil (appears white) when compared to normal channels B, D, and F. Tubes C and E indicate a wetting failure has occurred in these channels. There is an abnormal ratio of Dynabeads Cleanup Mix (brown) to Recovery Agent/Partitioning Oil (appears white) when compared to normal channels B, D, and F. Tubes A and H are empty.</p>

<p>After removing the Chromium™ Single Cell 3' Chip from the Chromium Controller: The image opposite illustrates clogs have occurred in the Gel Bead line (orange arrow) and the Sample line (yellow arrow) as evidenced by higher than usual residual volumes in the input wells.</p>	
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5.7. Chromium™ Controller Errors

If the Chromium Controller or 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 10x™ Chip 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 there is a 10x Gasket on the Chromium Chip. In the case the 10x Gasket installation was forgotten, install and try again. In the case a 10x Gasket was already installed, remove, reapply, and try again. If the error message is still received after trying either of these more than twice, contact support@10xgenomics.com for further assistance.
- c) **Pressure not at Setpoint:**
 - 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, it is likely one or more of the reagents was not loaded into the Chromium Chip. In this case, the Chromium Chip must be discarded. **Do not try running this Chromium Chip again as this will damage the Chromium Controller.**
- d) **CAUTION: Chip Holder not Present:** Eject the tray by pressing the eject button to check there is a 10x Chip Holder encasing the Chromium Chip. In the case the 10x Chip Holder was forgotten, install with a 10x Gasket in place, and try again. If the error message is still received after a 10x Chip Holder is confirmed as in place, contact support@10xgenomics.com for further assistance.
- e) **Invalid Chip CRC Value:** This indicates the Chromium Chip has encountered an error, should not be run, and must be discarded. Contact support@10xgenomics.com for further assistance.

[Click back to Running the Chromium Controller](#)

5.8. Glossary of Terms

10x™ Barcode

Defined DNA sequences that are added to each cDNA generated in a GEM so they can be distinguished and sorted during data analysis.

Chromium™ Single Cell 3' Chip

The Chromium Single Cell 3' Chip is a microfluidic chip specifically designed to run the Single Cell 3' Protocol in the Chromium Controller. The Single Cell 3' Chip is indicated by a red label at the top of the chip. Other chips used with the Chromium System include the Chromium Genome Chip.

Gel Beads

Gel Beads are the foundation of 10x Genomics® technology, and are beads functionalized with millions of copies of a 10x Barcoded primer. Gel Beads are provided in 8-reaction Gel Bead Strips.

GEM

GEM is an abbreviation of Gel Bead-In-EMulsion. In the Single Cell 3' Chip, a library of Single Cell 3' Gel Beads is combined with cells and a reverse transcriptase (RT) Master Mix to create single nanoliter reaction volumes partitioned by oil.

GemCode™ Technology

The GemCode Technology is the microfluidic chip-based technology that partitions cells across tens of thousands of GEMs. Upon isothermal incubation, the cDNA produced in each GEM contains a 10x Barcode that identifies them as having originated from the same sample partition.